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I am delighted to extend a warm welcome to each of you to our esteemed scientific journal dedicated to the exploration of Applied Research in Biological Sciences.

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Thank you for your unwavering commitment to advancing applied research in the biological sciences. I eagerly anticipate the wealth of transformative discoveries and insights that will emerge from your invaluable contributions.

Warm regards,

Afshar Mirzaei-Aghsaghali
Editor-in-Chief

RISK FACTORS OF CHILDREN'S ASTHMA (6-12 YEARS OLD) IN KHORRAMABAD, IRAN: A CASE CONTROL STUDY

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ABSTRACT

The aim of this study is to investigate the risk factors involved in the morbidity of asthma in Khorramabad city and its suburban areas. In the present case-control research, all the patients who had referred to the Shahid Rahimi Hospital and a pulmonary disease sub-specialist's office in Khorramabad in 2015 and had been diagnosed with asthma by a pulmonary disease sub-specialist and based on the criteria of Global Initiative for Asthma (GINA) were enrolled in the study. The census taking sampling method was used and all patients with immunodeficiency, congenital diseases, chronic allergic pulmonary diseases or sinusitis were excluded. The control group that matched the experimental group in terms of age, sex and the place of residence were selected from among those referring to the ophthalmological and dermatological clinics of the Shahid Rahimi Hospital. The data collection instrument used in this study was a questionnaire that included items related to the demographic information of the patients, and also questions regarding the patient's economic and social situation as well as the patient's clinical symptoms from birth until the present. All parents of the selected children signed written consents regarding their participation in the study. The data were analyzed using SPSS software. The mean age of the patients was 8.5 ± 2.1 and 60.3 percent of the patients were male. The average age of the mothers of asthmatic children was higher than the mothers of healthy children and this difference was statistically significant (P -value= 0.009). The mean duration of breastfeeding in healthy children was higher than the asthmatic children and this difference was Statistically significant (P -value= 0.001). There was observed a statistically significant relationship between a background of asthma or allergy among first-degree relatives and the development of asthma in the patients themselves (P -value < 0.05). The children of highly educated parents were less likely to develop allergic diseases and asthma, which could be attributed to the knowledge of the parents about various diseases. Given the risk factors, it can be concluded that acquired factors can play an important role in the occurrence of this disease in children.

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INTRODUCTION

Global Strategy for Asthma Management and Prevention Guidelines define asthma as “a chronic inflammatory disorder of the airways associated with increased airway hyper-responsiveness, recurrent episodes of wheezing, breathlessness, chest tightness, and coughing” [1]. Asthma, which typically begins in childhood and is the most common chronic disease of childhood, has reached epidemic proportions [2-4]. In the International Study of Asthma and Allergies in Childhood (ISAAC), the highest asthma prevalence was observed in westernized English-speaking countries (e.g., the United Kingdom, Australia, and New Zealand), with much lower prevalence rates in Eastern Europe, India, China, other countries in Asia, and Africa [5]. This observation has led to the belief that the rapid increases in asthma prevalence are more likely to be attributable to environmental than genetic factors. The rising prevalence in the United States and worldwide seems to be correlated with modern industrialization, suggesting that changes in the ambient environment may contribute to this increase in morbidity and mortality [6].

The responsible causes of initiating asthma are specific factors referred as allergens, which are present in the patients' surroundings originating from outdoor and indoor environment. Hence, allergens are divided into two categories; the outdoor allergens such as pollen grains, fungal spores, dust particles and non-specific irritants, and the indoor allergens such as House Dust Mites (HDMs), animal allergens, fungal allergens, insects, and rodent allergens, etc. [7].

In addition to sensitizing factors mentioned above, there are other non-sensitizing or irritating factors such as air pollution (primarily chemicals), cigarette and tobacco smokes, etc. present in both outdoor and indoor environment.

These irritating factors also play a role in the exacerbation of the allergic symptoms or may cause asthma and breathing problems temporarily [5, 8].

Considering the diversity of asthma risk factors in different regions of the world, much work remains to be done in defining the environmental factors that may cause asthma and that may trigger asthma exacerbations in individuals with the disease. Because of this issue, this study tries to investigate the risk factors involved in the morbidity of asthma in Khorramabad (a mountainous region in Lorestan province, southwest of Iran); so that, by identifying these factors and educating parents, the occurrence of this disease can be prevented as much as possible.

MATERIALS AND METHODS

In the present case-control research, all the patients who had referred to the Shahid Rahimi Hospital and a pulmonary disease sub-specialist's office in Khorramabad (a mountainous region located over 1000 meters above sea level in Lorestan province, southwest of Iran [Figure-1]) in 2015 and had been diagnosed with asthma by a pulmonary disease sub-specialist and based on the criteria of Global Initiative for Asthma (GINA) were enrolled in the study. GINA is the standard criteria used to diagnose asthma based on clinical symptoms and patient history and its items include: continuous wheezing in the chest more than once a month, wheezing and coughing after physical activity, the presence of coughs especially nighttime coughs without viral infection, wheezing that is independent of the effect of the season, continuation or worsening of symptoms after 3 to 6 years of age when encountering furry animals, chemical compounds, air fresheners and detergents, intense physical activity, viral infections of the respiratory tract, exposure to cigarette smoke, and exposure to emotional situations, remission of symptoms by medicines used for the treatment of asthma, and persistent colds, or the continuation of disease for more than 10 days. In the present research, only patients residing in Khorramabad and its suburban areas were studied. The census taking sampling method was used and all patients with immunodeficiency, congenital diseases, chronic allergic pulmonary diseases or sinusitis were excluded. Finally, 150 patients diagnosed with asthma were selected as the volume of the sample. The control group that matched the experimental group in terms of age, sex and place of residence were selected from among those referring to the ophthalmological and dermatological clinics of the Shahid Rahimi Hospital, provided that they had no backgrounds of chronic physical and mental illnesses.

The data collection instrument used in this study was a questionnaire that included items related to the demographic information of the patients, and also questions regarding the patient's economic and social situation as well as the patient's clinical symptoms from birth until the present. Based on reference books, factors listed in the questionnaire were the most important factors affecting asthma. The same data were collected from the members of the control group. All the diagnostic studies for the diagnosis of the disease were carried out by a pulmonary disease sub-specialist. All parents of the selected children signed written consents

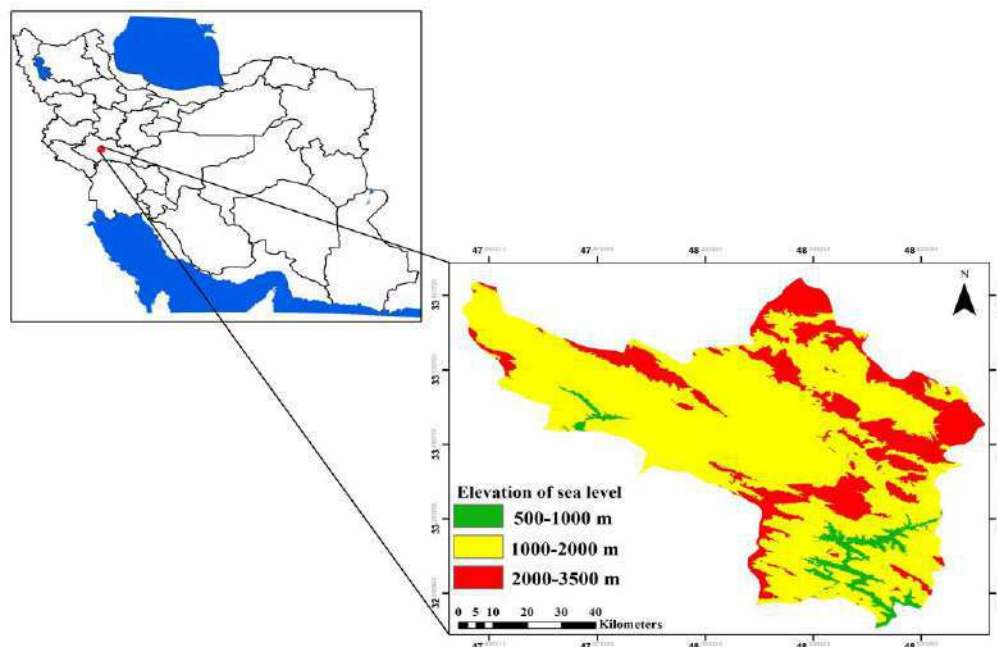


Fig: 1. Digital Elevation Mode (DEM) of Khorramabad city and its suburban areas

regarding their participation in the study. The data were analyzed using SPSS software. Descriptive statistical methods, the Chi-square test, and the Fisher's exact test, were used. In order to determine the intensity of the risk factors and the development of asthma, the odds ratio estimate was used with a confidence interval of 95%.

RESULTS

In the present research, 150 patients suffering from asthma and 150 children in the control group were studied. The mean age of the patients was 8.5 ± 2.1 and the mean age of the control group members was 8.1 ± 4.2 . The youngest patient was 4 years old and the oldest was 14 years old. 60.3% of the children were male and 39.7% were female, while the same figures in the control group were 61.9% and 38.1% respectively. The sex ration (male over female) of the patients suffering from asthma was 1.5 over 1. The average age of the mothers of asthmatic children (26 ± 5 years old) was higher than the mothers of healthy children and this difference was statistically significant (P -value= 0.009). The frequency distribution of the demographic characteristics of the patients and the control group is presented in [Table-1] in detail.

Table: 1. Frequency distribution of the demographics of the patients and the control group

Type of variable		Patients N (%)	Control group N (%)	P value
Sex	Male	94 (62.7)	88 (58.7)	
	Female	56 (37.3)	62 (41.3)	
Father's educational attainment	Illiterate	6 (4)	7 (4.7)	0.001
	Junior high school or less	55 (36.7)	8 (5.3)	
	High school or High school diploma	27 (18)	49 (32.6)	
	University	62 (41.3)	86 (57.3)	
Mother's educational attainment	Illiterate	19 (12.7)	3 (2)	0.001
	Junior high school or less	41 (27.3)	26 (17.3)	
	High school or High school diploma	49 (32.7)	52 (34.7)	
	University	41 (27.3)	69 (46)	
Father's Occupation	Office employee	63 (42)	68 (45.3)	0.161
	Self-employed	86 (57.3)	79 (52.7)	
	Unemployed	1 (0.7)	3 (2)	
Mother's Occupation	Office employee	15 (10)	10 (6.7)	0.231
	Self-employed	3 (2)	5 (3.3)	
	Housewife	132 (88)	135 (90)	
Place of residence	Urban areas	132 (88)	150 (100)	
	Rural areas	18 (12)	0 (0)	

The mean duration of breastfeeding in healthy children was higher than the asthmatic children and this difference was Statistically significant (P -value= 0.001). In the case group, 42% of mothers had vaginal delivery, the same figure in the control group was 46.7%, this difference was not statistically significant (P -value= 0.243). On the other hand, the average number of family members of asthmatic children (4.7 ± 2.1) was higher than the average number of family members of healthy children (3.9 ± 0.6) and this difference was statistically significant (P -value= 0.023).

One of the main objectives of this study was to investigate the relationship between asthma and having a familial background of asthma or allergy among first-degree relatives and to determine the intensity of this relationship. In this regard, 62 patients (41.3%) had a familial background of asthma among their first-degree relatives. The same figure in the control group was 34 cases (22.7%) and based on the chai squared test, this difference was statistically significant (P -value=0.004). The intensity of the relationship was determined using the odds ratio, which was calculated to be 3.96 (CI=0.48-7.61). Additionally, 71 patients (47.3%) had a familial background of allergy among their first-degree relatives. The same figure in the control group was 40 cases (26.7%) and based on the chai squared test, this difference was statistically significant (P -value=0.001). The intensity of the relationship was determined using the odds ratio, which was calculated to be 4.5 (CI=2.21-8.16).

In present study, tobacco consumption was higher among the parents of asthmatic children, but the difference was not statistically significant (P -value=0.093).

DISCUSSION

Since asthma is one of the most important health problems, identifying its risk factors is very important for diagnostic and preventive purposes [9]. In the present research, 150 asthmatic children who had referred to the Shahid Rahimi hospital of Khorramabad during one year were studied. Given the fact that the afore-mentioned hospital is the only center providing pulmonary disease sub-specialty services, this study can provide valuable information regarding the risk factors of asthma in a mountainous region.

Several factors are involved in the incidence of asthma among children, in some cases, several factors together lead to the exacerbation of this disease. As mentioned in the introduction, nowadays extensive environmental and genetic studies have investigated factors contributing to the exacerbation of asthma [5, 7, 8].

The parents play a very important role in the management of asthma in their children [9]. In this study, the educational attainment level of the parents of non-asthmatic children was significantly higher (especially the educational attainment level of the fathers). So considering the studies conducted in this regard [10, 11], it can be said that purposeful training for parents can have a very important role in preventing asthma.

The mother's age at the time of the pregnancy is one of the important factors in the birth of a healthy child. The mother's high age during pregnancy can be dangerous for the child (high age of the mother during pregnancy creates a series of psychological reactions and stresses which also affects the severity of the disease). In this study, the average age of the mothers of asthmatic children was significantly higher than the mothers of the control group [12]. In the present study, smoking was not recognized as a significant risk factor, but the number of smoking parents in the asthmatic group was higher. Haghbin et al conducted a study on this subject and found out that smoking together with underlying genetic factors play an important role in the pathogenesis of asthma in children [13], also in another study conducted by Gioviner et al smoking by the parents was recognized as a very important factor in the exacerbation of this disease [9].

Since in this study the number of city-dwellers were more than that of the inhabitants of rural areas, statistically the frequency of occurrence of asthma among urban-dwelling children was higher. Because this study was conducted in Khorramabad and its surrounding villages, 12% of the patients belonged to rural areas. In a study conducted by Zuo et al in Beijing, the percentage of children suffering from asthma in rural areas was less than in urban areas [14].

In the present study, the number of parents with a history of allergy (especially in fathers) was significantly higher in the asthmatic group. In studies conducted by Kim et al, a parental history of allergic diseases was recognized as the most important risk factor in the occurrence of asthma which confirms the results of the present study [15]. In terms of quality of life, frequency of asthma among children who live in crowded families was higher than children who live in smaller families. The results of a study conducted by Arash et al confirm the results obtained from the present study [16, 17].

The results of the present study show that there is no relationship between asthma and type of childbirth. A study conducted by Mohammadzadeh et al shows the same results [18]. In another study too, the type of childbirth was not related to the occurrence of asthma [19].

CONCLUSION

According to the findings of this study it can be said that educated parents protect their children better against allergy and asthma and this is due to their knowledge about the disease. Also, it can be said that acquired factors play an important role in the occurrence of asthma in children. So educating mothers during pregnancy and raising their awareness by brochures and other medias can be useful.

Because of some limitations, this research only studied the environmental factors contributing to asthma. We suggest an evaluation of both environmental and genetic factors in larger studies for further clarification. Since the best time to prevent asthma and other allergies is before birth, it is recommended that pregnant mothers, who have a history of asthma or allergies in their family, should avoid environmental pollution. Additionally, breastfeeding is strongly recommended during infancy, especially among families who are genetically predisposed to asthma.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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FINANCIAL DISCLOSURE

The authors report no financial interests or potential conflicts of interest.

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PROGNOSIS AND COMPLICATIONS OF TRACHEAL STENOSIS AND TRACHEAL RECONSTRUCTIVE SURGERY IN PATIENTS REFERRED TO AL-ZAHRA HOSPITAL IN ISFAHAN PROVINCE DURING A TEN-YEAR PERIOD: A SINGLE REFERRAL CENTER EXPERIENCE

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ABSTRACT

The aim of this study is, to evaluate the prognosis and complications of tracheal stenosis and tracheal reconstructive surgery. This descriptive study was conducted on patients with surgical repairing of tracheal stenosis in thorax surgery center of Al-Zahra hospital in Isfahan province (center of Iran) during a ten-year period (April 2003 to March 2013). Sampling method was census and information was collected through researcher by a questionnaire including demographic characteristics, post-operative complications, the number of need for bronchoscopy and the current status of patients, presence of tracheostomy at the time of presentation, intubation causes, intubation period, type of endotracheal tube, clinical symptoms of tracheal stenosis, time of beginning of symptom after extubation, length of removed part of trachea, complications, time of hospitalization, and the view of patients about their status after surgery. After collecting and completing information about patients, the data was analyzed through descriptive statistical techniques using SPSS software. Fifty-two patients with surgical repairing of post-intubation tracheal stenosis were studied, 75% of them were males. The mean age of patients was 39.15 ± 2.67 years old. The most common chief complaint was dyspnea in 39 patients (75%). Wound infection was recorded in 4 patients (7.7%), as well as dysfunction of vocal cord was recorded in 8 cases (15.4%) and anastomosis failure was recorded in 1 patient (1.9%). On the other hand, recurrence of stenosis occurred in 11 patients (21.2%). Failure of surgical treatment observed in 14 patients (3 cases dead during or after surgery and 11 cases required re-dilatation). The surgical treatment was successful in 38 other cases (73%). We note that by adding the 7 cases that were improved by re-dilatation the success rate of surgical treatment increase from 73% to 86.5%. Tracheal stenosis is one of the important complications of intubation. This complication is the most common cause of partial resection of trachea. So recognizing these complications is helpful for effective treatment planning.

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INTRODUCTION

One of the major complications of intubation is tracheal stenosis that caused physical discomforts and high therapeutic costs for patients. Intubation is one of acts that is often done for very ill patients, which can cause changes in the structure of the trachea and in some cases lead to tracheal stenosis [1]. Intubation-induced tracheal stenosis can complicate the treatment of patients and increase mortality risk in these patients [2]. This problem occurs at 19% of the tracheal intubation cases [3].

This disorder is the most common cause of partial removal of trachea [4]. Different factors create this complication; which one of the most important factors is the duration of intubation that there is no consensus about its role. Other important factors including: some underlying diseases (such as diabetes) and any factors that will delay repairing of the lesion [5]. The main pathophysiology of intubation-induced tracheal stenosis are cuff pressure of tracheal tube, chronic inflammation and fibrosis [6].

Intubation-induced tracheal stenosis is affected by patient factors [7], used tools [8] and expertness of the person who performs intubation [9]. So, accurate identification of each of these factors can play a main role in the prevention of Intubation-induced tracheal stenosis. The aims of this study are to evaluate the result and complications of tracheal stenosis and its repairing surgery, and to reduce the consequences and mortality rate of this disease.

MATERIALS AND METHODS

This descriptive study was conducted on patients with surgical repairing of tracheal stenosis in thorax surgery center of Al-Zahra hospital in Isfahan province (center of Iran) during a ten-year period (April 2003 to March 2013). Patients with incomplete medical profile and unavailable patients were excluded from the study. Sampling method was census and the medical records of all patients who underwent surgical repair of trachea was assessed. Information was collected through researcher by a questionnaire including demographic characteristics, post-operative complications, the number of need for bronchoscopy and the current status of patients (dyspnea, impaired daily function, irritative cough, hoarseness, having or not having a tracheostomy), presence of tracheostomy at the time of presentation, intubation causes (trauma, suicide, brain surgery and other causes), intubation period, type of endotracheal tube (with or without cuff), clinical symptoms of tracheal stenosis (dyspnea, cough, or stridor), time of beginning of symptom after extubation, length of removed part of trachea, complications (wound infection, recurrent stenosis, vocal cord dysfunction, pneumonia, anastomotic failure), time of hospitalization, and the view of patients about their status after surgery (much better, better, no matter, worse).

The validity of study was confirmed by expert panel. After collecting and completing information about patients, the data was analyzed through descriptive statistical techniques (standard mean scores, ratios, frequency percent and Fishers exact test).

RESULTS

We examined 64 patients using the inclusion criteria. Twelve patients were ineligible for the study because of incomplete medical profile and unavailable patients. Finally 52 patients were enrolled to this study. The mean age of patients was 39.15 ± 2.67 years old. Also, the 75% of patients were males and 25% of them were females. The frequency distribution of the demographic characteristics of the patients is presented in [Table-1] in detail.

Table: 1. Frequency distribution of the demographics of the patients

Type of variable	Patients N (%)	
Age groups	20-39	2 (3.8)
	40-59	19 (36.6)
	60-79	27 (51.9)
	>80	4 (7.7)
Sex	Male	39 (75)
	Female	13 (25)
Marital status	Married	43 (82.7)
	Single	9 (17.3)
Educational attainment	Illiterate	2 (3.8)
	Junior high school or less	7 (13.5)
	High school or High school diploma	10 (19.2)
	University	33 (63.5)
Occupation	Office employee	12 (23.1)
	Laborer	4 (7.7)
	Self-employed	10 (19.2)
	Unemployed	10 (19.2)
	Housewife	7 (13.5)
	Farmer or Stockbreeder	9 (17.3)

The results show that, 18 patients (34.6%) due to trauma, 1 case (1.9%) due to suicide, and 33 cases (63.5%) due to brain surgery were intubated, which based on age and state of them intubation was performed using cuffed endotracheal tube. Also, the chief complaints of patients were dyspnea in 39 patients (75%), coughing (without dyspnea) in 4 cases (7.7%), both symptoms (cough with dyspnea) in 5 (9.6%) cases and stridor in 1 (1.6%) case.

In addition, 25 patients (48%) had received tracheostomy before admission, that on average, 7.49 ± 3043 months after extubation were candidate for surgery (resection and anastomosis). On average, patients were intubated 21.69 ± 17.79 days. The average length of resected trachea was 2.44 ± 0.84 cm.

Wound infection was recorded in 4 patients (7.7%), as well as dysfunction of vocal cord was recorded in 8 cases (15.4%) and anastomosis failure (this was due to the long length of stenosis that required the lot release trachea and in ICU due to patient's agitation neck sutures was removed within 1 day) was recorded in 1 patient (1.9%). No case of pneumonia was reported. Recurrence of stenosis occurred in 11 patients (21.2%), that 2 cases of them had tracheostomy and 2 other cases had dyspnea without obvious dysfunction as well as 7 patients of them were improved after re-dilation. Also 3 deaths occurred during and after surgery that 1 case occurred during surgery due to cardiac arrest and 1 case occurred due to anastomotic disruption after surgery; and 1 case died due to heart attack in 1 day after surgery.

Bronchoscopy was performed in 21 patients after surgery, so that in ten patients bronchoscopy was performed just for evaluation of anastomosis status and location but bronchoscopic dilatation was performed in 11 other patients (3 times in 2 cases, 2 times in 1 case and 1 time in 8 cases).

Two patients (3.8%) that had sub glottis stenosis underwent extensive resection surgery, which due to restenosis after surgery were undergoing bronchoscopy, finally, tracheostomy performed for them after failure of bronchoscopy.

Also two patients (3.8%) had dyspnea on exertion (without impairment in their daily function). Three patients (5.8%) had hoarseness. None of the patients had irritative cough.

Investigating the view of patients about their status after surgery showed that, 26 cases (50%) believe that their status is much better, 18 cases (34.6%) believe that their status is better, 6 cases (11.5%) believe that there is no change and 2 cases (3.8%) believe that their status is worse than before surgery. Failure of surgical treatment observed in 14 patients (3 cases dead during and after surgery and 11 cases required re-dilatation). The surgical treatment was successful in 38 other cases (73%). We note that by adding the 7 cases that were improved by re-dilatation the success rate of surgical treatment increase from 73% to 86.5%.

DISCUSSION

Tracheal stenosis is a serious and life threatening disease [10] with various causes including intubation, tracheostomy, trauma [10, 11] tumors [10] burns [12] and idiopathic causes [13].

Despite the known etiology of post-intubation tracheal stenosis and the variety of preventive methods [14, 15], increased using of intubation for airway protection caused high incidence of tracheal stenosis [10, 13]. In fact, post-intubation tracheal stenosis is the result of regional ischemic necrosis of the airways [16].

Although tracheal injuries are accompanied by different symptoms but the diagnosis of these injuries is difficult in many cases. Appropriate radiographic tests like fluoroscopy, tomography and CT-scan can use for ruling out the possibility of tracheal damage in symptomatic patients (symptoms of airway obstruction) with normal lung fields on chest X-ray. Rarely, advanced techniques failed in diagnosis of atypical lesions, so bronchoscopy is indicated in this cases [17].

Evaluation of pulmonary function in patients with tracheal obstructive lesions, showed a high degree obstruction of airways. Sometimes this evaluation can determine the presence of parenchymal diseases, and in some cases the need for more extensive surgeries. Functional tests, especially Forced Expiratory Volume in one second (FEV1) and maximum expiratory flow-volume curve can provide suitable base for evaluation of the results.

Therapeutic methods of tracheal stenosis including endoscopy using laser, endoscopic dilatation, interposition graft, tracheal stenting, and resection and reconstruction (gold standard in benign tracheal stenosis treatment) [11, 15, 18]. On the other hand, post-intubation tracheal stenosis is the most common indication for resection and reconstruction [14, 15, 16], but in patients who are not appropriate candidates for surgery, tracheal stenting is a better treatment [10, 11].

As seen above, our finding about mean age of patients (the mean age of patients was 39.15 ± 2.67 years old) not confirmed the results of other studies (the mean age of the patients who required intubation in other studies, is

between 17 to 30 years old [9], which this finding can be due to selecting the study patients from cases who had underlying diseases and brain surgery that have usually old age.

Stenosis of airways after intubation affected by different factors such as intubation period, that based on our result (intubation period was 21 days, on average), we emphasize the necessity of appropriate planning for making decision aimed to reduce intubation period.

In present study, extubation to surgery lasted 7.49 ± 3043 months on average, which this long term is consistent with brain surgery (most common cause of intubation (65%)) and its complications that lead to long term movement disorder. In fact, inactivity will lead to shrinkage of airway and reduce the diameter of the airway, before clinical symptoms were appeared, in this circumstances fatal obstruction can be occur at any time [19].

Also, length of resected trachea is one of the factors that directly linked with recurrence of stenosis after surgery [20]. So we can said that the high success rate of this study can be caused by the length of resected trachea that was 2.44 ± 0.84 cm on average.

In the context of view of patients about their status after surgery, 84.6% of them feel health, that this finding consistent with success rate of surgical treatment that was 86.5%. Finally, according to this fact that the best way to manage the complications of tracheal stenosis surgery is prevent them, so recognizing these complications is helpful for effective treatment planning.

CONCLUSION

Tracheal stenosis is one of the important complications of intubation. This complication is the most common cause of partial resection of trachea [4]. So recognizing these complications is helpful for effective treatment planning.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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EFFECT OF MILK PLANT VITISVINIFERA EXTRACT ON NONSPECIFIC IMMUNITY OF RAINBOW TROUT (ONCORHYNCHUSMYKISS)

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ABSTRACT

Use of medicinal plants as compounds stimulating and strengthening the immune system of fish has been considered in recent years. However, there is a very little and inadequate knowledge in this area, unfortunately. The aim of this study was to investigate the impact of leaves and twigs extracts of *Vitisvinifera* plant on non-specific immunity of rainbow trout (*Oncorhynchusmykiss*). In this study, changes in the level of immunoglobulins IgG, total complement, lysozyme and peroxidase of fish fed by leaves and twigs of *Vitisvinifera* plant at doses of 100, 400 and 800 mg of drug per kilogram of commercial food for 28 days were examined. Changes in levels of immunoglobulin (IgM), total complement, and lysozyme activity in plasma of fish fed by nutrient supplements were not significant compared to the control group ($p < 0.05$). Peroxidase activity level in the plasma of fish fed by supplements of leaves and twigs of *Vitisvinifera* shows significant increase compared with experimental groups ($p < 0.05$). The results of this study suggest a positive impact of leaves and twigs of *Vitisvinifera* plant in relative increase in the immune system of fish.

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INTRODUCTION

The important role of immune system in maintaining the aquatic animal health and ensuring their survival and growth during the period of breeding and rearing has caused researchers to use a variety of stimulating chemical and natural compounds to strengthen the immune system. In the past decades, wide range of studies has been conducted regarding the use of medicinal plants in laboratory scale to strengthen the immune system of laboratory animals. The obtained results of these studies show a positive role of many of these medical plants and herbs in strengthening the immune system of animals [1].

For example, a study conducted on the stimulating role of medical plants of *Thymus vulgaris* and geranium *Plargvnyvm* in strengthening the immune system of laboratory animals indicate that the positive effect of these medicines in strengthening of their immune system [2]. Jafarian et al (2002) showed that the use of extract of medicinal plant of arveneh in the diet of laboratory animals can strengthen and improve the cellular and humoral immune system. Use of medicinal plants is one of the new approaches of using these compounds in pharmacology to treat and cope with viral infections [3,4], bacterial infections [5], fungal infections [1] and even preventing the spread protozoan parasites [6]. The use of medicinal plants as anti-fungal compounds [7] and anti-bacterial compounds, and as compounds stimulating the immune system [8] has old history to enhance the immune system of the fish. However, many of the known species of plants have adverse effects on consumers. For example, *Achilleatalagonica* Boiss plant and *A. tenuifolia* Lam plant from the family of yarrow plant, as one of the most important medicinal plants, are extremely toxic and deadly to artemia [7]. Milk thistle plant as member of Chicory and with scientific name of *silybummariamum*, and English name of Milk thistle has *silymarin* complex that has extraordinary medicinal properties and its role in strengthening the immune system has been proven in laboratory animals [1,7]. However, the operating mechanism of milk thistle extract as an immune system stimulant has not been described well, and its effect on aquatics has not been studied so far. The aim of this study was investigate the impact of milk thistle extract on the immune system of rainbow trout (*Oncorhynchusmykiss*).

MATERIALS AND METHODS

One hundred and twenty rainbow trout fish that their appearance was healthy (85.5 ± 15 g) were transferred to the laboratory of breeding and rearing of fish. Fish were kept in 12 tanks of 1000 liters equipped with aeration with designing system of semi-closed circuit with 10% replacement of water in the distribution day for 15 days so that they can fully adapt themselves to laboratory conditions. During this time, the fish was fed by commercial diet. The experiment of impact of leaves and twigs of *Vitisvinifera* plant on indicators of non-acquired immunity of rainbow trout over 28 days and in a completely randomized was designed with four treatments, control group fish and fish fed by different doses of leaf and twig of *Vitisvinifera* plant, and each treatment with three replications. Food was supplied weekly and freshly by adding powdered supplement of leaves and twigs of *Vitisvinifera* in 100, 400 and 800 mg per Kg of food with powder of commercial food. After the beginning of the experiment, 3 fish were selected randomly from each tank (in total 9 fish from each treatment) on 14 and 28 days of fishing and after the anesthetizing by extract of clove powder (1: 5000) from their caudal vein and using syringes covered with EDTA, their blood was collected. After centrifugation of blood samples, the plasma was isolated in a centrifuge device with the power of 6000 g for 15 minutes at 4 °C and it was stored in the freezer of -78 °C until the final tests. In measuring of peroxidase activity level of plasma, 15 mL plasma was diluted with 35 ml of HBSS buffer free from magnesium and calcium. Then, 50 ml of solution (TMB) and 5 mM of hydrogen peroxide was added so that solution turns blue, then after 2 minutes, by adding 50 ml of sulfuric acid, the colored reaction was stopped and the color of solution was changed to light yellow. In the next stage, the absorbance was measured at wavelength of 450 nm and after measuring by absorbance of the standard solution, result is expressed in terms of international unit of ml. Measurement of complement of CH50 was done by kit prepared from the company of Tehran Bahar Afshan and based on Radial Immuno Diffusion method. The activity level of Lysozyme was also measured by turbidity test and suspension of *Mvramydaz Micrococcus* and lysodeikticus enzyme. The turbidity level was also measured at a wavelength of 670. The immunoglobulin IgM of plasma was also measured by kit prepared from Tehran Bahar Afshan Company and Hitachi autoanalyzer.

Statistical analysis

Statistical analysis was done by using MINITAB 13 software and tables were drawn using EXCEL 2003 software. Statistical analysis was performed by ANOVA. In addition, the significant level of means was done by Tukey test at the 95%.

RESULTS

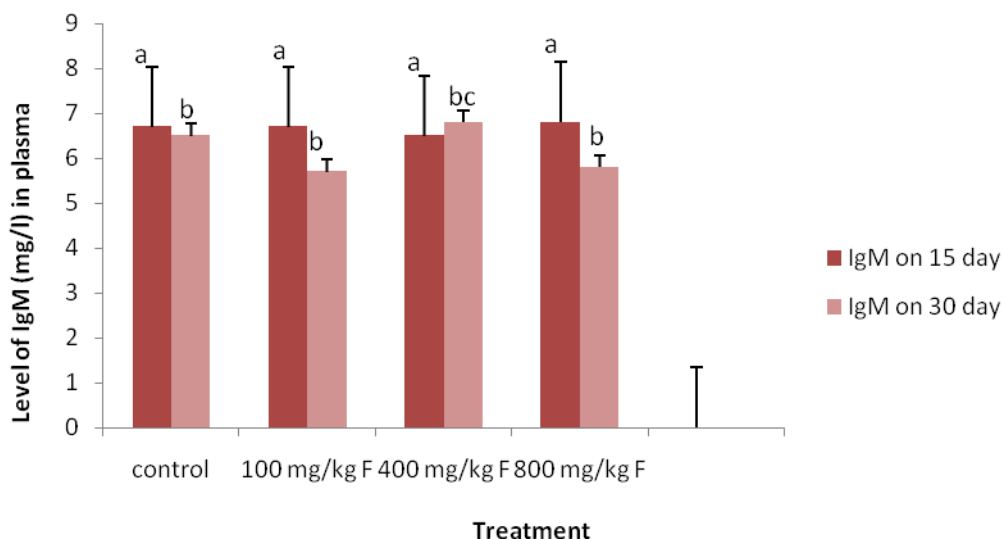


Fig. 1. Changes in immunoglobulin level in fish treated by diet containing the leaves and twigs extract of *Vitisvinifera*

Changes in level of immunoglobulin (IgM) in plasma of fish fed by a diet supplemented are not significant compared with the control group ($p < 0.05$).

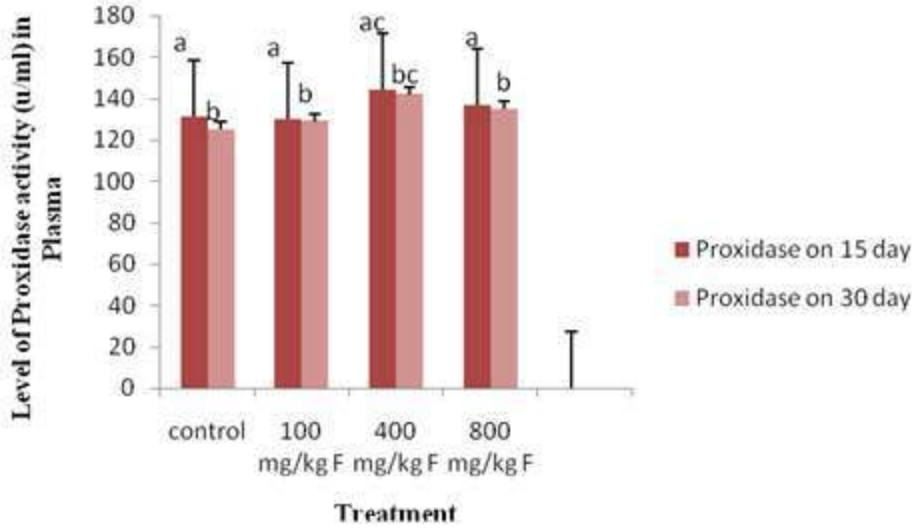


Fig. 2. Changes in the level of peroxidase activity in fish treated by diet containing the leaves and twigs extract of *Vitisvinifera*

Changes in peroxidase activity level in plasma of fish fed by treated by diet containing the leaves and twigs extract of *Vitisvinifera* (400 mg of leaves and twigs extract of *Vitisvinifera* per one kg of food) were significant compared with the control group ($p < 0.05$).

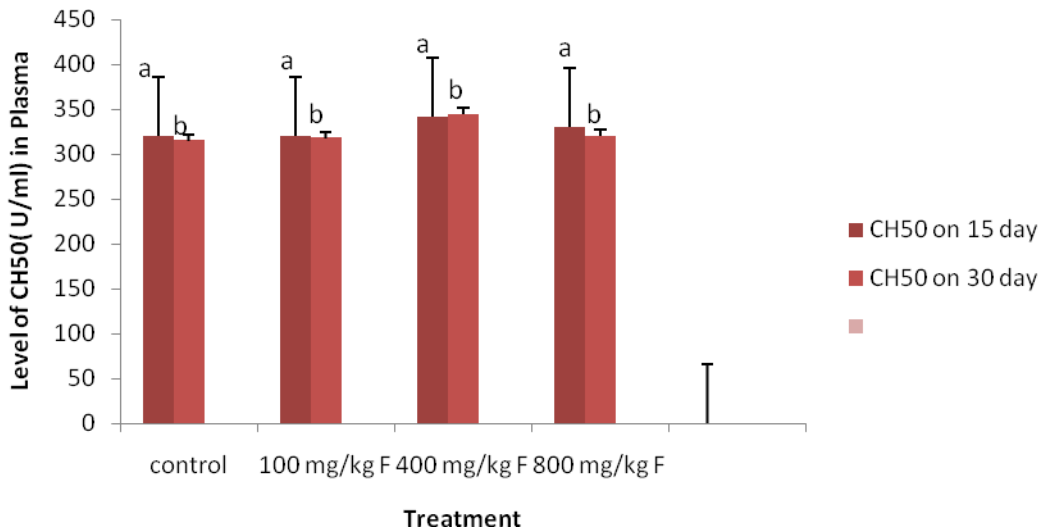


Fig. 3. Changes in the level of total complement in fish treated by diet containing the leaves and twigs extract of *Vitisvinifera*

Total complement level in plasma of fish fed by treated by diet containing the leaves and twigs extract of *Vitisvinifera* (400 mg of leaves and twigs extract of *Vitisvinifera* per one kg of food) was not significant compared with other experimental groups ($p < 0.05$).

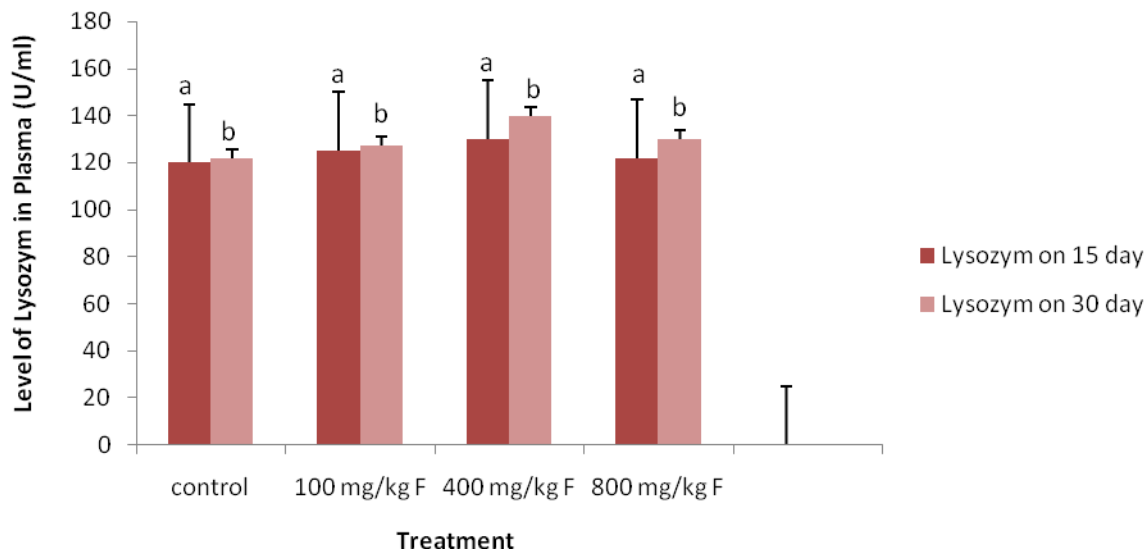


Fig. 4. Changes in the activity level of lysozyme in fish treated by diet containing the leaves and twigs extract of *Vitisvinifera*

Changes in activity level of lysozyme in plasma of fish fed by treated by diet containing the leaves and twigs extract of *Vitisvinifera* (400 mg of leaves and twigs extract of *Vitisvinifera* per one kg of food) were not significant ($p < 0.05$).

DISCUSSION

Maintaining the health and enhancing the immune system of fish against pathogens is one of the most important factors to increase their production at a macro level. Therefore, many fisheries researchers have conducted studies to achieve this important goal in recent decades. One method to increase the immune system of fish is to use of compounds stimulating the immune system called as immune-stimulants. These compounds include different types of chemical synthetic materials, and probiotics is a natural compounds having plant origin. By inhibiting 5-lipoxygenase and production of leukotrienes and free radicals of kupffer cells in rat liver, silymarin extract reduces inflammation of the liver [9] and prevents from the occurrence of cell damage and hemolysis of red blood cells in laboratory animals that have experimental poisoning [9, 10].

Medicinal use of silymarin can prevent inflammation of the brain cells and damage to central nervous system in laboratory animals [9]. The leaves and twigs of the *Vitisvinifera* plant can also inhibit the release of Milo peroxidase. This herbal medicine has anti-cancer property [10]. A significant increase was not observed ($p < 0.05$) in the IgM immunoglobulin level rainbow trout fish of *O. mykiss* fed by leaves and twigs of *Vitisvinifera* plant compared with control group was. David showed that the adding a mixture of sunflower seeds and vitamin in the diet of *Channastriata* fish increases the antibody and resistance of this fish against *Aphanomyces* invadans. Adding a dietary supplement containing extracts of *Catharanthus roseus* to Indian carp of *Labeorohita* increased immune response of this fish [10]. Lysozyme activity level in fish fed by leaves and twigs of *Vitisvinifera* plant was relatively increased, but this increase was not statistically significant compared to the control group ($p < 0.05$). A significant increase in lysozyme activity level in a common carp of *Cyprinus carpio* fed by a mixture of traditional Chinese herbal medicines has also been reported [11]. Peroxidase activity level in experimental treatments fish, especially in fish fed by leaves and twigs of *Vitisvinifera* plant (400 mg per kilogram of leaves and twigs of *Vitisvinifera* plant), was significantly increased ($p < 0.05$). In addition, despite the relative increase in the total complement level in this fish, no difference was found in this fish compared with the control group ($p < 0.05$). The strengthening of immune system of Japanese yellow tail fish fed by nutritional supplements containing extracts of *Quillajasaponin* plant also demonstrated it [12]. The use of extracts of medicinal plants of *Rheum officinale*, *Andrographis paniculata*, *Lonicera japonica*, *Isatis indigotica* in diet of carp enhanced immune system of them [11]. Using turmeric powder and garlic in the diet of Indian larvae carp of *Catla catla* increased their resistance to disease and thus increased their survival rates in the rearing the fish period [13]. Based on the results, it can be

said that use of extracts of medicinal plants and vaccination of fish simultaneously can increase their specific immune against many pathogenic bacterial factors, including the *A. hydrophila* [12].

Despite the lack of a significant increase in immunoglobulin level in plasma of fish treated by leaves and twigs of the *Vitisvinifera* plant compared with control group fish, and an increase in peroxidase activity level in experimental treatment fish, especially in fish fed by food supplement of leaves and twigs of *Vitisvinifera* (400 mg per kilogram of leaves and twigs of *Vitisvinifera* plant), and the relative increase in the level of total complement and lysozyme activity in this species compared with the control group fish, it can be concluded that the leaves and twigs of the *Vitisvinifera* can have a positive impact on the immune system of fish.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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INVESTIGATION THE ROLE OF MEPXH1 (HIS139ARG) POLYMORPHISM ON NUMBER OF EXACERBATIONS AND DISEASE SEVERITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN A SMOKER POPULATION

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ABSTRACT

The purpose of this study was to examine investigation the role of mEPXH1 (His139Arg) polymorphisms on number of exacerbations and disease severity in chronic obstructive pulmonary disease in a smoker population. Chronic obstructive pulmonary disease (COPD) has become the fourth most common single cause of morbidity, and its prevalence is increasing worldwide. It is a syndrome composed of chronic bronchitis, small airways disease (bronchiolitis), and emphysema, in varying proportions between affected individuals. The study was performed cohort and prospectively. The population consist of 213 patients with COPD disease. Genotyping of mEPXH1 was performed using multiplex PCR. Data analysis included, Pearson's *r* correlations, regression analysis, ANOVA analyses, Tukey, test for comparison and SPSS software (package of Spss / pc + + ver18). The results showed that there is not relationship between polymorphisms of mEPXH1 and number of exacerbations. According the results, there is not significant relationship between polymorphisms of mEPXH1 and disease severity. Also there is not significant relationship between mEPXH1 and disease in COPD patients on basis parameters of spirometry and oxidative stress in COPD patients.

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KEY WORDS

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Chronic Obstructive Pulmonary
Disease (COPD)

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the sixth leading cause of morbidity and mortality in the western world and the prevalence of the disease is increasing as the population ages [1]. Cigarette smoking is the major environmental contributor to the disease in western societies yet only, 15% of cigarette smokers develop the disease, indicating that genetic factors play a part in determining susceptibility. Although cigarette smoking is considered the major environmental risk factor for the development of COPD, only 20–30% of chronic smokers develop severe impairment of lung function associated with this pathogenesis [2]. These individual differences in susceptibility to tobacco smoke injury may be related to genetic factors. During the last three decades, research studies reported that the imbalance of the protease–antiprotease and the oxidant–antioxidant systems is the major factor causing emphysema and COPD [3]. With the notable exception of severe α 1-antitrypsin (α 1-AT) deficiency, these genetic factors are poorly understood. Given that .95% of those who develop COPD are smokers and oxidative stress is thought to be important in the pathogenesis of the disease, genetic variation in enzymes that protect the lung against smoke-induced oxidative stress has been a significant focus of study. Microsomal epoxide hydrolase (EPHX1), an enzyme involved in the first-pass metabolism of epoxide intermediates, has received particular attention as two functional variants of the gene, which confer slow and fast metabolic activity, have been identified [4]. In order to replicate a subset of these previous genetic associations in COPD, a case–control study was performed in 492 Caucasian current or former smokers with and without COPD. The current authors chose single nucleotide polymorphisms (SNPs) in three relatively well-studied interesting candidate genes: tumor necrosis factor (TNF- α), b2-adrenoreceptor (ADRB2) and microsomal epoxide hydroxylase (EPHX1). A

systematic literature review and meta-analysis of previous studies was also performed Tyr113His polymorphism with a recessive odds ratio (OR) of 3.5 [5], whereas subsequent studies failed to confirm this [6]. The aim of this study was to examine investigation the role of mEPXH1 (His139Arg) polymorphisms on number of exacerbations and disease severity in chronic obstructive pulmonary disease in a smoker population.

MATERIALS AND METHODS

The study was performed cohort and prospectively. The population consist of 213 patients with COPD disease. In this study, patients candidate for COPD were selected in Beasat Clinic of Kerman in Iran. Inclusion criteria for COPD were chronic airway symptoms and signs such as coughing, breathlessness, wheezing, and chronic airway obstruction. COPD phenotype identification was based on chest radiographic and high-resolution computerized tomography density findings.

DNA preparation

For genotyping, 10 ml blood was drawn into an EDTA tube and stored at -20 C until DNA extraction was carried out. Genomic DNA was isolated from whole peripheral blood using the Salting out [7].

mEPXH1 Genotyping

mEPXH1 (His139Arg) polymorphism was detrmind as described before by authors [8]. To examine the polymorphism of mEPXH1 a simultaneous amplification of genes of interest in the same reaction was performed using a multiplex polymerase chain reaction (PCR) as described in the literature [9,10,11].

Table: 1. The profile sequences and position-specific of primers mEPHX1

Primer	Sequence	GC%	Tm (°C)	Band size
Forward Common	5'-TGG CAG GAC TCA ATA TCT AGG CTC TG-3'	50	67.9	240 bp
Reverse Wild	5'-ATC AGC AAG GGC TTC GGG GTA T-3'	54.5	64	
Reverse Mutant	5'-ATC AGC AAG GGC TTC GGG GTA C-3'	59.1	65	

Statistical analysis

Data analysis included, pearson's r correlations, regression analysis, ANOVA analyses, Tukey: test for comparsion and SPSS software (package of Spss / pc + + ver18).

Demographics results

- (1) Of the 213 patients enrolled in the study 162 were smoker and 51 were Nonsmoker (male:female ratio 163:50)
- (2) The education level of 213 subjects were studied, 109 cases were lack education and 104 cases were Educated.

Determination mEPXH1 genotypes (Exon 4)

Figure- 1 shows different genotypes of mEPXH1 after the Multiplex PCR. Despite the band with primers forward common and Reverse G indicative GG genotype (Homozygote). Despite the band with primers forward common and Reverse A indicative AA genotype (Wild type) and band with primers Forward common, Reverse G and Reverse A indicative GA genotype (Heterozygote).

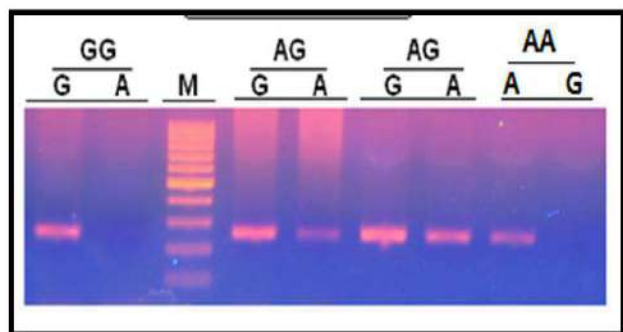


Fig: 1. The various genotypes of His139Arg polymorphisms (mEPXH1 gen) in Agarose gel

Table: 2. Determination various genotype of His139Arg polymorphisms (mEPXH1 gen)

Genotype	Band Fragment (bp)	Phenotype (Gene expression)
GG (Homozygot)	240 (only G)	High
AG (Heterozygot)	240 (Both A ,G)	Intermediate
AA (Wild type)	240 (only A)	Low

RESULTS

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and number of exacerbations in COPD patients

Table- 3 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and number of exacerbations in COPD patients. There is not significant relationship between polymorphisms of His139Arg and number of exacerbations in COPD patients.

Table: 3. The relationship between polymorphisms of His139Arg and number of exacerbations in COPD patients

Exacerbation/yr.	Frequency n (%)	mEPXH1 exon-4 genotype			P-value
		His/His	His/Arg	Arg/Arg	
No	n (%)	48 (47)	42(41)	13(12)	0.857
1-2	n (%)	30(45)	29(43)	8(12)	
3-4	n (%)	23(54)	14(32)	6(14)	

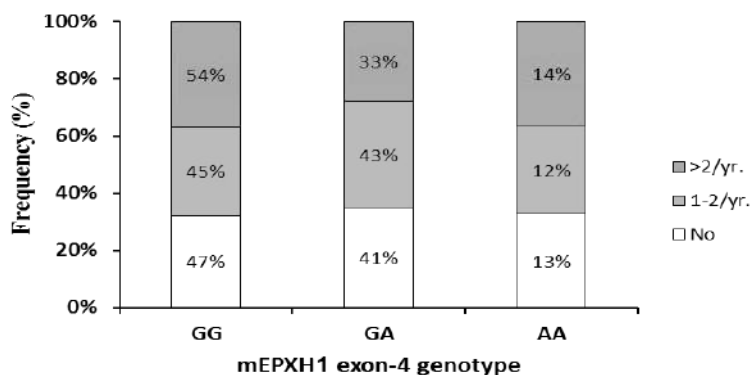


Fig: 2. The relationship between polymorphisms of His139Arg and number of exacerbations in COPD patients

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients

Table 4 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients. There is not significant relationship between polymorphisms of His139Arg and disease severity in COPD patients.

Table: 4. The relationship between polymorphisms of His139Arg and disease severity in COPD patients

mEPXH1 exon-4 genotype	FEV1<50 n(%)	FEV1>50 n(%)	OR (95% C.I)	P-value
His/His	50(46)	51(49)	-	ns
His/Arg	46(43)	39(37)	0.8 (0.5-1.5)	
Arg/Arg	12(11)	15(14)	1.2 (0.2-2.9)	

ns : Non-significant

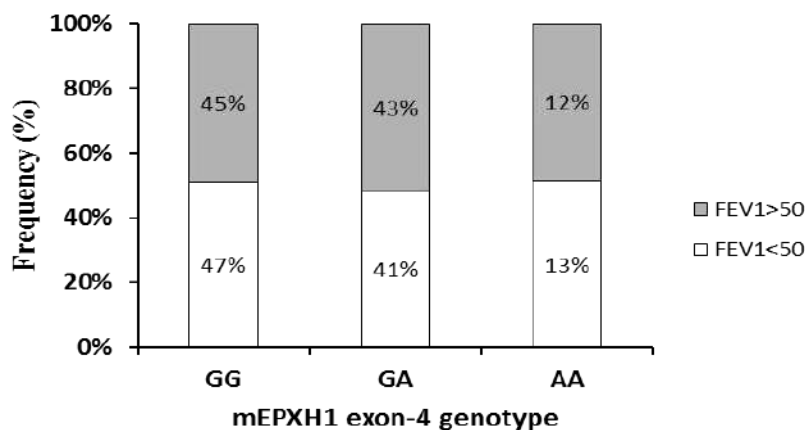


Fig: 3. The relationship between polymorphisms of His139Arg and disease severity in COPD patients

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in presence of confounding factors in COPD patients

Table- 5 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients in presence of confounding factors (sex, age, smoking and BMI). There is not significant relationship between polymorphisms of His139Arg and disease severity in presence of confounding factors in COPD patients.

Table: 5. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in presence of confounding factors in COPD patients.

Variable	B	P-value	OR (95% CI)
mEPXH 1 Exon4 genotype			
His/His		0.592	
His/Arg	-0.122	0.686	0.8 (0.5-1.6)
Arg/Arg	0.341	0.447	1.4 (0.6-3.4)
Sex	-0.594	0.135	0.5 (0.3-1.2)
Age	-0.001	0.92	0.99 (0.98-1.02)
Smoking Status	0.056	0.784	1.06 (0.71-1.58)
BMI	0.042	0.194	1.04 (0.98-1.11)

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients on basis parameters of gender

Table -6 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients on the basis parameters of gender. There is not significant relationship between polymorphisms of His139Arg and disease severity in COPD patients. But there is a protective effect against the disease, so that men with genotype AA low (Arg / Arg) show the severity high of the disease.

Table: 6. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients on basis parameters of gender

Sex	mEPXH1 exon-4 genotype				OR
	COPD Stage	His/His	His/Arg	Arg/Arg	
Male	n (%)	40(51)	32(41)	6(8)	2.0
	n (%)	44(52)	29(34)	12(14)	
Female	n (%)	10(33)	14(47)	6(20)	0.2
	n (%)	7(35)	10(50)	3(15)	

OR : Odd Ratio

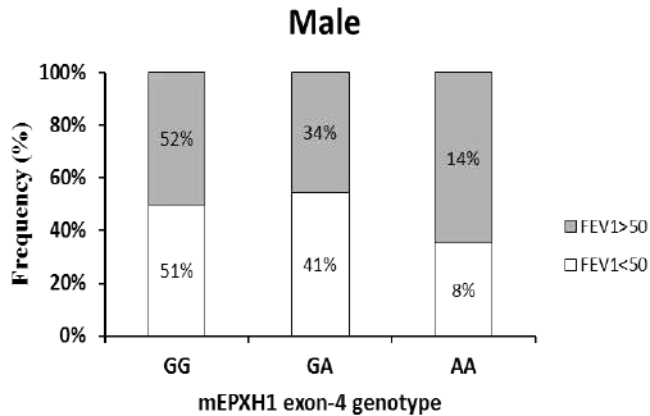


Fig. 4. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients on basis parameter Male

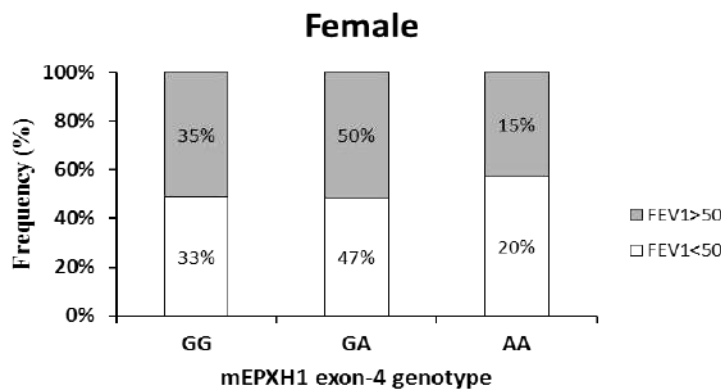


Fig. 5. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease severity in COPD patients on basis parameter Female

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on basis parameters of spirometry

Table- 7 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on basis parameters of spirometry (FEV₁, FEV₁/FVC and FEF₂₅₋₇₅). There is not significant relationship between polymorphisms of His139Arg and disease in COPD patients on basis parameters of spirometry.

Table: 7. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on basis parameters of spirometry

mEPXH1 exon-4 genotype		N	Mean (95% CI)	P- value
FEV ₁	Wilde	101	49.0(45.4-52.5)	-
	Hetro	85	50.7(46.9-54.4)	ns.
	Mutant	27	51.0(43.2-58.8)	ns.
FEV ₁ /FVC	Wilde	101	73.5(71.6-75.3)	-
	Hetro	85	74.0(71.8-76.2)	ns.
	Mutant	27	76.6(73.2-79.9)	ns.
FEF ₂₅₋₇₅	Wilde	100	37.5(32.2-42.7)	-
	Hetro	85	39.0(34.1-43.8)	ns.
	Mutant	26	38.6(30.8-46.3)	ns.

FEV₁: Forced Expiratory Volume in first second. FVC: Forced Vital Capacity
FEF_{25-75%}: Mean forced Expiratory Flow during the middle half of FVC

Relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on basis parameters of oxidative stress

Table- 8 shows the results relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on basis parameters of oxidative stress (LPO, SOD, TAC). There is not significant relationship between polymorphisms of His139Arg and disease in COPD patients on basis parameters of oxidative stress.

Table: 8. The relationship between polymorphisms of His139Arg (mEPXH1 gen Exon 4) and disease in COPD patients on the basis parameters of oxidative stress

mEPXH1 exon-4 genotype		N	Mean (95% CI)	P- value
LPO	Wilde	101	0.2(0.15-0.23)	-
	Hetro	85	0.2(0.12-0.20)	ns.
	Mutant	26	0.2(0.13-0.27)	ns.
SOD	Wilde	60	2.6(2.50-2.76)	-
	Hetro	45	2.7(2.57-2.85)	ns.
	Mutant	18	2.7(2.45-2.88)	ns.
TAC	Wilde	101	1.4(1.35-1.50)	-
	Hetro	85	1.4(1.32-1.50)	ns.
	Mutant	26	1.4(1.25-1.57)	ns.

LPO: Lipid peroxidation SOD: Superoxide dismutase TAC: Total antioxidant capacity

DISCUSSION

The purpose of this study was investigation the role of mEPXH1 (His139Arg) polymorphism on number of exacerbations and disease severity in chronic obstructive pulmonary disease in a smoker population. Of the 213 patients enrolled in the study 163 were male and 50 were female. The frequency of different genotypes mEPXH1 (His139Arg) was including 47.4 % of His/His, 40 %, His/Arg and 12.7% Arg/Arg. Erkisi et al (2010) reported in Turkey the abundance of His/His 53.7%, His/Arg 39% and Arg/Arg 7.3% [12]. Cheng et al (2004) reports the abundance of His/His 65.6%, His/Arg 31.3% and Arg/Arg 3.3% [9]. The results of this study show the there is not significant relationship between polymorphisms of His139Arg and number of exacerbations in COPD patients. According the results, there is not significant relationship between Heterozygote genotype and FEV1 <50 (OR= 0.83; 95%CI: 0.77-1.5) and homozygous genotypes and FEV1 <50 (OR= 1.2; 95%CI: 0.22-2.9) in COPD patients. The results of this study show the there is not significant relationship between polymorphisms of His139Arg and disease in COPD patients on basis parameters of spirometry (FEV₁, FEV₁/FVC and FEF₂₅₋₇₅) and oxidative stress (LPO, SOD, TAC). These results are in compliant with result Cheng et al (2004), Smith and Harrison (1997) and Park (2007) [9, 13, 14]. Cheng et al reported the there is not significant relationship between polymorphisms of mEPXH1 His139Arg and risk of COPD in COPD patients ((OR_{His/Arg}= 0.6; 95%CI: 0.3-1.1) and (OR_{Arg/Arg}=0.4; 95%CI: 0.1-2.6)) [9]. Sandford et al (2001) indicated the there is significant relationship between combining two polymorphisms mEPXH1 His139Arg, His113Tyr and reduced lung function in COPD patients on basis parameters of spirometry [15]. Yoshikawa et al (2000) finding the there is significant relationship between polymorphisms of His113Tyr and disease severity in COPD patients (OR=2.9; 95%CI: 1.1-7.4, p=0.025) [16]. Hu et al (2008) reported the there is significant relationship between Homozygous mEPXH1 and increased risk of COPD disease [17]. Although in some studies shown relationship between polymorphisms mEPXH1 (His139Arg) and COPD disease, but in this study there is not significant relationship between polymorphisms of His113Tyr and number of exacerbations, disease severity and susceptibility to disease in COPD patients.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ACKNOWLEDGEMENT

None.

FINANCIAL DISCLOSURE

None.

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CONSTRUCTION OF A RECOMBINANT BACMID DNA ENCODING VIRAL PROTEIN-2 OF CANINE PARVOVIRUS USING SITE-SPECIFIC TRANSPOSITION MECHANISM

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ABSTRACT

Background and Aim: Canine parvovirus (CPV) is a small non-enveloped ssDNA virus composed of viral capsid proteins 1, 2 and 3 (VP1, VP2 and VP3). This virus has a natural affinity to cancer cells via VP2 ligands/transferrin receptors (TfRs) attachment. It has shown that VP1 and VP3 are unnecessary for capsid formation and consequently the VP2 alone is sufficient for assembly of canine parvo-virus like particle (CP-VLP) for therapeutic aims. So, in this research our purpose was to construct a recombinant bacmid shuttle vector expressing VP2 of CPV using site-specific transposition mechanism in a Bac-to-Bac baculovirus expression system. **Methods:** The mini-Tn7 transposone located in pFastBac1 donor vector containing expression cassette of CPV-VP2 was used in this experimental study that had constructed in our previous study. Firstly, the presence of gene of interest in pFastBac1 donor vector was evaluated by PCR and enzymatic digestion analysis. Then the confirmed pVP2FastBac1 plasmid was transferred into *E. coli* DH10Bac competent cells and the site-specific transposition of VP2 into a bacmid shuttle vector was accomplished using helper plasmid. Finally, the accuracy of transposition process was evaluated by a PCR panel using specific primers and PUC/M13 universal primers. **Results:** The presence of the gene of interest in pFastBac1 donor vector was confirmed by PCR and enzymatic digestion analysis and VP2-containing recombinant bacmid was subsequently constructed successfully by site-specific transposition mechanism and verified by the mentioned PCR panel. **Conclusions:** In this study, we used the Bac-to-Bac system for site-specific transposition of VP2 gene from pVP2FastBac1 to a baculovirus derived bacmid shuttle vector. The constructed recombinant bacmid can express recombinant VP2 protein in insect cells.

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KEY WORDS

Canine parvovirus, VP2, Baculovirus, Bacmid

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INTRODUCTION

The *Parvoviridae* family consists of small, icosahedral, non-enveloped viruses that contain linear single-stranded DNA (ssDNA) genomes about 5000 nucleotides long. Canine parvovirus (CPV) belongs to *Parvovirus* genus of this family and first emerged in the late 1970s as the cause of a new disease in dogs and is now prevalent in dogs worldwide [1,2,3]. CPV particles have a diameter of 25 nm and are composed of three proteins, viral protein-1 (VP1), viral protein-2 (VP2), and viral protein-3 (VP3) [4]. VP2 is the major component of the viral capsid. About 90% of the protein in the capsid is VP2, and 10% is VP1, which contains the entire VP2 sequence and 154 additional residues at its N-terminus. The third protein, VP3, is produced after intracellular proteolytic cleavage, which removes approximately 25 amino acids from the N-terminus of VP2. A wild-type capsid contains 60 subunits primarily of the VP2, along with a few VP1 and VP3 subunits [5]. CPV has a natural affinity to cancer cells via VP2 ligands/transferrin receptors (TfRs) attachment. In fact, the VP2 protein of CPV is the main part of attachment ligands for entry into specific and cancerous cells through transferrin receptors (TfRs) [6,7]. Yuan et al. (2001) claimed that VP2 can assemble into capsid-like structures and the expression of VP2 alone can result in assembly of a typically-sized virus like particle (VLP) for therapeutic aims. However, the importance of VP2 protein of canine parvovirus in binding to human cancer cells and production of veterinary detection kits for detecting the virus and also in vaccination has motivated a lot of research on production of this protein [8].

One of the best systems, considered in production of recombinant proteins, is the use of baculoviruses in insect cell expression system. The recombinant baculovirus and insect cell expression system provides high levels of recombinant proteins that undergo post-translational modifications like glycosylation. Therefore, the application of such system allows large quantity production of a desirable protein, in the native conformation [9,10]. There are various methods for construction of recombinant baculoviral vectors. One method is the use of Bac-to-Bac baculovirus expression vector system (BEVS) with an efficient site-specific transposition mechanism to generate recombinant baculovirus. This system has two major components. The pFastBac donor plasmid vector into which the gene(s) of interest will be cloned and has an expression cassette. The second component is the baculovirus shuttle vector (bacmid) into which the expression cassette will be transposed via recombinant pFastBac, constructed [11]. In this study, we tried to generate the second component of BEVS system, through construction of a recombinant bacmid DNA encoding VP2 of canine parvovirus, using site-specific transposition mechanism. This construct can be used to produce large scale of VP2 protein in insect cell.

MATERIALS AND METHODS

Plasmids, bacterial strains

The pFastBac1 (Invitrogen, USA) was used as the transfer vector. The *E. coli* strain DH5 α (Invitrogen, USA) was used for amplifying recombinant pFastBac1 donor plasmid vector. The gene of interest (VP2 gene) located in eukaryotic expression cassette of mini-Tn7 transposone of pVP2FastBac1 (generated in previous study) was available in this work. The *E. coli* strain DH10Bac (Invitrogen, USA) containing the baculovirus modified DNA (bacmid shuttle vector) with a mini-attTn7 target site and helper plasmid was used as an appropriate strain to perform the transposition process. The helper plasmid harbored by DH10Bac strains, confers resistance to tetracycline and encodes enzymes needed for transposition of the gene of interest into the bacmid.

Plasmid extraction

After the selection of proper colonies by blue/white screening, the recombinant plasmids were extracted from 1500 μ l of bacterial cell cultures using a Roche commercial kit (Germany) according to the manufacturer's instructions.

Evaluation of VP2 gene existence in pFastBac1 donor plasmid vector

The mini-Tn7 transposone located in pFastBac1 donor vector containing expression cassette of CPV-VP2 was used in this experimental study that had constructed in our previous study. So, the presence of gene of interest in expression cassette of pFastBac1 donor plasmid vector was evaluated by PCR and enzymatic digestion analysis (triple digestion using EcoRV and HindIII enzymes and also double digestion using BamHI and EcoRI) and the fragments produced were analyzed according to NEBcutter software pattern. Finally, the accuracy of the VP2 gene ORF in recombinant pFastBac1 was evaluated and confirmed by sequencing process and the analysis of sequencing results was accomplished by Chromas software, version 1.45 (data not shown).

Construction of a recombinant bacmid

The VP2-containing recombinant pFastBac1 donor plasmid was transferred into the *E. coli* DH₁₀Bac competent cells. After the transformation process, incubation for 4-6 hours was accomplished for site-specific transposition of the VP2 expression cassette from the transposing vector into the bacmid shuttle vector, leading to *lacZ* gene disruption. The presence of helper plasmid is required in this process. The transformed cells were cultured on a LB agar plate containing kanamycin (50 μ g/ml), gentamicin (7 μ g/ml), tetracycline (10 μ g/ml), X-gal (100 μ g/ml) and isopropylthio- β -galactoside (IPTG, 40 μ g/ml) and incubated at 37°C for 16h. The bacmid DNA was isolated from the overnight cultures by alkaline lysis purification method according to the general and current protocols.

It is notable that bacmid DNA is a high-molecular-weight plasmid (~ 135 kbp) and we must take care not to shear it. Over-drying, mechanically resuspension and storing the purified bacmid DNA at -20°C (as repeated freezing and thawing) may shear the DNA and gentle tapping of the bottom of the tube and storing the purified bacmid DNA at +4°C is recommended.

Analysis of recombinant bacmid DNA

The evaluation of VP2 gene existence in bacmid DNA is not performed as other plasmids. The enzymatic digestion analysis is not convenient and a PCR only by using specific primers is not sufficient. So, the transposition process accuracy and/or VP2 gene existence in bacmid DNA was evaluated by a PCR panel using both VP2 specific primers and PUC/M₁₃ universal primers (Table 1). In fact, the PCR using specific primers shows the accuracy of DH₁₀Bac transformation by recombinant pFastBac1 and the PCR using PUC/M₁₃ universal primers indicates the accuracy of recombination through site-specific transposition mechanism. The evaluation of VP2 orientation in recombinant bacmid is also indicated by the PCR using both VP2 specific and PUC/M₁₃ universal primers.

Table: 1. Details of amplified region and primer sets used for PCR analysis of recombinant bacmid

Primer Pairs	Sequence (5' to 3')	Fragment Size (bp)	The Amplified Region
pUC/M ₁₃ F VP2 specific R	5'-GTTTTCCCAGTCACGAC-3' 5'-TTAATATAATTTTCTAGGTGCTAGT-3'	3400	Tn7 R + Polyhedrin promoter + VP2 gene
VP2 specific F pUC/M ₁₃ R	5'-ATGAGTGATGGAGCAGTTCAAC-3' 5'-CAGGAAACAGCTATGAC-3'	2450	VP2 gene + Tn7 L
pUC/M ₁₃ F pUC/M ₁₃ R	5'-GTTTTCCCAGTCACGAC-3' 5'-CAGGAAACAGCTATGAC-3'	4000	Tn7 R + Polyhedrin promoter + VP2 gene + Tn7 L
VP2 specific F VP2 specific R	5'-ATGAGTGATGGAGCAGTTCAAC-3' 5'-TTAATATAATTTTCTAGGTGCTAGT-3'	1750	VP2 gene

- All the Fragment Sizes are Expressed Approximately and Has Been Calculated According to the Sequence date of the Bacmid DNA

Polymerase chain reaction programs

PCR reaction was performed in a tube containing 5 μ L of 10x PCR buffer, 1 μ L of dNTP mix (0.2 mM for each), 1.5 μ L of MgCl₂ (1.5 mM), 1 μ L of each primer with the concentration of 10 μ M or 10 pmol/ μ L (10 pmol for each), 1-2 μ L of template DNA, 1 unit of Taq DNA polymerase (Fermentas, Vilnius, Lithuania) and water nuclease-free up to 50 μ L final volume. Amplification reactions were performed in Biorad thermocycler (USA) and the PCR program included the following steps for specific primers: denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 45 sec (denaturation), 67°C for 60 sec (annealing), 72°C for 80 sec (extension), and a final extension at 72°C for 10 min. For PUC/M₁₃ universal primers the program included the following steps: denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 45 sec (denaturation), 63°C for 45 sec (annealing), 72°C for 300 sec (extension), and a final extension at 72°C for 7 min. Finally, the PCR program included the following steps for VP2 specific and PUC/M₁₃ universal primers: denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 45 sec (denaturation), 63°C for 45 sec (annealing), 72°C for 210 sec (extension), and a final extension at 72°C for 10 min. PCR products were analyzed by electrophoresis using 1% (w/v) agarose gel, stained with safe view (Kiangene, IRI).

RESULTS

Confirmation of VP2 gene existence in pFastBac1 donor plasmid vector

The presence of the gene of interest in expression cassette of pFastBac1 donor vector was confirmed by enzymatic digestion [Figures- 1(a) 1(b)] and PCR [Figure- 1b]. Triple digestion using EcoRV and HindIII enzymes and also double digestion using BamHI and EcoRI were accomplished and the fragments produced were analyzed and verified according to NEBcutter software pattern.

Analysis of recombinant bacmid DNA construct

After the verification of VP2 gene existence in expression cassette of pFastBac1, the transformation of *E. coli* DH₁₀Bac cells was accomplished successfully by VP2-containing recombinant pFastBac1 donor plasmid vector. Subsequently, the site-specific transposition of VP2 expression cassette from the transposing vector into the bacmid shuttle vector was performed with the presence of helper plasmid. After plating the cells on LB agar, the colonies containing recombinant bacmid were visible as large white colonies among the blue ones harboring the unaltered bacmids. The selected white colonies were restreaked onto a LB agar plate to ensure if they have true white phenotype. Since verification of the high molecular weight recombinant bacmid DNA is not convenient by digestion, at first a PCR using PUC/M₁₃ universal primers was performed to ensure that recombination has been done in selected colonies. All the white colonies showed a 4000 bp fragment, indicating the successful recombination in them. The results of amplification in non-recombinant bacmids of blue colonies (as the negative control) using M13/pUC primers showed a 300 bp fragment, indicating the lack of recombination performance [Figure -2].

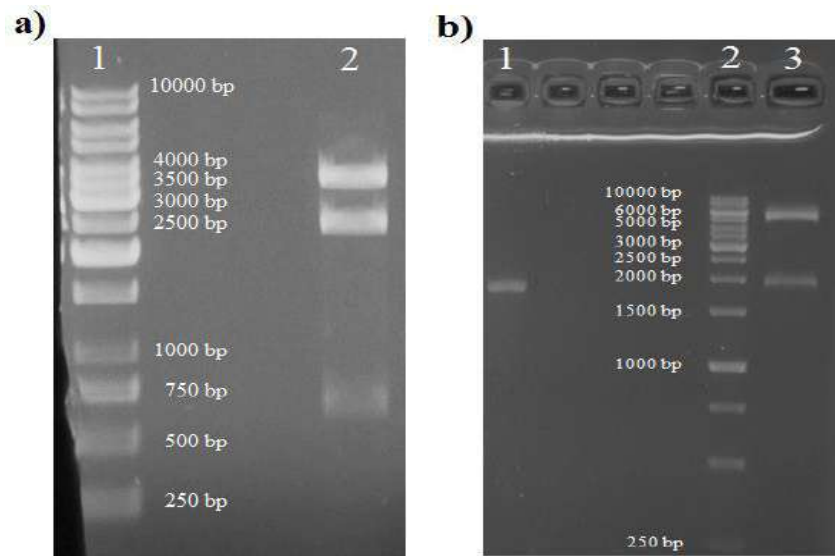


Fig: 1. a) Enzymatic triple digestion of recombinant pFastBac1 vector (*EcoRV/HindIII*). Lane 1: GeneRuler 1 Kb DNA ladder (Fermentas, Vilnius, Lithuania). Lane 2: Expected ~ 600 bp, ~ 2500 bp and ~ 3500 bp fragments. b) PCR and Enzymatic double digestion of recombinant pFastBac1 vector (*BamHI/EcoRI*). Lane 1: VP2 gene expected fragment (1755 bp) obtained from confirmatory PCR on recombinant pFastBac1 vector. Lane 2: GeneRuler 1 Kb DNA ladder (Fermentas, Vilnius, Lithuania). Lane 3: revealed expected 1775 bp VP2 fragment and ~ 5000 bp linearized vector.

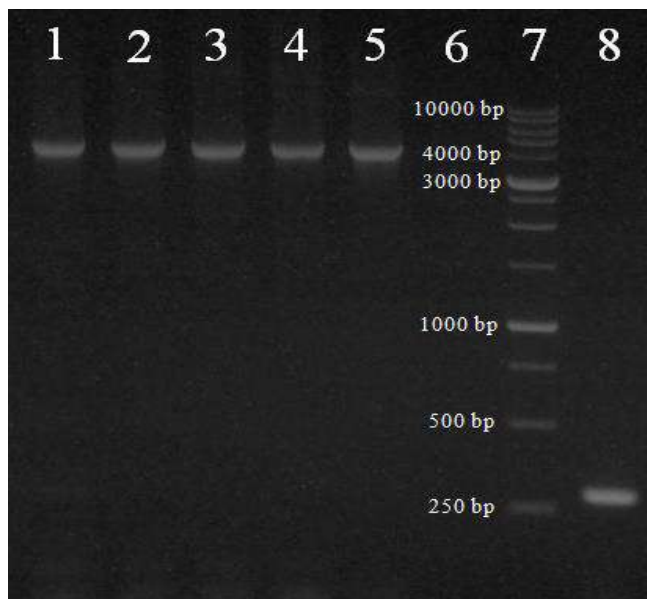


Fig: 2. Primary verification of recombination process through transposition mechanism by PCR, using PUC/M13 universal primers. Lanes 1-5: The expected fragment (~4000 bp) including Tn7 R + Polyhedrin promoter + VP2 gene + Tn7 L produced by PCR of recombinant bacmids extracted from white colonies. Lane 6: Negative control of PCR (without template). Lane 7: 1 kb DNA size marker (Yekta Tajhiz Azma, Iran). Lane 8: The expected fragment (~300 bp) produced by PCR of non-recombinant bacmid extracted from a blue colony as a negative control.

In the next step, a PCR panel was performed using VP2 specific and PUC/M₁₃ universal primers to ensure that proper transposition of the gene of interest has been done in recombinant bacmids.

The bacmid DNA contains M₁₃ forward and reverse priming sites, flanking the Tn7 mini-att site within the LacZ α -complementation region. The panel of PCR was done using PUC/M₁₃ universal forward and reverse primers, VP2 gene specific forward and reverse primers, VP2 gene specific forward primer and PUC/M₁₃ universal reverse primer and finally VP2 gene specific reverse primer and PUC/M₁₃ universal forward primer, respectively. PCR of nonrecombinant bacmid extracted from a blue colony as the negative control generated an expected ~300 bp fragment using PUC/M₁₃ universal forward and reverse primers [Figure-3].

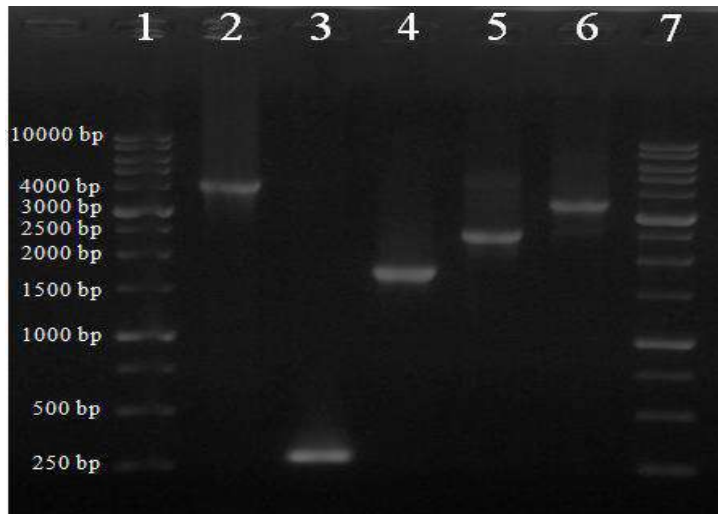


Fig. 3. The panel of PCR performed to confirm proper transposition of VP2 into the bacmid extracted from a white colony. Lanes 1 and 7: 1 kb DNA size marker (Yekta Tajhiz Azma, Iran). Lane 2: The expected PCR product generated using PUC/M13 universal primers (~4000 bp) including Tn7 R + Polyhedrin promoter + VP2 gene + Tn7 L fragments. Lane 3: The expected PCR product generated using PUC/M13 universal primers (~300 bp) by the non-recombinant bacmid extracted from a blue colony as a negative control. Lane 4: The PCR product generated using VP2 gene specific forward and reverse primers (1755 bp) including VP2 gene fragment. Lane 5: The PCR product generated using VP2 gene specific forward and PUC/M13 universal reverse primers (~2500 bp) including VP2 gene + Tn7 L fragments. Lane 6: The PCR product generated using PUC/M13 universal forward and VP2 gene specific reverse primers (~3500 bp) including Tn7 R + Polyhedrin promoter + VP2 gene fragments.

The results indicated the accuracy of recombination through site-specific transposition mechanism and the accuracy of VP2 orientation in the recombinant bacmid.

DISCUSSION

The expression of eukaryotic genes using baculovirus expression vectors takes advantages of their protein synthesis machinery and facilitates proper folding and post-translational modifications including glycosylation, acetylation, oligomerization and proteolysis. The Bac-to-Bac Baculovirus Expression System provides a rapid and efficient method to generate recombinant baculoviruses. This method was developed by researchers at Monsanto, and is based on site-specific transposition of an expression cassette into a baculovirus shuttle vector (bacmid) propagated in *E. coli*. The first major component of the system is a pFastBac vector into which the gene(s) of interest will be cloned. Depending on the pFastBac vector selected, expression of gene(s) of interest is controlled by the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) polyhedrin (PH) or p10 promoter for high-level expression in insect cells. This expression cassette is flanked by the left and right arms of Tn7, and also contains a gentamicin resistance gene and a SV40 polyadenylation signal to form a mini Tn7 [11,12]. The second major component of the System is the DH10Bac *E. coli* strain that is used as the host for pFastBac vector. DH10Bac cells contain a baculovirus shuttle vector (bacmid) with a mini-attTn7 target site and a helper plasmid. After the generation of recombinant pFastBac construct, once the pFastBac expression plasmid is transferred into DH10Bac cells, transposition occurs between the mini-Tn7 element on the pFastBac vector and the mini-attTn7 target site on the bacmid to generate a recombinant bacmid. This transposition reaction occurs in the presence of transposition proteins supplied by the helper plasmid. Once the transposition reaction is performed, we can isolate the high molecular weight recombinant bacmid DNA and transfect the bacmid DNA into insect cells to generate a recombinant baculovirus that can be used for preliminary expression experiments. After the baculoviral stock is amplified and titered, this high-titer stock can be used to infect insect cells for large-scale expression of the recombinant protein of interest [13,14].

Using the Bac-to-Bac Baculovirus Expression System to generate a recombinant baculovirus provides the following advantages over the traditional method using homologous recombination: a) Requires less than 2 weeks to identify and purify a recombinant baculovirus as compared to the 4-6 weeks required to generate a recombinant baculovirus using homologous recombination. b) Reduces the need for multiple rounds of plaque purification as the recombinant virus DNA isolated from selected colonies is not mixed with parental, non-recombinant virus. c)

Permits rapid and simultaneous isolation of multiple recombinant baculoviruses, and is suited for the expression of protein variants for structure/function studies [14].

CONCLUSION

In this study our purpose was to construct a recombinant bacmid DNA encoding viral protein-2 (VP2) of canine parvovirus using site-specific transposition mechanism. This recombinant baculoviral vector was constructed successfully under the control of polyhedrin promoter. We used the Bac-to-Bac system for site-specific transposition of VP2 gene from pVP2FastBac1 to baculovirus derived bacmid shuttle vector. The recombinant bacmid constructed here will transfect into the cultured Sf₉ (*Spodoptera frugiperda*) insect cell line to produce VP2 protein for therapeutic aims.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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FINANCIAL DISCLOSURE

None.

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AN INVESTIGATION ON TOXIGENICITY AND ANALYTICAL COMPARISON OF OCHRATOXIN AND CITRININ IN LABORATORY CULTURE MEDIA

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ABSTRACT

One of the most serious problems facing the quality of food and feed, is the presence of mycotoxins which are produced by different species of the genus *Aspergillus*. Ochratoxin and citrinin (CIT) are of the greatest concerns as they are highly toxic and carcinogenic compounds. Samples were collected by settling plates, in northern Iran, and pure culture isolation was performed upto toxin measurement in culture media, which had been prepared by merging culture in separately prepared culture media incubation. The amount of toxin was measured by extracting solutions using Direct Competitive enzyme-linked immunosorbent assay (ELISA). The greatest amount of CIT was produced by *A.niger* (1655.91ppb), and the highest amount of ochratoxin produced by *A.carbonarius* (8.007ppb) that played an important role among all the conducted isolates. In contrast the lowest amount of CIT was produced by *A.foetidus* (0.060ppb), the lowest ochratoxin was produced by *A.spIV* isolates (1.835ppb). Considering the maximum limits CIT (200ppb) and for ochratoxin (5ppb) in food and feed including abbreviated new drug applications (ANDAs), the maximum amount of CIT which was produced by *A.niger* was much more than the universal standard limits. The greatest amount of ochratoxin produced by *A.carbonarius* and also was more than the standard limits. Since methods for controlling mycotoxins are highly preventive, the analytical comparison of toxins, including aflatoxin, ochratoxin and CIT was intended to be elucidated in this study.

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KEY WORDS

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Culture media

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INTRODUCTION

Fungal growth is one of the main causes of food spoilage, which not only makes great economic losses, but also threatens human and animal health, particularly through the synthesis of mycotoxins. Citrinin is a polyketide mycotoxin produced by several species of the genera *Aspergillus*, *Penicillium* and *Monascus*. Some of the fungi producing citrinins (CITs) are also able to produce mycotoxins ochratoxin A (OTA) or aflatoxin. CIT is generally formed after harvest under storage conditions and it occurs mainly in grains and cereals. It can also occur with other products of plant origin and in spoiled dairy products. However, in various studies on OTA, references have been made to the concomitant occurrence of CIT in a given food or feed materials, Meanly when CIT was found in a sample, it always occurred with OTA [1]. CIT has a conjugated, planar structure which gives it's a natural fluorescence, the highest fluorescence is produced by a non-ionized CIT molecule at pH 2.5 [2]. For the growth of the CIT, which produces fungi on grain, it is necessary to have a humidity of at least 16.5 – 19.5%. This CIT is practically insoluble in cold water and soluble in aqueous sodium hydroxide, sodium carbonate, or sodium acetate, methanol, acetonitrile, ethanol, and most other polar organic solvents [3]. One of the most serious problems facing the quality of food and feed, is presence of mycotoxins [4]. CIT is a nephrotoxic mycotoxin produced by several species of the genera *Aspergillus*, and it has also been reported to be involved in human disease. However, there are limited evidences for the carcinogenicity that was concluded by the International Agency for Research on Cancer. During studying antibiotic agents in mid 20th century, the interests in CIT arose when its broad antibacterial activity was identified. However, this interest decreased when its mammalian toxicity was demonstrated [4]. A large number of CIT derivatives have been isolated from different fungal species in search of antitumor compounds indicating that CIT might be a precursor of novel active compounds [5]. Fungal growth and mycotoxin production during storage are generally spot processes significantly affected by crop variety agronomic

practices and weather conditions during harvest, post harvest, drying, cleaning and storage and processing conditions as well as the toxigenic potential of the mold species. Consequently, the distribution of mycotoxins in a lot of agricultural products is heterogeneous. This sampling is the largest source of variability associated with mycotoxin analysis and the most crucial step in obtaining reliable results [6].

OTA is a toxin naturally produced by several species of *Aspergillus* and *Penicillium*. These mold species are capable of growing in different climates and on different plants. Thus, contamination of food crops with OTA can occur worldwide. This toxin has a nephrotoxic, immunosuppressive, teratogenic and carcinogenic properties [7]. Many studies have shown that cereals and cereal products are the main sources of OTA [8,9]. It has also been detected in other products such as green coffee, milk, wine and grape juice [10]. OTA can be found in a wide range of human foods. The toxicology and human health risks of OTA have been assessed at both European and international levels by the European Commission Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), who have established tolerable intakes of OTA from food [11]. The kidney is the major site of OTA-induced toxicity, where it acts principally on the middle (S2) and terminal (S3) segments of the proximal convoluted tubules [12]. OTA has been shown to be nephrotoxic in all monogastric species tested, although there are species differences in sensitivity to nephrotoxic effects [13]. Once absorbed, OTA readily binds to serum albumin and is distributed in the blood predominantly in a bound form [14]. OTA binds strongly to human serum albumin and shares a common binding site with other known anionic compounds, including warfarin, naproxen and phenylbutazone, giving rise to the possibility of OTA–drug interactions [15]. Clinical chemistry parameters have been used to monitor the effects of OTA on renal function. Studies have shown that OTA exposure can lead to increased urine volume, blood urea nitrogen [15], urinary glucose, and proteinuria. As well as to reductions in the activity of OTA 313 enzymes such as alkaline phosphatase, leucine aminopeptidase and γ -glutamyl transferase, in the kidney [16]. The biochemistry of OTA results primarily from its structural similarity to the essential amino acid, phenylalanine (Phe). The principal effect appears to be inhibition of protein synthesis, although secondary effects, such as inhibition of RNA and DNA synthesis have also been implicated in its mechanism of action. The chemical structure of OTA consists of a 5'-chlorinated-3,4-dihydro-3-methylisocoumarin moiety linked to L-Phe [17]. A number of epidemiological studies have identified OTA as a likely etiological agent responsible for a fatal kidney disease primarily affecting rural populations in the central Balkan peninsula, including Bosnia and Herzegovina, Bulgaria, Croatia, Romania, Serbia and Slovenia. The disease, referred to as Balkan endemic nephropathy (BEN), is characterized by tubular degeneration, interstitial fibrosis and hyalinization of the glomeruli and a slowly developing impairment of renal function with a progressive decrease in kidney size [18]. Although OTA is a contaminant of food and feed worldwide, it has been detected at high levels in a wide range of foods and in human blood samples taken from areas where BEN is endemic [19]. OTA has been implicated in the development of cancers of the human urinary tract because of the higher incidence of urinary tract tumors in humans in regions where BEN is endemic [20]. The mechanism by which OTA induces tumor formation remains controversial and is an area of active study. Formation of DNA adducts is thought by some researchers to be an important event in the tumorigenicity of OTA and it is postulated that OTA or one of its metabolites may act through a genotoxic mechanism involving direct covalent binding to DNA. However, numerous studies yet have investigated the transformation of OTA into a reactive intermediate and its possible role in tumorigenesis [21]. Data on the possible effects of OTA on the immune system are limited. A number of *in vivo* and *in vitro* studies suggest that OTA may affect both humoral and cell-mediated immunity, although the reported effects were generally observed at higher doses than those capable of causing nephrotoxicity. At very high doses (0.5–80 mg/kg body weight) OTA has been shown to cause gross changes to organs of the immune system, such as reductions in thymus size in mice, rats and chickens [22] and necrosis of cells in the spleen and lymph nodes in rats [23].

Scientific evaluations now generally become the basis for recommendations regarding the international regulation on mycotoxins (ochratoxin and CIT) by the Codex Committee on Food Additives and Contaminants (CCFAC) as well as the European Union. Regulations may include guidelines regarding maximum residue levels or procedural guidelines aimed at prevention by using a Hazard Analysis of Critical Control Points (HACCP), or a combination of them. The overall process needs to be transparent and should aid in the development of harmonized mycotoxin regulations control procedures. Ideally, such guidelines are acceptable to countries producing as well as those importing food commodities [24].

MATERIALS AND METHODS

performed. Fifty square acres of agricultural areas and processing plants sampled by settling plates in a group The following sample agenda for the closed (indoor) and open (outdoor) positions based on the CBS rules was setting, were taken by six plates

containing malt extract agar, yeast extract agar, Czapek yeast extract agar, Czapek agar, Sabouraud dextrose agar and potato dextrose agar confounded with chloramphenicol (100ppm) were used to withdraw a sample group. All plates were aerobically incubated at $2 \pm 25\text{ }^{\circ}\text{C}$ and consistently checked in the range of 3, 7 and 15 days to be withdrawn. The culture plates were subcultured in the tubes containing agar slant bott from the growth media of malt extract agar, yeast extract agar, potato dextrose agar, corn meal agar, Sabouraud dextrose agar, Czapek yeast extract agar and Czapek doxs agar and incubated according to the previously mentioned program. Of *Aspergillus* colonies on selective agar plates those containing Czapek doxs agar, Czapek yeast extract agar (with and without 20% sucrose), malt extract agar and Czapek doxs agar (with and without 20% sucrose) according to ICPA identification rules grown at $2 \pm 25\text{ }^{\circ}\text{C}$, after 3, 7 or 14 days were reviewed and each sample was provided with slide on substrates of Czapek doxs agar and Czapek yeast extract of 20% sucrose to provide a normative growth model.

Preparing extracts obtained from isolates grown in liquid medium to perform more motivate and abundant extract of each isolate grown were taken on Czapek extract broth then a 50ml Falcon tube containing liquid medium Czapek doxs broth with 2% of malt extract to enhance growings, and tubes with 200RPM at $3 \pm 25\text{ }^{\circ}\text{C}$ and in the light - darkness were incubated after a 7-14 days float or sink in a liquid mass on the same field small Germ tubes using centrifugation with 3000RPM for 15 min precipitated then separated with sterile filter paper from the growth medium till each to be harvested. The mass was dried for 48h in a desiccator and then 2g of biomass was harvested, dried and then 2g of each major mold was transferred in to a 15ml Falcon tube three times in a row (every $1 \pm 5\text{ min}$), mixed with 5 ml of liquid nitrogen and a stirring glass Pearls every 25 min combination. Falcon tubes with 5ml of sample buffer and added cold acetone of 1ml, were centrifuged with 3000RPM for 15min. Separation toke place supernatant of coarse sediment removal and other tubes were kept in the notation for synchronization. The size of each protein mixture obtained from *Aspergillus* isolates and all samples were measured by the Bradford method. To size 0.5mg/ml the concentrated sample dilution was aligned and the diluted samples were again concentrated by Bradford method until all the juice extract samples with 0.5 mg/ml of protein were attained.

A competitive enzyme immunoassay for the quantitative analysis of CIT in cereals and feed (RIDA SCREEN® FAST assay) was used to quantify of mycotoxin CIT formed by the *Aspergillus* species. *Aspergillus* species are able to produce CIT and/or OTA, therefore both mycotoxins often appear together and it would be possible to detect the mycotoxins rapidly and with accuracy. Firstly the extracts were filtered through whatman No.1 filter, 1ml of the deionized water was diluted and 50 μl of the filtrate per well was used in the tests as a basis for the antigen-antibody reaction in the microtiter wells coated with the aimed mycotoxins. Afterwards, the standards, respective sample solutions and anti- mycotoxins antibodies, were added concomitantly. Free and immobilized mycotoxins competed for the mycotoxins antibody binding sites (competitive enzyme immunoassay) after a washing step. Secondary antibodies of 100 μl labeled with peroxidase were added to bind to the bound anti- mycotoxins antibodies while no unbound enzyme conjugated. Secondary antibodies were then removed in washing step, 100 μl substrate/chromogen was added to the wells, bound enzyme conjugate (secondary antibodies labeled with peroxidase) converted the chromogen into a blue color product and then turning by addition of 100 μl stop solution lead to a color change to yellow. The measurement was made photometrically at 450nm, and the absorbance lead inversely proportional to the mycotoxins concentration in the sample.

RESULTS AND DISCUSSION

The measurement revealed that the mean CIT was produced by the studied species, with most of the toxin produced by *A.niger* (1655.91 ppb) and at lower levels by *A.niveus* (951.81ppb), *A.ochraceus* (883.29ppb), *A.parasiticus* (873.01ppb), *A.fumigatus*(868.25ppb), *A.ostianus* (577.84ppb), *A.af.flavus* (522.44ppb), *A.flavus*(522.42ppb), *A.alliaceus* (311.73ppb), *A.sojae* (245.99ppb), *A.awamori*(216.27ppb), *A.terreus* (198.8ppb), *A.af.terreus* (198.5ppb), *S.ornate* (53.33ppb), *A.melleus* (44.54ppb), *A.unguis* (32.39ppb), *A.wentii* (8.04ppb), *A.candidus* (2.74ppb), *A.carbonarius*(1.62ppb), *A.foetidus* (0.061ppb), *A.SP3*, *A.spIV*, *A.spV*, *A.af.nidulans* and *A.spVI* species did not produce CIT in culture media [Figure-1].

According to the measurements average amounts of ochratoxin and CIT were produced by *Aspergillus* species, *A.niger*, *A.niveus*, *A.ochraceus*, *A.parasiticus*, *A.fumigatus*, *A.ostianus*, *A.af.flavus*, *A.flavus*, *A.alliaceus*, *A.sojae*, *A.awamori*, *A.terreus*, *A.af.terreus*, *S.ornate*, *A.melleus*, *A.wentii*, *A.candidus*, *A.carbonarius* and *A.foetidus* isolates which produced both ochratoxin and citrinin. This showed that there was a significant relationship among *Aspergillus* species enabling them to produce more than one toxin at a time.

Considering the conducted studies and the results obtained by ELISA and relative distribution, the number of samples per obtained *Aspergillus* species isolates for culture media was determined, and the most frequent ones were identified to be *A.foetidus*, *A.ostianus*, *A.spV*, *A.unguis*, *A.candidus*, *A.awamori*, *A.melleus*, *A.wentii*, and *A.niveus* with a prevalence of (36%) as the most frequent the measurements of mycotoxins averaged CIT, ochratoxin in culture media, indicated that the most CIT was produced by *A.niger* (1655.91ppb), for ochratoxin the highest concentration was produced by *A.carbonarius* (8.007ppb). In contrast, the lowest CIT (0.061ppb) produced by *A.foetidus*, the lowest ochratoxin (1.835ppb) was prepared by *A.spIV* isolates. According to maximum CIT limits (200ppb) and maximum Ochratoxin limits (5ppb) in Europe, Asia, America eventually Latin America, New Zealand, Africa, Canada and the Middle East for food products and animal feed including, wheat, corn, barley, rice, flour, Black wheat, oats, red rice, fruit and nuts, especially related to the genus *Aspergillus* and *Penicillium* were determined.

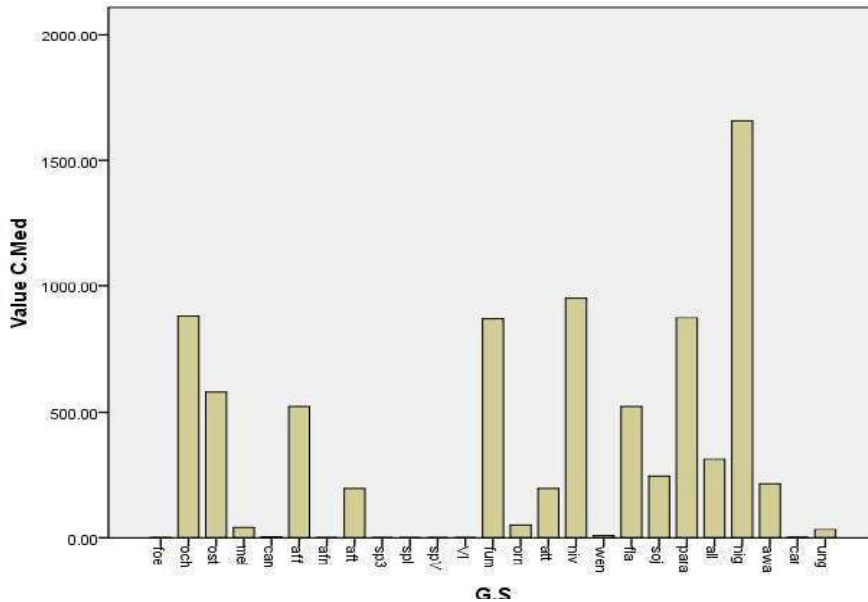


Fig: 1. Measurement of CIT average in culture media of each *Aspergillus* species.

The measurement revealed that the mean ochratoxin was produced by studied species, with most of the toxin produced by *A.carbonarius* (8.007ppb) and at lower levels by *A.melleus* (6.14ppb), *A.niveus* (5.033ppb), *A.ostianus* (4.619ppb), *A.spV* (4.285ppb), *A.awamori* (4.241ppb), *A.ochraceus* (3.897ppb), *A.foetidus* (3.645ppb), *A.niger* (3.535ppb), *A.spVI* (3.285ppb), *A.alliaceus* (3.243ppb), *A.flavus* (3.225ppb), *A.af.nidulans* (3.149ppb), *A.Fumigatus* (3.133ppb), *A.wentii*(3.055ppb), *S.ornata* (2.959ppb), *A.af.terreus* (2.941ppb), *A.sp3*(2.733ppb),*A.parasiticus* (2.725ppb), *A.terreus* (2.551ppb), *A.af.flavus* (2.349ppb), *A.candidus* (1.967ppb), *A.sojae* (1.939ppb), *A.spIV* (1.835ppb) and *A.unguis* species did not produce OTA in culture media [Figure-2].

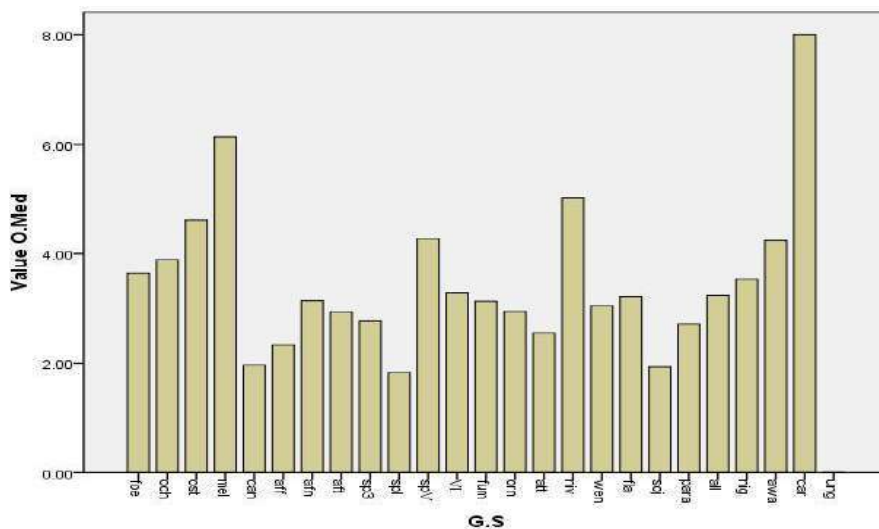


Fig: 2. Measurement of ochratoxin average in culture media of each *Aspergillus* species.

According to the previous researches in relation to the measurement of ochratoxin and CIT and the subsequent analysis, OTA produced by species in *A.carbonarius*, *A.ochraceus* and *A.niger* in grain samples were examined, however, our recent measurements performed in this study revealed that *A.niger* (3.535ppb), *A.carbonarius* (8.006ppb), *A.ochraceus* (3.897ppb) produced OTA in culture media [25]. Pioneer studies were carried out in order to determine the possible presence of OTA and CIT in game sausages, semi-dry sausages and fermented dry-meat products, randomly taken from individual producers and the market. OTA was quantified using ELISA,

while CIT was quantified using high performance liquid chromatography (HPLC)-fluorescence detector. In 90 samples, the fungi most frequently isolated from dry-cured meat products were of *Penicillium* species, while *Aspergillus* was isolated from only one sample. As much as 68.88% of the samples were positive for mycotoxins. Finally, the analysis of different types of meat products resulted in the OTA identification in 64.44%, CIT identification in 4.44% of the samples. The maximum OTA concentrations established in the commercial sausage samples equalled to 7.83 µg/kg. Although OTA was detected in all the three types of products at different percentage, mutual differences were not statistically significant [26,27]. In Spain, samples of aromatic and/or medicinal were herbs sold screened, using an ELISA (LOD = 16.5µg/kg) and 61% of them were found to be contaminated with CIT (up to 355 µg/kg in ginkgo leaves), in two samples the highest concentrations of CIT contamination, the toxin co-occurred with OTA, AFB1. In another study in different storage centers in India, seed samples of medicinal plants were found to have curative properties for various human diseases, and 20% of samples were contaminated with CIT at a concentration between 10 and 760 µg/kg [28]. One of the first comprehensive studies on the occurrence of Citrinin was done on grain samples associated with lung problems in farmers and silo operators were collected from farm storages and analyzed. The grain had been stored under damp conditions, resulting in heating and spoilage. After development of an appropriate screening method, more samples were found to contain CIT (0.07 to 80mg/kg). The contaminated samples included wheat, oats, barley and rye. All samples positive for CIT were also contaminated with OTA. Studies In Europe have been mainly carried out in Southeastern European countries, where the occurrence of CIT has been linked to the so-called Balkan endemic nephropathy [29]. In a study on wheat samples (for food use) from the Czech Republic were analyzed shortly after harvest. In the mentioned study, there was only one sample positive for CIT, which had a low content, not exceeding the limit of quantification (LOQ) 1.5µg/kg. The same samples had an OTA content of 4.7µg/kg. The authors also analyzed barley samples destined for malt production. One of the samples was offered to a malt house, but not accepted due to a higher content of admixtures and impurities and a moldy smell. This sample contained the highest CIT content (93.6µg/kg) and also contained OTA (31.4µg/kg). Barley and wheat for feed use were also analyzed by these authors and CIT was found in only few barley samples a concentration of 13.2 µg/kg [30]. In another study on CIT in grains for food use with through LC-MS/MS method. In the mentioned study, CIT was detected in one wheat sample at a concentration of 0.19µg/kg, together with OTA and also in two buckwheat samples at concentrations of 0.55 and 0.62µg/kg, together with OTA, in one third of rice samples [31]. A methodology was described by Molinié et al. (2005) for simultaneous extraction/purification of OTA and CIT with a recovery for CIT of 80% and an LOD of 0.5 µg/kg. They confirmed that for breakfast cereals, it Citrinin was presenting its content was higher than that of OTA. They analyzed samples, of which 69% were contaminated with OTA at 0.2-8.8µg/kg and 18% were contaminated with CIT in the range of 1.5_42 µg/kg. When CIT was found in a sample, it always occurred with OTA. The sample with the highest CIT concentration (42µg/kg) contained OTA at a concentration of 4.1µg/kg. The (ELISAs) for CIT detection have been reported in wheat, barley, maize, RMR, and other grains, with LODs ranging from 2 to 15000 µg/kg. According to the analysis using ELISA methods, CIT was produced in the range of (0.060-1655.91ppb) in the culture media by *A.niger* (1655.91ppb) and by *A.foetidus* (0.060ppb) [32].

CONCLUSION

Mycotoxins are very hazardous to animals and humans. Mycotoxicoses cause severe problems. The risk of contamination by mycotoxins such as citrinin, ochratoxin is an important food and feed safety concern. Domestic animals are exposed to mycotoxins, significant caution shall be carried on using animal products such as milk, eggs and meat. According to the measurements average amounts of ochratoxin and citrinin were produced by *Aspergillus* species, there was a significant relationship among *Aspergillus* species taxonomic criteria enabling them to produce more than one toxin at a time, and also a significant relationship was seen among isolates which produced both of citrinin and ochratoxin as well. After development of an appropriate screening method, a significant relationship was seen among *A.niger*, *A.niveus*, *A.ochraceus*, *A.parasiticus*, *A.fumigatus*, *A.ostianus*, *A.af.flavus*, *A.flavus*, *A.alliaceus*, *A.sojae*, *A.awamori*, *A.terreus*, *A.af.terreus*, *S.ornate*, *A.melleus*, *A.wentii*, *A.candidus*, *A.carbonarius* and *A.foetidus* isolate which produced both of CIT and ochratoxin as well. Considering the conducted studies and the results obtained by ELISA and relative distribution, the number of samples per obtained *Aspergillus* species isolates for culture media was determined, based on the measurements and the statistical analysis, the maximum amount of CIT in culture media (1655.91ppb) was produced in *A.niger*. In fact *A.niger* was the most important species and the highest CIT producer causing it to exceed the universal and local standard limits. The greatest amount of ochratoxin produced by *A.carbonarius* (8.007ppb) was more than the standard limits. When CIT was found in a sample, it always occurred with OTA confirmed that it Citrinin was presenting its content was higher than that of OTA. It can be concluded that since methods for controlling

mycotoxins are largely preventive, those mycotoxins that are known or suspected to cause human disease have been focused in this study. The occurrence of 2 mycotoxins (CIT and OTA) was investigated in mycobiome samples collected from 3 provinces of the northern region. Our results confirm that there is a great need to measure the average amount of OTA, and CIT were produced by native *Aspergillus* species and showed too, there was a significant relationship among *Aspergillus* species enabling them to produce more than one toxin at a time.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FINANCIAL DISCLOSURE

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DETECTION OF HBV VIRUS NUCLEIC ACID BY SEROLOGICAL METHODS

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ABSTRACT

Since Cumitech 18 was published in 1984, significant advances have occurred in the understanding of the viruses that cause hepatitis. These advances include laboratory diagnosis, prevention, and the treatment of viral hepatitis. Hepatitis (liver inflammation) has many etiologies, one of which is viral infection. Although the differences in the clinical course of infection with each of the hepatitis viruses give some indication as to the viral etiology, diagnosis is usually laboratory based. Laboratory diagnosis of these infections is based on serological or nucleic acid detection techniques, because members of this group of viruses grow either poorly or not at all in cell culture. Therefore, protection against the hepatitis viruses will require developing an effective vaccine for each individual agent. In this article from valid sources of Google Scholar, search for, in this paper, which is convenient and fast method for detection of viruses to you introduce the readers.

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INTRODUCTION

HBV is a 42-nm spherical particle consisting of a 7-nm outer shell and a 27-nm inner core that encloses the viral DNA and DNA polymerase. surface component of the complete virion, sometimes referred to as the Dane particle, contains hepatitis B surface antigen (HBsAg), and the inner core component contains hepatitis B core antigen (HBcAg) [1]. In addition to complete virus particles, numerous HBsAg-containing, 22-nm spherical particles and tubular structures of the same diameter and variable length circulate in the blood of HBV-infected individuals. HBV strains have been classified into nine different subtypes, ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+, and adrq-, according to the antigenic determinants and subdeterminants of their HBsAg HBV has also been classified into six genotypes, A to F, based on nucleotide sequence differences [2]. HBcAg, unlike HBsAg, is not directly detectable in the serum, but removal of the outer HBsAg component of the complete HBV particle with nonionic detergents results in the release of serologically reactive HBcAg. Solubilization with sodium dodecyl sulfate and 2-mercaptoethanol of either the inner HBcAg component of HBV particles or core components from infected liver results in the appearance of hepatitis B e antigen (HBeAg) and the disappearance of HBcAg reactivity [3], suggesting that HBeAg is a structural component of the inner core of the HBV particle. HBeAg is also present as a readily detectable soluble protein in the serum of HBV-infected individuals who have high levels of viremia. During HBV infection, HBsAg, HBcAg, and HBeAg can induce an antibody response in the host with the development of anti-HBs, anti-HBc, and anti-HBe, respectively. The inner core component of the HBV virion contains an endogenous DNA polymerase with reverse transcriptase activity and a unique viral genome that consists of partially single-stranded, noncovalently closed circular DNA and is only 3,200 nucleotides (nt) in length [4]. The complete nucleotide sequence of the HBV genome and the coding sequences for all of the HBV proteins have been identified. The unique characteristics of HBV classify it as the prototype member of the hepadnavirus family of hepatitis B-like viruses, which includes hepatitis viruses infecting woodchucks, Beechey ground squirrels and Pekin ducks. These viruses have several characteristics in common,

including ultrastructure, antigenic composition (except for the duck virus), DNA size and structure, DNA polymerase, a tropism for liver, an association with persistent infection, and, for the primary hepatocellular woodchuck carcinoma [5]. virus and HBV, Transmission of HBV in humans is primarily through intimate physical contact or, less frequently, through exchange of blood products, such as transfusion or hemodialysis [6].

Clinical findings

The incubation period for HBV is usually about 3 months, but varies from 4.5 to 180 days. Approximately 30 to 40% of adults infected with HBV develop symptomatic acute hepatitis, and fulminant hepatitis resulting in death occurs in 1 to 3% of these individuals [7]. At least 50% of persons infected with HBV exhibit a transient subclinical infection [8]. In symptomatic HBV infection, the onset of acute hepatitis is preceded by a short prodromal phase characterized by fever, fatigue, malaise, anorexia, myalgia, nausea, and vomiting. The icteric phase usually begins within 10 days of the initial prodromal symptoms and is characterized by the appearance of dark, goldenbrown urine followed several days later by pale stools and jaundice [9]. A chronic HBsAg carrier state develops in 5 to 10% of adults after either symptomatic or subclinical acute hepatitis B (54). Up to 90% of infants who acquire HBV infection from HBsAg-positive mothers become chronically infected. Chronic HBV infection may progress to chronic persistent hepatitis, chronic active hepatitis with or without cirrhosis, or even primary hepatocellular carcinoma [10].

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Serological Markers Andimmunodiagnosis

Because HBV is not readily propagated in cell cultures or in animals other than high-order primates, the diagnosis of HBV infection is accomplished with sensitive immunoassays for the identification of HBV-associated antigens and antibodies in the blood [11]. Third-generation immunoassays including RIAs, EIAs, microparticle enzyme immunoassays (MEIAs), and a reversed passive hemagglutination (RPHA) test are commercially available for the detection of HBsAg, anti-HBs, anti-HBc, IgM anti-HBc, HBeAg, and anti-HBe. Additional serum markers for HBV infection include the following: DNA polymerase, HBcAg, and HBV DNA (see "Nucleic Acid Diagnosis"). Tests for the presence of these markers do not add substantially to the information provided by the commercially available assays for HBeAg and are thus not included in most routine diagnostic evaluations. Measuring HBV DNA levels may be useful in chronic hepatitis B patients who are receiving antiviral therapy. This complicated system of HBV-associated serological markers may define the phase of infection, degree of infectivity, prognosis, and immune status of the patient. HBsAg is the first serological marker to appear after HBV infection. HBsAg becomes detectable during the late incubation period, 2 to 5 weeks before the onset of symptoms, and persists for 1 to 5 months in symptomatic acute hepatitis B. HBsAg is the first serological marker to appear after HBV infection. HBsAg becomes detectable during the late incubation period, 2 to 5 weeks before the onset of symptoms, and persists for 1 to 5 months in symptomatic acute hepatitis B. Although HBsAg is detectable in the sera of most patients with symptomatic acute hepatitis B, it may be undetectable by current third-generation assays in more than 10% of these cases. HBsAg is frequently absent, or present for only days, in transient subclinical HBV infection. In chronic HBV infection, HBsAg usually persists at high titer for a minimum of 6 months to as long as decades [12]. The presence of HBsAg should always be considered indicative of ongoing HBV infection. Some individuals with chronic HBV infection have undetectable levels of circulating HBsAg. Such persons are referred to as "low-level carriers". HBeAg appears at the same time as or shortly after HBsAg, and its presence correlates with early and still active disease as well as high infectivity in acute HBV infection [13]. HBeAg persists for 1 to 9 weeks after the onset of symptoms in symptomatic acute hepatitis, and although it may occasionally remain detectable for several weeks after the clearance of HBsAg, it usually disappears before HBsAg. Persistence of HBeAg for more than 10 weeks after the onset of illness predicts the development of chronic HBV infection. HBeAg remains detectable for several months to years in HBsAg-positive chronic HBV infection and will eventually disappear in many patients with persistent HBsAg. HBsAg-positive chronic HBsAg carriers tend to be young, to have developed the carrier state within the previous 10 years, to have some evidence of chronic liver disease, and to have high titers of hepatitis B virions [14].

Mother-to-infant transmission of HBV infection is highly associated with the presence of HBeAg. Anti-HBc appears shortly after the appearance of HBsAg and HBeAg and shortly before or at the onset of clinical

symptoms. In acute hepatitis, anti-HBc is frequently the only detectable serological marker of HBV infection other than anti-HBe [15]. This serological pattern may occur during the acute phase in persons who do not develop detectable HBsAg and HBeAg, or during the convalescent phase after the disappearance of HBsAg but before the appearance of anti-HBs. Anti-HBc, alone or together with anti-HBe, is sometimes observed in individuals who have recovered from past HBV infection but did not develop or subsequently lost detectable anti-HBs. Anti-HBc initially consists of both IgG and IgM antibodies, but IgM anti-HBc decreases in titer and disappears within several months, to one or more years after acute infection, depending on the sensitivity of the assay [16]. Since IgG anti-HBc can persist for years or even a lifetime, the detection of IgM anti-HBc distinguishes HBsAg-negative, anti-HBc-positive patients with current or recent acute HBV infection from those with past infection. IgM anti-HBc has been demonstrated in more than 50% of acute hepatitis patients who are negative for both HBsAg and anti-HAV IgM but are positive for total anti-HBc, regardless of the presence or absence of anti-HBs. In chronic infections the initial anti-HBc response also consists of both IgG and IgM antibodies, but unlike the situation in acute infection, IgM anti-HBc titers decrease very slowly and low titers persist for years while total anti-HBc titers remain very high. Anti-HBc may be the only detectable serological marker in chronically infected HBsAg-negative individuals. The demonstration of IgM anti-HBc and high titers of total anti-HBc in these low-level carriers is indicative of chronic HBV infection. Because IgM anti-HBc can be detected in chronic HBV infection, its presence does not always distinguish between acute and chronic hepatitis B [17]. However, the sensitivity of commercial RIAs, EIAs, and MEIAs for IgM anti-HBc has been adjusted to a level where patients with current or recent acute HBV infection are IgM anti-HBc positive, but those with chronic infection are negative or only weakly positive [18]. Anti-HBe usually becomes detectable 2 to 6 weeks after the disappearance of HBeAg and 2 to 4 weeks before the clearance of HBsAg, and it may persist for months or years after acute infection. Anti-HBe positivity after acute infection has a duration generally less than that of anti-HBc and anti-HBs and may persist for only 6 months or less in about 5% of acute HBV infections. Early appearance of anti-HBe often predicts an uncomplicated course of acute hepatitis, whereas a delay of more than 6 weeks after the clearance of HBeAg may indicate a prolonged course or development of chronic hepatitis. Seroconversion from HBeAg to anti-HBe frequently occurs several years after the development of HBsAg-positive chronic hepatitis and is usually accompanied by the spontaneous resolution of chronic liver disease. Anti-HBe-positive chronic HBsAg carriers tend to be older than HBeAg-positive carriers, tend to have been chronically infected for many years, tend to have little or no evidence of chronic liver disease, and tend to be minimally infectious. Anti-HBs usually appears several months after the disappearance of HBsAg in acute hepatitis, although it may be present together with HBsAg as early as the onset of symptoms in 6% of cases. Seroconversion from HBsAg to anti-HBs usually reflects termination of HBV infection, clinical recovery, and immunity against reinfection by any HBsAg subtype by virtue of the common antigenic determinant. Anti-HBs persists for years after recovery from acute infection and is usually detected together with anti-HBc and anti-HBe, although it may be found alone in some individuals who have recovered from a past HBV infection with the subsequent loss of detectable anti-HBe and anti-HBc [19]. Anti-HBs is also the only detectable marker in individuals who have been immunized with the hepatitis B vaccine rather than infected with the virus. Anti-HBs is not usually detectable during chronic HBsAg-positive hepatitis, but it may occasionally be present due to previous or subsequent infection with a different HBsAg subtype or as a result of antigen-antibody complexes [20]. Tests for the detection of HBsAg, anti-HBc, and anti-HBs should be performed routinely for the laboratory diagnosis of current or past HBV infection. IgM anti-HBc, HBeAg, and anti-HBe determinations should only be performed when indicated by the results of HBsAg, anti-HBc, and anti-HBs assays. IgM anti-HBc testing can be used to distinguish between acute and chronic HBV infection in HBsAg-positive individuals [21]. HBeAg and anti-HBe detection should be attempted on HBsAg-positive sera when the relative infectivity of blood is of clinical significance or when early seroconversion from HBeAg to anti-HBe after acute HBV infection is used as a prognostic indicator of transient rather than chronic infection [22]. The detection of IgM anti-HBc or anti-HBe may be diagnostic of recent or current acute HBV infection when it is found together with total anti-HBc as the only detectable HBV-associated serological markers during the “window” phase of the infection between the disappearance of HBsAg and the appearance of anti-HBs. HBeAg and anti-HBe determinations may have therapeutic importance in chronic HBsAg-positive hepatitis because it has been suggested that antiviral therapy be reserved for HBeAg-positive patients with chronic liver disease [23].

Nucleic Acid diagnosis

Nucleic acid hybridization assays have been developed for the detection of HBV DNA in the serum of patients with HBV infection. Two (research use only) hybridization assays for the quantitation of HBV DNA in human serum are commercially available: a liquid-phase molecular hybridization assay (Genostics HBV DNA Assay; Abbott Laboratories) and a “sandwich” nucleic acid hybridization assay (Quantiplex HBV-DNA Assay; Chiron

Corporation, Emeryville, Calif.). Slot or dot blot hybridization assays have a detection limit of about 5 pg of HBV DNA per ml, corresponding to approximately 1.5×10^6 viral genomes per ml [24].

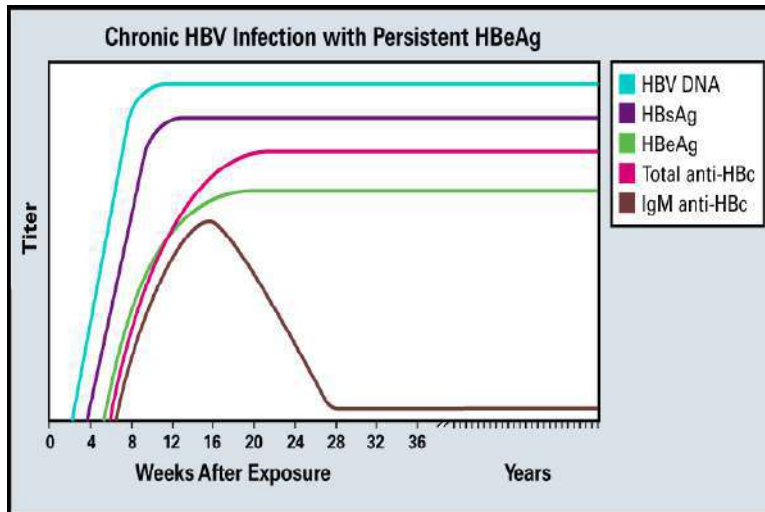


Fig. 1. Virologic and Serologic Markers in Patients who Progress to Chronic Hepatitis B Virus Infection with Persistent HBeAg.

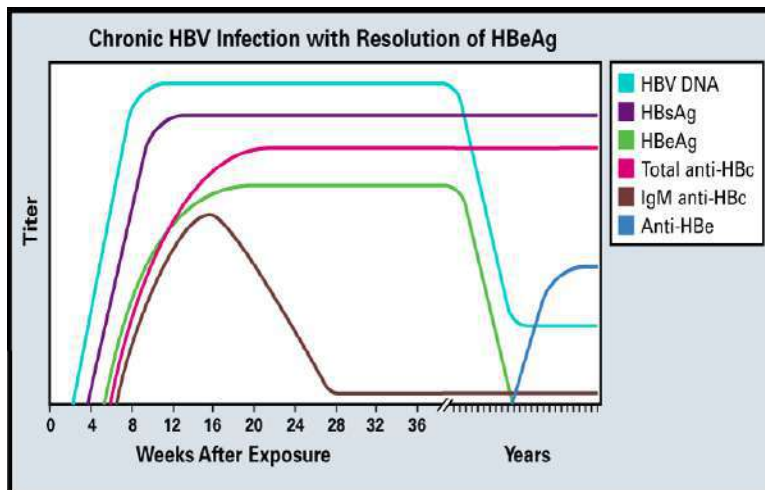


Fig. 2. Virologic and Serologic Markers in Patients who Progress to Chronic Hepatitis B Virus Infection with Resolution of HBeAg

The branchedchain DNA (bDNA) hybridization assay from Chiron Corporation can detect as few as 700,000 HBV DNA equivalents per ml (approximately 2.5 pg/ml) [25]. And the liquid-phase hybridization assay from Abbott has a detection limit of 400,000 HBV DNA equivalents per ml (approximately 1.5 pg/ml). The loss of detectable HBV DNA by a solution hybridization assay is an earlier indicator of response to treatment than loss [26]. PCR has been shown to detect as little as 10^3 pg of HBV DNA per ml, or approximately 100 to 1000 HBV DNA equivalents per ml, and is currently the most sensitive method available for the detection of HBV DNA in the serum. Most HBsAg-positive individuals have detectable HBV DNA in the serum, especially those who are also HBeAg positive. HBeAg-positive individuals usually have high levels of HBV DNA in the serum that are easily detectable by hybridization assays and PCR. HBsAg-positive individuals who are negative for HBeAg usually have lower levels of HBV DNA in the serum that may be undetectable by hybridization assays, but most of these individuals have detectable HBV DNA by PCR. HBeAg-negative patients with detectable serum HBV DNA have higher serum alanine aminotransferase [27].

levels than those who are PCR negative. HBV DNA may persist for several months after the clearance of HBsAg from patients with acute HBV infection. HBV DNA is also detectable in some patients with chronic HBV infection who are anti-HBc positive but HBsAg negative. Finally, in patients with chronic hepatitis B who become HBsAg negative following IFN- α treatment, HBV DNA may remain detectable by PCR for many months after the clearance of HBsAg. Thus, the PCR assay for HBV DNA appears to be more sensitive than HBsAg assays for detecting low-level viremia. The clinical significance of low levels of HBV DNA in HBsAg-negative patients is uncertain because HBV DNA eventually becomes undetectable in most of these patients [28].

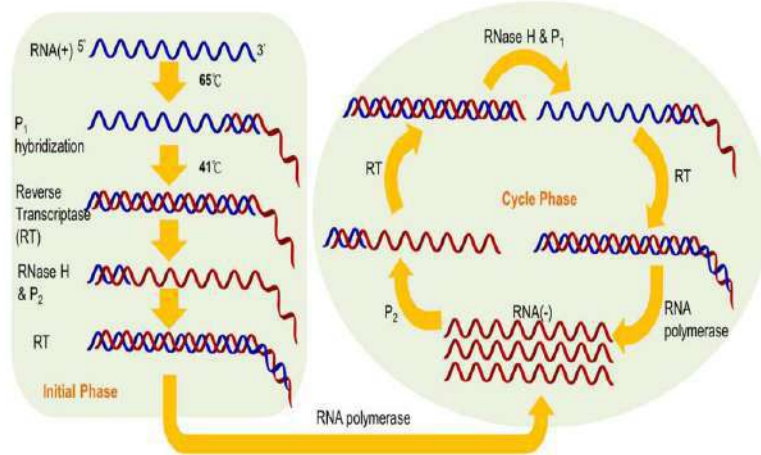


Fig: 3. Schematic representation of the NASBA amplification.

CONCLUSION

There is no completely satisfactory therapy for chronic delta hepatitis. Early studies indicated that a 3- to 4-month course of IFN- α suppressed HDV replication and reduced liver disease in some patients, but almost all patients relapsed after therapy was discontinued [29]. Subsequent studies with longer courses of IFN- α have shown more promising results. Approximately one-half of patients with chronic delta hepatitis who were treated with 9 million units of IFN- α three times a week for 48 weeks had a complete response to treatment [30]. A positive response includes a return of serum alanine aminotransferase levels to normal, clearance of HDV RNA from the serum by hybridization assay, and histologic improvement on liver biopsy. Half of the patients who had a biochemical response to treatment still had normal alanine aminotransferase levels more than. However, none of the treated patients had a sustained clearance of HDV RNA from the serum. While IFN therapy may be helpful in many patients with chronic delta hepatitis, liver transplantation may be the only treatment option for patients with fulminant delta hepatitis or with advanced disease and decompensated cirrhosis. Because HDV can establish infection and cause disease only in individuals who are also infected with HBV, prevention of HBV infection is the most effective way to prevent HDV infection. Persons who are immune to HBV as the result of past infection or vaccination with the hepatitis B vaccine are also protected against HDV infection. Individuals at high risk for HDV infection who are susceptible to HBV infection should receive the hepatitis B vaccine to prevent coinfection with HBV and HDV. Postexposure prophylaxis following percutaneous or permucosal exposure to both HBV and HDV is the same as that for exposure to HBV alone [31]. Prevention of HDV superinfection in persons with chronic HBV infection is more difficult because it must depend on avoiding percutaneous or permucosal exposure to HDV [32].

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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THE INTERACTION OF TRAIT EMOTIONAL INTELLIGENCE AND ACADEMIC KNOWLEDGE ON JOB PERFORMANCE OF PROFESSIONAL TRANSLATORS

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ABSTRACT

Evidence supports the fact that Trait Emotional Intelligence (EI) is a predictor of better academic performance. Also, emotionally intelligent people are regarded to better function in workplace settings. Evidence shows that the interaction of EI and academic knowledge could influence the performance of individuals in workplace environments. In this regard, translators' job seems to be highly influenced by this combination. In fact, academic knowledge, professional and psychological conditions of translators may directly influence their translation job. The present causal-comparative study aimed at investigating the interaction of professional translators' Trait Emotional Intelligence and academic knowledge on their job performance. Using purposive sampling method, a sample of 100 professional translators was selected. The participants were divided into two groups containing individuals with and without an academic degree in translation. Petrides & Furnham's (2001) Trait Emotional Intelligence Questionnaire (TEIQue) was administered to collect data on the participants' EI. The participants were asked to translate a text to assess the quality of their translation. To do this, Waddington's model D (2001) was used as the marking system. For data analysis, Two-way ANOVA statistical procedure was used to investigate the research hypothesis. The study results showed that the interaction of translators' Trait Emotional Intelligence and academic knowledge does not predict their translation quality. However, this finding could not be sufficient to reject the hypothesis about the influence of EI on job performance of translators. One may imply that certain subscales of EI may affect performance in workplace settings and the role of individuals' EI besides their academic and professional knowledge could not be underestimated, as is the case of professional translators' job.

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Trait Emotional Intelligence, Job performance, Academic knowledge, Translation quality

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INTRODUCTION

A combination of individual differences and traits including translators' knowledge, professional and psychological conditions may directly influence their translation quality [1-3]. On the one hand, the basic step of a planned and organized approach for training competent translators is the mastery over theoretical frameworks which is made possible by flourishing translation theories and new ideas towards the process of translation [4]. Over the past decades, few studies have directly examined the relationship between individuals' level of education and their job performance. Years of work experience do not moderate the education-performance relationship because work experience indicates the quantity, not the quality of the job done; yet, education influences some dimensions of performance [5]. For instance, it was found that professional translators without an academic degree in Translation do not focus on the revision stage which can challenge the quality of their translation; on the contrary, qualified translators and academics recognize the significance of this stage. Thus, the academic status of the translator should be taken into account as one of the elements of his/her sense of responsibility which influences the quality of their job [6].

Furthermore, to perform a better job, a translator is expected to have good knowledge of other fields [7]; translators are expected to have a very good knowledge of all the domains with which their work interferes. That is why translation students expect to receive solid and complex training, including various specific knowledge and skills associated with their profession, which enables them to face the challenges of translating in real world situations [8]. On the other hand, translation has gone hand in hand with so many factors and should be studied along other fields, and it is not separate from them. Psychology is one of these fields. Emotions and being intelligent about them is an important factor, and may heavily influence the translation job [9]. The study of Trait Emotional Intelligence is one

of the most important dimensions of intelligence [10]. Trait Emotional Intelligence and Emotional Quotient (EQ level) are among the factors contributing to the task of translation [2]. It is hypothesized that emotional intelligence influences the success with which employees interact with the strategies they use and their overall job performance. Preliminary findings also suggest that emotional intelligence positively contributes to different aspects of workplace performance [11].

Translation is an intelligent activity, requiring creative problem-solving in novel, textual, social, and cultural conditions [12]. It is widely accepted that translation can be viewed as a problem-solving process in which the translator encounters problems of various sources and uses tools and resources to solve them. What makes Trait Emotional Intelligence important is that people with Trait Emotional Intelligence are able to control their feelings, utilize their affective data to lead their thoughts and activities and provide themselves with more opportunities to think and be more creative and to aim their emotions at solving problems which, in turn, link to successful translation and the quality of translation [13,14]. That is why the present study approaches Trait Emotional Intelligence in terms of job performance of the translators.

The process of translation needs to engage in a great deal of decision-making activities [15]. Considering the process of decision-making in translation as a particularly complex procedure, Willis suggested that the translator should pay attention to factors that lead to choices, rather than on the choices themselves [16]. There is also a general consensus among translation researchers that the decision-making process also plays a salient role in translators' performance and the quality of their translation [17]. The behavior, psychological disposition [14], cognitive, emotional [18] and individual characteristics [19,20,21,22] influence decision-making process. Furthermore, "according to popular opinion and work-place testimonials, Trait Emotional Intelligence increases performance and productivity" [23].

Therefore, the necessity of investigating translators' Trait Emotional Intelligence is of great importance to predict their job performance; For instance, there is a positive relationship between EQ and the quality of children's literature translation [2]. Briefly speaking, translators' degree in translation and academic knowledge positively affect the quality of the translation product, the process of translating and producing a good translation [3,6,24]. On the other hand, investigating Trait Emotional Intelligence is necessary to gain a deeper understanding of the translation process [6]. Also, Trait Emotional Intelligence has proved to be correlated with successful task performance [7]. Translation as a career places a higher emphasize on Trait Emotional Intelligence abilities, which may successfully predicts translators' job performance [25]. Trait Emotional Intelligence influence academic achievement [26] and academic performance [11,23, 25, 27] as well as writing performance [28]. Job-relevant knowledge is a direct determinant of job performance on the one hand and individual differences in traits are an antecedent of job knowledge on the other hand [29]. Hence, emotional intelligence may only affect job performance through its effect on knowledge. This rationale highlights the importance of investigating the interaction of translators' Trait Emotional Intelligence and their academic knowledge and the impact which it exerts on and their job performance.

The focus of the present study was to investigate whether the interaction of translators' Trait Emotional Intelligence and academic knowledge influence their job performance. This study was designed to seek answers to the following question:

Does the interaction of translator's academic knowledge and Trait Emotional Intelligence significantly influence their translation quality?

Based on the aforementioned research question, the following hypothesis was formulated:

The interaction of translator's academic knowledge and Trait Emotional Intelligence significantly influence their translation quality.

The influence of translator's academic knowledge on the quality of their translations has been investigated in some studies. For instance, it was revealed that higher-quality of translations and better performance of translators is related to their training and experience [30]. Translators' academic knowledge enables them to produce a 'good' translation [24]. The education of translators enhances the quality of their work [31]. It was also reported that the translators' level of education influences their translation quality [32]. Some professional translators, mainly without academic degree in Translation, ignore or underestimate the role of revision on the translation product while qualified translators recognize the importance of the revision stage [6]. Translators' knowledge of pragmatics is one of the crucial elements to enhance the quality of their translation [33]. Finally, the translators'

academic knowledge plays an important role in predicting the quality of their job. Being aware of theoretical frameworks and having knowledge of theories of translation may affect the quality of the translation product [1]. Trait Emotional Intelligence is a completely new topic in translation studies and very little research investigated the impact it exerts on translation quality. It seems that Trait Emotional Intelligence may directly or indirectly influence the process and product of translation; however, one study provided evidence that there was not a significant relationship between Trait Emotional Intelligence and the quality of the translation among the students of translation studies [2]. On the contrary, there is a difference between Trait Emotional Intelligence scores of literary and non-literary translators in terms of emotion regulation. People with higher EI are able to handle the affective nature of texts much better. It is both necessary and desirable to investigate translators' Trait Emotional Intelligence to gain a deeper understanding of translation processes [20]. It was also demonstrated that a positive relationship exists between EQ and the quality of translation [2]. Also, the level of Trait Emotional Intelligence might be one of the possible factors manipulating the translators' mind. There was a positive and significant relationship between the learners' EQ and their success in oral translation performance [34]. Another study stressed the importance of Trait Emotional Intelligence in the work of interpreters; Emotionally Intelligent interpreters would not necessarily be better interpreters, but they would definitely be a more adjusted professional with a higher level of success in their career. The study further suggested that Trait Emotional Intelligence should be introduced in academic courses or in the training of interpreters [35].

To the best of our knowledge, very few studies have primarily investigated the interaction between Trait Emotional Intelligence and academic knowledge and the impact it exerts on translators' job performance. High educational background had a strong correlation with Trait Emotional Intelligence [36]. Moreover, job-relevant knowledge is a direct determinant of job performance on the one hand and individual differences in traits are an antecedent of job knowledge on the other hand. Hence, emotional intelligence may only affect job performance through its effect on knowledge. This rationale highlights the importance of investigating the interaction of translators' Trait Emotional Intelligence and their academic knowledge and the impact which it exerts on and their job performance [29].

MATERIALS AND METHODS

Design

The design of this quantitative study was causal-comparative in nature.

Instruments

- 1) Trait Emotional Intelligence Questionnaire (TEIQue-SF) was used for measuring the participants' global Trait Emotional Intelligence or EQ which was designed and validated by Petrides & Furnham (2003).
- 2) To assess translation quality of the participants, Waddington's translation quality assessment model D was used. This model is designed and validated by Waddington (2001).

Participants

The study participants were a group of professional translators, working in official and non-official translation centers, and freelance translators. The inclusion criterion was: 2-7 years of experience. Individuals with both Bachelors' and Masters' degrees in translation were excluded from the study to minimize the influence of their knowledge and proficiency. Using purposive sampling method, 100 translators were selected. They were divided into two groups, each containing 50 subjects. Group A and group B contained subjects with and without a degree in translation, respectively.

Data collection procedures

The study participants completed the TEIQue-SF questionnaire and the total EQ scores were computed. Then, both groups were asked to complete a translation task in 30 minutes. The translations were assessed and scored based on Waddington's model D. To do so, a paragraph from the Orwell's 1984 novel was given to be translated into Persian. The scores were entered in SPSS version 21.0 for statistical analysis.

Data analysis

After data entry, the appropriate statistical analysis including descriptive statistics (Frequency, Percentage, Mean and Standard Deviation) were used to analyze demographic data; in order to investigate the hypothesis, inferential statistics including Two-way ANOVA was used.

RESULTS

Reliability of the measures

The Chronbach's alpha was estimated to be 0.86 and 0.82 for the translated version of Petrides and Furnham's TEIQue and Waddington's model D, respectively.

Results of the descriptive and inferential statistics of variables

The mean age of the participants was 28.2 years (SD 6.8). **Table- 1** summarizes the results of descriptive statistics of the variables.

Table :1. Descriptive statistics of variables

	Workplace	Gender	Academic experience	Years of experience	Age
N	100	100	100	100	100
Mean				4.0404	28.2828
Std. Deviation				1.95312	6.83036

Table- 2 reveals the results of total EQ of participants in each group. Mean total EQ of the participants in groups 1 and 2 was 97.9(SD 15.02) and 105.3(SD 17.01). In our study, participants without a degree in translation enjoyed a higher EQ.

Table: 2. Descriptive Statistics of variables (Total EQ)

		N	Mean	SD
Group A	Total EQ	50	97.96	15.021
Group B	Total EQ	50	105.36	17.012

Tables- 3 and 4 present the results of scoring the translations based on Waddington's model D. This scoring system makes use of two subjective (holistic) and objective (error analysis) scoring methods. According to table 3, mean holistic, objective and total score of participants in group 1 was 5.3(SD 1.1), 5.7(SD 1.3) and 5.5 (SD 1). On the other hand, mean holistic, objective and total score of participants in group 2 was 5.1(SD 0.9), 5 (SD 1) and 5.1(SD 0.9). Mean total score in groups 1 and 2 was finally calculated to be 5.5(SD 1) and 5.1(SD 0.9).

Table: 3. Descriptive statistics of variables (Total score)

		N	Mean	SD
Group A	Holistic	50	5.32	1.114
	Objective	50	5.71	1.343
	Total score	50	5.59	1.078
Group B	Holistic	50	5.18	0.983
	Objective	50	5.08	1.083
	Total score	50	5.11	0.911

Table: 4. Mean and Std. Deviation of variables (Total score)

		N	Mean	SD
Total score	Group A	50	5.59	1.078
	Group B	50	5.11	0.911

For investigating the interaction of translators' Trait Emotional Intelligence and academic knowledge on their job performance, Two-way ANOVA was run. According to **Table- 5**, there is no significant relationship between the interaction of translators' Trait Emotional Intelligence and academic knowledge on their translation quality (sig = 0.49).

Table: 5. Results of Two-way ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	57.825 ^a	59	0.980	0.858	0.708
Intercept	2116.029	1	2116.029	1852.87	0.000
TOTALEQ	39.226	46	0.853	0.747	0.831
Academic	1.732	1	1.732	1.517	0.225

TOTALEQ*academic	13.318	12	1.110	0.972	0.491
Error	45.681	40	1.142		
Total	2968.860	100			
Corrected Total	103.506	99			

a. R Squared = 0.559 (Adjusted R Squared = -0.092)

DISCUSSION

Hypothesis: The interaction of translator's academic knowledge and Trait Emotional Intelligence significantly influence their translation quality.

The results of Two-way ANOVA reveal that the interaction of translator's Trait Emotional Intelligence and academic knowledge does not significantly influence their translation quality ($p > 0.05$). This finding could be predicted as no significant relationship was found between translators' Trait Emotional Intelligence and their translation quality [36]. On the other hand, the fact that Trait Emotional Intelligence and academic knowledge influence each other has been proposed in very few studies. The researcher believes that this should be further studied to determine exactly how and why these two variables may interact with each other and consequently impact the translators' translation quality and job performance.

Previous studies indicated that translators' degree in translation, academic knowledge and academic knowledge positively affect the quality of the translation product and producing a good translation. On the other hand, the translators' psychological conditions including their Trait Emotional Intelligence may influence their job performance. Trait Emotional Intelligence is considered as a factor which contributes to performing processes such as problem-solving and decision-making that are embedded in translation career. Translators' Trait Emotional Intelligence was found to affect the quality of the translation product and producing a good translation; while another study did not find a significant relationship between total Trait Emotional Intelligence and translation quality [2]. On the contrary, it was found that there is a difference between Trait Emotional Intelligence scores of literary and non-literary translators [20]. There is a positive relationship between EQ and the quality of translation [2]. Furthermore, some authors stressed the importance of Trait Emotional Intelligence for success in oral translation performance [35, 37]. Although no relationship was found in the present study between the interaction of Trait Emotional Intelligence and academic knowledge on job performance of translators, it is suggested that the concept of Trait Emotional Intelligence should be considered in assessing job performance of translators with academic knowledge in translation; this, for sure, leaves the subject open to further research and discussion.

CONCLUSION

Even though very few studies have investigated this relationship, they have found that Trait Emotional Intelligence and academic knowledge influence each other and this fact may be used as evidence to suggest that this interaction may indirectly affect translation quality. Results of Two-way ANOVA in our sample rejected the hypothesis of the study; although this result is not in agreement with similar studies. For instance, our results are not in agreement with the results of a similar study, which found that Trait Emotional Intelligence and academic knowledge interact with each other to influence job performance.

The conclusions drawn indicate that translators can acquire a higher quality translation through further training in academic courses and problem-solving and decision-making strategies included in the academic environment. What is more, translation educators should not ignore the important role of Trait Emotional Intelligence in the act of translation. It is also recommended that the translation educational system develops a comprehensive curriculum for the benefit of all students with respect to their Trait Emotional Intelligence.

CONFLICT OF INTEREST

None declared.

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EVALUATION OF ASPERGILLUS SPP CONTAMINATION IN THE GILAN PROVINCE, IS STORED AND FRESH HARVESTED RICE CROPS

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ABSTRACT

Introduction: World attention on food safety are increasingly developed. Polished rice grains as a staple food in terms of consumption may be contaminated to different types of fungi. This study aimed to investigate the incidence / prevalence of fungal pollutions in the rice cropped seeds in Gilan province agricultural area that they have been put argument to evaluate. **Material and Methods:** In this study randomized sampling of fresh rice crops and stored facilities were applied, cultured in a series of steps on Saboraud, s agar, Czapek agar medium are of choice for *Aspergillus* spp studies concomitantly macroscopic and microscopic compartments characteristics following diagnostic log with the keys to identify species designated as *Aspergillus* species were reported by ICPA. **Results:** According to three times enumeration the total number of colonies grown on agar plates containing specifications; The greatest surge number of colonies were observed in samples of 2013 by a week of incubation at 25°C (20×10⁵), while of 2014 the largest number of colonies were on 2nd week day of incubation 25°C (7×10⁵). The resultant findings showed that 50% of the tested samples were contaminated to *Aspergillus* spp, only 10% were related to black and more belonging to subgenus *circumdati* and section *nigri*. **Conclusion:** Which indicate that recently taken and stored samples came into microbial incubation and counts must not neglect. The comparison ratio showed that the black colored ones were repeatedly more isolateable, especially *A.niger* (57.14% & 12.5%), *A.carbonarus* (21.42% & 18.75%) and *A.foetidus* (3.16% & 37.5%).

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Aspergillus section nigri, Rice grains, Pollutions, Gilan province

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INTRODUCTION

Mycotoxins are natural toxic secondary metabolites produced by fungi. For centuries, the quality of many products has been reduced by mycotoxins, toxins that are produced by molds in many agricultural products, but especially in grains are observed after harvest during storage even when processed, concentrate and feed the animals [1,2]. Fungal contamination of rice, directly used in human food, if there are suitable conditions of the possibility of producing mycotoxins, endanger human health even failures and pollution, of pollution fungal effect, is high and at least 20% cereals is corrupted and unusable [3]. Crops contamination, especially rice to mycotoxins encounter to the wide range of their effects on the human and animal body parts as well as economic losses, make important issues in the field of food hygiene and public health too [4]. Rice is the most important cereal food consume by the most people of the world, especially the Eastern even Middle east. Half of the world population, are dependent on rice as a main food. Iran with having rice cultivation and annual production of close to 2/5 million tons, occupies a global rank 23th in production and 13th in taking it to be allocated. In Iran, particularly in the northern provinces rice is the staple food of a million people, in the preparation of food Such as cooked rice, dried fruit, odor and Processed food made from rice flour along with its waste used in animal feed [5]. The rice three major fungi pollutants as follows: *Aspergillus*, *Fusarium* and *Penicillium*. These fungi with attacks on agricultural products cause discoloration, reduce marketability, friability, vulnerability and pollution as they have toxins. One of the important factors for rice contamination, is moisture percent of the product, weather condition and storage quality are the most important and determinative factors for rice contamination [6].

MATERIALS AND METHODS

In this research randomly 30 samples of stored and freshly harvested rice (2013:14 and 2014:16 samples) from 9 cities in Gilan (Kelachay, Roodsar, Langerood, Lahijan, Rezvanshahr, Rasht, Anzali, Khomam, Khoshkebijar) for each 10 ton by 100 g sampling were obtained and performed with CBS standard sampling techniques, after sampling process and transfer the samples to the laboratory, were coded, weighing 20 gr of each sample divided in 2:10 gr preparations the first 10 gr milled for mycotoxins

measurement the second transferred to 40 ml of saline solution then centrifuged for fifteen minutes at a speed of 2000 rpm for two time, after homogenization the falcon contents entered into the appropriate test tubes and centrifuged (15 minutes at 3000 rpm) till supernatants were evacuated tubes and the resulting residues to transfer, in microtubes after by above steps into the culture plates of sabouraud agar medium containing 2% malt be used. Using as sterile wab of samples subculture in prepared agar media plates and the all were coded, incubated in $2 \pm 25^{\circ}\text{C}$ for a period of 3,7 and 14 days plates were evaluated for growth, colony count and the results were recorded. During the study, colonies grown on environment marked the days listed and transferred by sterile scalpel with a volume of 5mm^3 to micro tubes containing 1ml of saline solution and stored at $4-8^{\circ}\text{C}$ for the second phase culture were kept. The second phase began cultivating as follows: subculture on agaris used for the first cultures and transfer to micro-tubes the plates were incubated, and according to plan cultures checked and likewise above transferred to the micro-tubes containing saline and were stored at $4-8^{\circ}\text{C}$. The third culture of stored colonies in the second stage was used in order to obtain a single colony using the needle and plates containing single colonies were separated for the next step into account for the final determination by slide culture used which considered selective culture for samples, to research the *Aspergillus* was the target respectively Czapek agar preparing slide culture and the designated culture time results were examined then slides prepared using lactophenol staining and the standard diagnostic method by ICPA rule using optical microscopy and identification of key reviews and the final result were recorded [7].

RESULTS

Of rice samples harvested in 1392 (2013), the number of colonies counted on the fourteenth day has been 8×10^5 . The tested samples produced in 1393 (2014) showed a number of counted colonies 7×10^5 . The samples grown colonies harvesting results as 3 different species of the black *Aspergilli* belonging to subgenus *Circumdati* section *Nigri* obtained as follows:

A.foetidus from rice samples obtained in 2013 and 2014 in Roodsar (6 &1), Rezvanshahr (10&0), Rasht (0&1), Anzali (3&0), Khomam (0&2), Jafarood (4&0) cities. *A.niger* in 2013 and 2014 rice samples in cities Roodsar (0&1), Rezvanshahr (0&5), Khoshkebijar (0&2), Anzali (1&0), Jafarood (1&0) and *A.carbonarus* of 2013 and 2014 rice in cities Roodsar (1&1), Rezvanshahr (1&0), Khoshkebijar (0&1), Rasht (1&0) and Anzali (1&0) were obtained as well as the other circumdati sections and species, **Figure-1**.

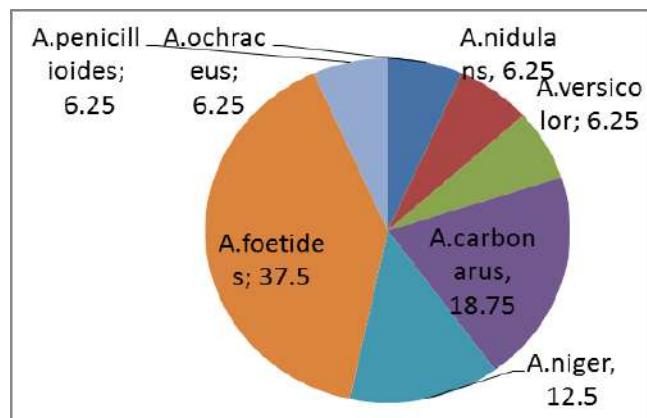


Fig:1. Frequency of *Aspergillus* species isolated from samples

DISCUSSION AND CONCLUSION

With regard to three times moulds grown colonies count averages on agar plates the greatest number of colonies could be harvested in seventh day of incubation (20×10^5) in comparison to on the third day of incubation (12×10^5) as on the fourteenth day (8×10^5) ves versa for the stored rice samples while of the fresh samples the greatest number of grown colonies count averages can be harvested on the fourteenth day of incubation of (7×10^5) and in the third day by 5×10^5 and the seventh day only 1×10^5 as colonies are recyclable which indicates that it should determine the microbial load of amples per incubation and counting procedures, recently obtained samples considered to have a priority to stored samples always be able to have more microbial load.

The sub-genus of the *Aspergillus* sector "Black" showed only 3 species *A.niger*, *A.foetidus* and *A.carbonarus*, *A.niger* (of storage to the 57.14%) in the most sampled cities found and its concentration on fresh samples were

12.5%. *A.foetidus* have been obtained in the vast majority of collecting samples almost has been found repeatedly in a density of 3.16%, in each instance more than a colony has been harvested routinely. The fresh samples (37.5%) ability to separate more *A.niger* as the main species has been notified. Among the cities studied for the fresh rice samples Rezvanshahr and then Roodsar have the most grown appearance colonial seen. *A.carbonarus* in all stored samples (21.42%) more over than fresh samples (18.75%) have been found. *A.carbonarus* from the most cities were found, but lesser than *A.foetidus* and *A.niger* in terms of density, due to colonies have been identified. The resultant findings shows that 50% of examined samples contains Aspergillus pollution, while only 10% were Black Aspergillii and moreover were from subgenus circumdati and section nigri, although the black Aspergillii highly contamination and density ratio, especially *A.niger*, *A.carbonarus* and *A.foetidus*, after them non-black Aspergillii especially *A.versicolors*, *A.parasiticus*, *Aglaucus*, then finally *A.flavus* have often been identified.

abundance fungal pollution in the rice samples of Mazandaran province although only from 7 samples fungal elements in PDA were isolated and from 100 tested samples and 93% for a while at least one plate from one of sampled grain contamination has been shown of which Aspergillus (43.96%), Cladosporium (13.96%), Alternaria (10.21%), Penicillium (4.79%) were the most reliable pollutants. The most common agents isolated (Aspergillus, Cladosporium, Alternaria and Penicillium) including toxins production potential to food and in case of inaccuracies in terms of maintenance Perhaps it will rise more and more by the time pass. reporting among isolated Aspergillii *A.flavus* and *A.fumigatus* were the most frequent and some fungal genera isolated as rice pollutants in this study as well as in other studies in Iran in other vegetable seeds including Wheat, Sorghum and Maize have also been reported repeatedly. Several studies in other countries in the field of rice fungal contamination indicating in a lot of results the study are consistent to us likely the other Iranian or international researchers [7], a survey on Thai's polished rice microflora reported genus Aspergillus, Penicillium and mucoral fungi as the most common have been identified as the same way by [8], Aspergillus and Penicillium for the rice most common storage fungi counts [8], reviewed Vietnamese rice and showed Aspergillus (43.8%), Fusarium (21.9%) and Penicillium (10.9%) as the most common isolates above mentioned [9], in their study on 196 moldy rice sample reported of the genus Aspergillus, Penicillium, Alternaria, Mucor separation. In their study on the stored rice for a year long, from 18 different ecosystems in India, stated that Aspergillus basically causes rice surface fungal contamination reflecting grains surface sterilization influences on lesser and very limited separating them. Taligoola et al (2004), also reviewed imported rice grains and ground rice, 60 fungal species in 30 different genus were separated that *A.candidus*, *A.flavus*, *A.niger*, *Urotium amsteloidami*, *U.rubrum*, *Penicillium citrinum* etc. As the most common isolated agents. The mean of various fungi, basically Penicillium, Aspergillus, Mucor and Rhizopus species are the rice grains, main pollutant fungi whereas kept in step in case of high humidity, their penetration into grains and in areas with higher rainfall with more moisture was seen too much more. In areas of the Mazandaran province west region also found out similarities in the case of above and our findings for the east areas of the Gilan province especially reasonably due to the rainfall and higher humidity similarities thus genus Aspergillus and Penicillium have been the main causes of pollution likewise in this study also separated and probably indicate of seeds contamination to dust and also inappropriate storage conditions thus increasing influence of these elements in rice and products food consumption [8]. Despite the importance of fresh, stored and ready to use rice pollution because that almost all harvested and stored rice in Iran purchase and sale of small batches in the country as well as and local consumers, performs and used, state rice fungal contamination study is very little are more about toxicogen set fungi and mycotoxins is focused. Based on existing standards the maximum allowed rice moisture is 14% [8,9], proved the relationship between insect invasion and especially rice infection increase to *A.flavus*, this type of pollution can because of long time improper rice storage have been shown [9]. In stored rice conditions of high humidity Aspergillii can cause the most contamination, however, a major share of contamination areas surface colonization introduced. Rice deep tissue infection can be evidence of surface colonization, rice well cooking before consumption, risks caused by the colonization will be resolved, but if proper storage conditions were optimum for fungal invasiveness, provide rice inner tissue mycotoxin production possibility and increases the risk of complications. However, a lot of studies have been yielded comprehensive study of the various components of the rice fungal flora and ecosystems of infection has not been done, findings show that the fungal pollution cases, contamination with mycotoxins in all samples is zero or close to zero. These results confirm the national standards country reports on the lack of mycotoxin contamination of imported rice complies given that some species are able to produce mycotoxins, these studies in examining the quality of the food in the country is necessary, to determine the types of fungi that infect and the ability to produce toxins by the fungi and measuring mycotoxins in food is too much important while rice production quantity is important but health and maintain quality, especially before consumption are a more important issue. Many fungal elements isolated from consuming rice, can directly play a role in the pathogenesis

of human and on the other hand, some of them if you do not care when storing at home and of conditions, is capable of producing mycotoxins what have adverse effects on human health and well being. Therefore, identification of fungal contamination as a first step to determine the health of the consume prepared rice is recommended and must also be assessed as a grain health standard.

CONFLICT OF INTEREST

None declared.

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YEARS OF PROFESSIONAL EXPERIENCE AND DEGREE IN TRANSLATION: PREDICTORS OF TRANSLATION QUALITY

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ABSTRACT

The gap between theory and practice is better felt when assessing quality of translation of professional translators with and without an academic degree in Translation. The present study aimed at investigating whether translators' years of professional experience and degree in Translation predict their translation quality. Following a causal-comparative study, 100 professional translators were selected using purposive sampling method. The participants were divided into two groups each containing individuals with and without a degree in translation, respectively. The participants were asked to translate a paragraph to assess their translation quality. For data analysis, appropriate statistical procedures including correlation and regression were used. Results showed that both degree in Translation and years of professional experience significantly predict translation quality. Also, the interaction of translators' years of professional experience and degree in Translation significantly affect their translation quality. An implication could be that besides providing translators with academic knowledge of theories, ample practical training in translation is necessary as a prerequisite for a competent translator. Another implication is that both professional and non-professional translators with a degree in Translation should acknowledge that they still need academic and practical experience, respectively.

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INTRODUCTION

Recent studies made it obvious that translation has always gone hand in hand with so many factors. There are a lot of questions about why two translators with the same age and academic background are different from each other in their task of translation [1, 2, 3]. The basic step of a planned and organized approach toward training competent translators is the mastery over theoretical frameworks [4]. A brief review of the literature indicates that translators' degree in translation positively affect the quality of their translation, the process of translating and producing a good translation [3, 5, 6]. Considering the relationship between individuals' level of education and their job performance, it is believed that years of work experience do not moderate the education-performance relationship since work experience indicates the quantity not the quality; however, some dimensions of performance are influenced by education [7]. Professional translators without an academic degree in Translation do not focus on the revision stage which can challenge the quality of their translation. On the contrary, qualified translators and academics recognize the significance of this stage. Thus, the academic status of translators should be taken into account as one of the elements of their sense of responsibility which influences the process and the quality of translation [5].

On the other hand, it has been argued that investigating years of professional experience is necessary to gain a deeper understanding of the translation process [5]. Also, years of professional experience has proved to be correlated with successful task performance [8]. As certain career fields highly emphasize years of professional experience than others [9], further research was needed to focus on a specific years of professional experience construct which successfully predicts translators' job performance.

While people tend to specialize in as few as narrow areas as possible, translators are expected to have a very good knowledge of all the domains with which their work interferes. That is why translation students expect to receive solid and complex training meant to enable them to face the challenges of translating in real world situations. Most of the students expect their teachers to give them the opportunity to acquire various specific knowledge and skills associated with their future profession [10]. In this respect, professional translators have been provided with ample

experience of translating different types of texts from various fields of study and seem to be more confident than non-professionals with only an academic experience, when facing such conditions.

To sum up, degree in translation and years of professional experience are among those factors that seem to affect the translator's performance and his/her translation quality. A main point which makes the present study significant is that there has not been much research in Translation Studies regarding the effect of translators' degree in translation, years of professional experience and the interaction of these two factors on their translation quality. Therefore, the focus of the present study was to investigate whether translators' degree in translation and years of professional experience predict their translation quality.

This study was designed to seek answers to the following question:

- Does translators' degree in translation and years of professional experience significantly predict their translation quality?

Based on the research question, the following hypothesis was formulated:

- Translators' degree in translation and years of professional experience significantly predict their translation quality.

The concept of *quality* has been approached from different perspectives, taking into account, different factors which claimed or seemed to influence translation quality. For instance, study revealed that higher-quality of translations and better performance of translators is related to their training and experience [11]. Another study found that there is a positive correlation between being a risk-taker and the quality of translation among BA students of Translation Studies [1]. One study showed that translator's personality had no impact on translation quality of narrative texts [12].

In the field of Translation Studies, some studies have focused on the influence of translator's degree in translation on the quality of their translations. For instance, the role of the translator's degree in translation have been implicitly recognized which enable him to produce a 'good' translation and to facilitate quality control [6]. The education of translators is of paramount importance in enhancing the quality of their work. She emphasized that the need for integrating the aspect of terminology science is obvious while for experienced translators without any translator-specific education, the connection between various aspects of terminology science and their everyday work comes more or less as a revelation [13]. Cultural assessment in clinical Psychiatry, formulated by the committee on cultural psychiatry group for advancement of Psychiatry also found that the translator's level of education influences the translation quality so that a translator with a low educational level might have difficulty translating some specialized texts including psychiatry [14]. In addition, one of the crucial elements to enhance the quality of translation is knowledge of pragmatics, more specifically when, how, and in what situation the target language is used in the society [15]. The results of another study shed more light on the positive effects of incorporating teaching functional translation theories on translation quality of translator trainees [4]. The translator's degree in translation may play an important role in predicting the quality of his/her translation. Translation trainees being aware of theoretical frameworks and having knowledge of theories of translation may affect the quality of the translation product [1]. Finally, regarding the translators' years of professional experience, years of work experience did not moderate the education-performance relationship due to the fact that work experience indicates the quantity not the quality; nevertheless, some dimensions of performance are influenced by education [7]. For instance, some professional translators, mainly without a degree in Translation, seem to ignore or underestimate the role of revision as pertains to the quality of the translation product while qualified translators acknowledge the importance of the revision stage [5].

MATERIALS AND METHODS

Design

The design of this quantitative study was causal-comparative in nature. In this design, cause was identified as the effect of translators' degree in translation and years of professional experience, and the impact of this interaction on the translation quality remained to be investigated.

Instruments

Waddington's translation quality assessment model D was used to determine participants' translation quality. This model is designed and validated by Waddington (2001). Model D actually consists of combining error analysis and holistic methods of Waddington's model in a proportion of 70/30, respectively.

Participants

The population under study was a group of professional translators, working in official and non-official translation centers, and freelance translators. For selection and homogeneity purposes, subjects were selected to be translators with 2-7 years of experience. Individuals with both Bachelors' and Masters' degrees in translation and those who have studied or lived abroad were excluded from the study to minimize the impact of their degree in translation and proficiency level. 100 translators selected using purposive sampling method participated in the study. They were divided into two groups and each group contained 50 subjects. Group 1 and group 2 contained translators with and without a degree in translation, respectively.

Data collection procedures

First, the study participants answered the demographic questions. Then both groups were asked to complete a translation task in 30 minutes. The translations were assessed and scored based on Waddington's model D. To do so, a paragraph containing 232 words was given to be translated into Persian; the text was part of George Orwell's 1984 novel. The scores were entered in SPSS 21.0 for statistical analysis.

Data analysis

After data entry, the appropriate statistical analysis including descriptive statistics (Frequency, Percentage, Mean and Standard Deviation) were used to analyze demographic data; in order to investigate the hypothesis, inferential statistics including correlation was used.

RESULTS

Reliability of the measures

The reliability of the measures and instruments was estimated as a preliminary step by Chronbach's alpha which supported the previous reliability findings of the instrument [16, 17]. The reliability of Waddington's model D was calculated to be 0.82.

Results of the descriptive and inferential statistical analysis

First the normality of the scores was initially checked. The normal Q-Q Plot showed that the distribution of scores was reasonably normal. Mean age of the participants was 28.2 years (SD 6.8). **Table- 1** summarizes the results of descriptive statistics of the variables. **Table- 2** and **Table- 3** present the results of scoring the translations based on Waddington's model D. As explained in part 3, this scoring system makes use of two subjective (holistic) and objective (error analysis) scoring methods, respectively. According to **Table- 2**, mean holistic, objective and total score of participants in group 1 was 5.3, 5.7 and 5.5 with (SD 1.1), (SD 1.3) and (SD 1), respectively. On the other hand, mean holistic, objective and total score of participants in group 2 was 5.1, 5 and 5.1 with (SD 0.9), (SD 1) and (SD 0.9), respectively. Mean total score in groups 1 and 2 was finally calculated to be 5.5 and 5.1 with (SD 1) and (SD 0.9), respectively.

Table: 1. Descriptive Statistics of variables

	Years of experience	Age
N	100	100
Mean	4.0404	28.2828
Std. Deviation	1.95312	6.83036

Table :2. Descriptive Statistics of variables (Total score)

		N	Mean	SD
Group 1	Holistic	50	5.320	1.11465
	Objective	50	5.712	1.34387
	Total score	50	5.594	1.07847
Group 2	Holistic	50	5.180	0.98333
	Objective	50	5.082	1.08377
	Total score	50	5.111	0.91117

Table: 3. Mean and Std. Deviation of variables (Total score)

	N	Mean	SD
Total score	Group 1	50	5.5944
	Group 2	50	5.1114

For investigating the effect of translators' degree in translation, years of professional experience and the interaction of their degree in translation and years of professional experience on their translation quality correlation and regression statistical procedure was run the results of which are presented in [table- 4](#) and [Table-5](#). According to these two tables, degree in translation and professional experience significantly predict translation quality. Also, the interaction of translators' years of professional experience and degree in translation significantly affect their translation quality. An implication could be that besides providing translators with academic knowledge and theories, practical training in translation is necessary as a prerequisite for a competent translator.

Table: 4. Results of correlations

	Score	
Degree	Pearson	-0.237*
	Correlation	
	Sig. (2-tailed)	0.017
	N	100
Years of experience	Pearson	0.342**
	Correlation	
	Sig. (2-tailed)	0.001
	N	99

Table :5. Results of regression

Model	Unstandardized Coefficients		t	Sig.
	B	Std. Error		
(Constant)	5.299	0.396	13.367	0.000
Years of experience	0.156	0.050	3.132	0.002
Degree	-0.397	0.193	-2.053	0.043

DISCUSSION

Hypothesis: Translators' degree in translation and years of professional experience significantly predict their translation quality.

The results of correlation coefficients and regression procedure revealed that the hypothesis of the study could not be rejected ($p < 0.05$). While some studies showed that, years of work experience did not moderate the education-performance relationship due to the fact that work experience indicates the quantity not the quality; nevertheless, some dimensions of performance are influenced by education. The findings of the present study support the fact that years of experience in translation could predict translation quality. Taking into account the previous studies which indicate that academic knowledge and having a degree in translation significantly affect translation quality [17], it seems that both *degree in translation* and *professional experience* should be regarded as two wings necessary for a translator to fly with and they should not be considered as two factors which form a dichotomy; instead they form a unity.

CONCLUSION

The conclusions drawn indicate that translators can acquire a higher quality translation through further training in translation courses, translation theories and techniques included in the academic environment. Another implication of this study is that a translation task could be better completed by translators with degree in translation in Translation Studies. What is more, we should not ignore the important role of years of professional experience in the act of translation. In other words, besides providing translators with academic knowledge and theories, practical training in translation is necessary as a prerequisite for a competent translator. Translation students and trainees need to be provided with ample practice in translating different texts during their courses so that they can practice the theory and theorize the practice to become a competent translator.

CONFLICT OF INTEREST

None declared.

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EVALUATION OF ASPERGILLUS SPP REFLECTING TO SUBGENUS CIRCUMDATI SECTION FLAVI CONTAMINATION IN THE GILAN PROVINCE, IS STORED AND FRESH HARVESTED RICE CROPS

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ABSTRACT

Introduction: World attentions on food safety are increasingly developed. Polished rice grains as a staple food in terms of consumption may be contaminated to different types of fungi. This study aimed to investigate the incidence / prevalence of fungal pollutions in the rice cropped seeds in Gilan province agricultural area that they have been put argument to evaluate. **Material and Methods:** In this study randomized sampling of fresh rice crops and stored facilities were applied, cultured in a series of steps on Saboraud, s agar, Czapek agar medium are of choice for *Aspergillus* spp studies concomitantly macroscopic and microscopic compartments characteristics following diagnostic log with the keys to identify species designated as *Aspergillus* species were reported by ICPA. **Results:** According to three times enumeration the total number of colonies grown on agar plates containing specifications; The greatest surge number of colonies were observed in samples of 2013 by a week of incubation at 25°C (20×10⁵), while of 2014 the largest number of colonies were on 2nd week day of incubation 25 °c(7×10⁵). The resultant findings showed that 50% of the tested samples were contaminated to *Aspergillus* spp, 40% of this pollution were non-black and more belonging to the subgenus *circumdati* and section *flavi*. **Conclusion:** Which indicate that recently taken and stored samples came into microbial incubation and counts must not neglected, followed by non-black colored isolates, especially *A.parasiticus* (64.28%&6.25%), *A.glaucus*(57.1%&0), *A.flavus* (42.8%&0), *A.ochraceus*(14.28%&6.25%), *A.penicillioides*(14.28%&6.25%), *A.terreus*(7.14%&0), *A.nidulans* (7.14% & 6.25%), *A.oryza* (7.14% & 0) *A.versicolor* (3/16% & 6/25%).

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INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by fungi. For centuries, the quality of many agricultural products has been reduced by mycotoxins, natural toxins that are produced by molds in many agricultural products, but especially in grains and oilseeds, and sometimes both, are observed after harvest during storage even when food is processed, concentrate and feed the animals [1]. Fungal contamination of rice directly, used in human food, if there are suitable conditions for the development or indirectly in effect, of the possibility of producing mycotoxins, endanger human health. Failures and corruption of grain and, products, of pollution fungal effect, is high and at least 20% cereals is corrupted and unusable [2,3]. Crops contamination, especially rice to mycotoxins by the wide range of their effects on the human and animal body parts as well as economic losses, this pollution make important issues in the field of food and public health as well [4]. Rice is the most important cereal food consume by the most people of the world, especially the Eastern even Middle east. Half of the world population, are dependent on rice as a main food. Iran with having rice cultivation and annual production of close to 2/5 million tons, occupies a global rank 23th in production and 13th in taking it to be allocated. In Iran, particularly in the northern provinces rice is the staple food of a million people, in the preparation of food Such as cooked rice, dried fruit, odor and Processed food made from rice flour along with its waste used in animal feed [5]. The rice three fungi major pollutants as follows: *Aspergillus*, *Fusarium* and *Penicillium*. These fungi with attacks on agricultural products cause discoloration, reduce marketability, friability, vulnerability and pollution as they have toxins. Despite the inherent resistance of rice against fungal infections, this product in the course of cultivation, care, harvesting and first challenge exposure infection caused by fungi. One of the important factors for rice contamination, is the moisture percent of the product. Weather condition and storage quality are the two important and determinative factors for rice contamination by mycotoxins [6,7].

MATERIALS AND METHODS

In this research randomly 30 samples of stored and freshly harvested rice (2013:14 and 2014:16 samples) from 9 cities in Gilan (Kelachay, Roodsar, Langerood, Lahijan, Rezvanshahr, Rasht, Anzali, Khomam, Khoshkebijar) for each 10 ton by 100 gr sampling were obtained and performed with CBS standard sampling techniques, after sampling process and transfer the samples to the laboratory, were coded, weighing 20gr of each sample divided in 2:10 gr preparations the first 10 gr milled for mycotoxins measurement the second transferred to 40ml of saline solution then centrifuged for fifteen minutes at a speed of 2000 rpm for two time, after homogenization the falcon contents enter into the appropriate test tubes and centrifuged (15 minutes at 3000rpm) till supernatants were evacuated tubes and the resulting residues to transfer, in microtubes after by above steps into the culture plates of sabouraud agar medium containing 2% malt be used. Using as sterile wab of samples subculture in prepared agar media plates and the all were coded, incubated in $25 \pm 2^\circ\text{C}$ for a period of 3, 7 and 14 days plates were evaluated for growth, colony count and the results were recorded. During the study, colonies grown on environment marked the days listed and transferred by sterile scalpel with a volume of 5mm^3 to micro tubes containing 1ml of saline solution and stored at $4-8^\circ\text{C}$ for the second phase culture were kept. The second phase began cultivating as follows: subroud agar is used for the first cultures and transfer to micro-tubes the plates were incubated, and according to plan cultures checked and likewise above transferred to the micro-tubes containing saline and were stored at $4-8^\circ\text{C}$. The third culture of stored colonies in the second stage was used in order to obtain a single colony using the needle and plates containing single colonies were separated for the next step into account for the final determination by slide culture used which considered selective culture for samples, to research the *Aspergillus* was the target respectively Czapek agar preparing slide culture and the designated culture time results were examined then slides prepared using lactophenol staining and the standard diagnostic method by ICPA rule using optical microscopy and identification of key reviews and the final results was recorded [8].

RESULTS

Of rice samples harvested in (2013), the number of colonies counted on the seventh day have been 20×10^5 . The tested samples produced in (2014), showed a number of counted colonies 1×10^5 . The samples grown colonies harvesting resulted as 12 different species of the genus Non-black *Aspergillus* obtained as follows: *A.versicolor* isolated from rice samples of 2013 and 2014 in cities Roodsar (9 & 1), Rezvanshahr (9 & 0), Langerood (1 & 0), Khoshkebijar (1 & 0) and Anzali (1 & 0). *A.parasiticus* harvested from rice samples of 2013 and 2014 in cities Rezvanshahr (4 & 0), Roodsar (3 & 1), Langerood (1 & 0) and Anzali (1 & 0) obtained. *A.glaucus* only obtained in the samples of rice belonging to 2013 in the cities; Rezvanshahr (4), Roodsar (2) and Anzali (2), *A.flavus* only rice of 2013 in the cities Roodsar (4) and Anzali (2) as well, *A.fumigatus* only in 2013 and obtained from the cities Roodsar (2) and Rezvanshahr (2) too. *A.penlicillioides* reflected in the rice samples of 2013 and 2014 in cities Rezvanshahr (1 & 0), Khoshkebijar (1 & 0), Khomam (0 & 1) and Anzali (0 & 1) in contrast *A.oryza* only obtained in the rice samples of 2013 in the cities Langerood (1), Lahijan (1), Rezvanshahr (1) and Anzali (1) unlikely *A.ochraceus* harvested from rice in 2013 and 2014 in cities Roodsar (0 & 1), Rezvanshahr (1 & 0) and Khoshkebijar (1 & 0). *A.nidulans* isolated from samples of 2013 and 2014 in Roodsar (2 & 0), Langerood (1 & 0), Khomam (0 & 1) and Anzali (1 & 0), *A.terreus* only seen in the samples of rice belonging to 2013 in the Khoshkebijar (1), even *A.ostianous* only isolated in 2013 rice samples obtained in Rezvanshahr (1) city and *A.eurotium* seen only in 2013 Roodsar (1) rice samples (Figure 1) were obtained as well as another circumdati section Nigri's species. **Figure-1**

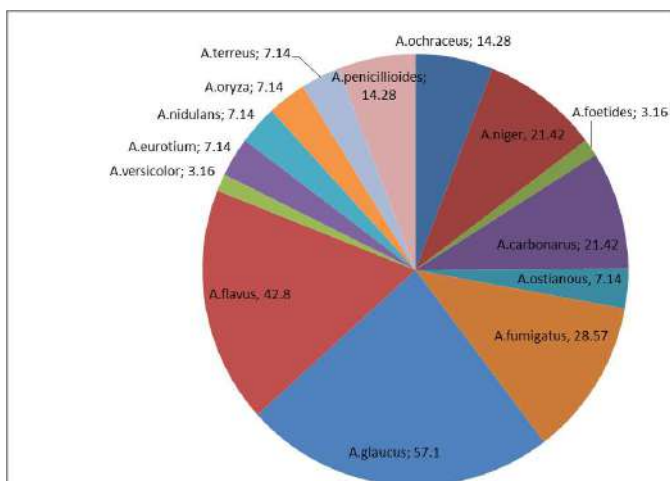


Fig:1. Frequency of Aspergillus species isolated from samples

DISCUSSION AND CONCLUSION

With regard to three times moulds grown colonies count averages on agar plates the greatest number of colonies could be harvested in seventh day of incubation (20×10^5) in comparison to on the third day of incubation (12×10^5) as on the fourteenth day (8×10^5) ves versa for the stored rice samples while of the fresh samples the greatest number of grown colonies count averages can be harvested on the fourteenth day of incubation (7×10^5) and in the third day by 5×10^5 and the seventh day only 1×10^5 as colonies are recyclable which indicates that it should determine the microbial load of amples per incubation and counting procedures, recently obtained samples considered to have a priority to stored samples always be able to have more microbial load. The stored samples survey the most *A.versicolor* with frequency 3.16 % and fresh samples up to 6.25%, because more than a colony there have been isolated in any of these plates. The most of these isolates reserved by the Rezvanshahr and Roodsar cities and gained have seen about most conducted cities sampled. *A.flavus* only isolated in store samples with a frequency of 42.8 % and the Roodsar city showed more than other cities have been founded the mould. *A.glucus* (57.1%) obtained from Rezvanshahr then Roodsar more than other cities. *A.fumigatus* (28.57 %) of the city Rezvanshahr and Roodsar were more than the other cities. *A.ostianous* (7.14%) only from the city Rezvanshahr and *A.terreus* (7.14%) only in the city Khoshkebijar, *A.oryzae* (7.14%) from the four cities Langerood, Lahijan, Rezvanshahr and Anzali have been found. *A.eurotium* with frequency 7.14% only seen in the city Roodsar and the above list did not get and were not identified of fresh rice samples.

In contrast *A.parasiticus* (of the storage samples 64.28% and in fresh samples 6.25%) in the four cities Rezvanshahr, Roodsar, Langerood and Anzali where have been found. Resulted frequency for *A.penicillioides* (in the storage samples 14.28% and in fresh samples 6.25%) noticed by the four cities khomam, Rezvanshahr, Anzali and Khoshkebijar. *A.ochraceus* frequency in the storage samples 14.28% in comparison to fresh samples 6.25% calculated for the three cities Roodsar, Khoshkebijar and Rezvanshahr and been found. *A.nidulans* (of storage 7.14% and fresh samples 6.25%) isolated from cities Roodsar, Langerood, khomam and Anzali.

The resultant findings show that 50% of examining samples contains Aspergillus pollution, which Non-black Aspergillii have been 40% of this pollution as causative agents, and more belonging to subgenus circumdati and section flavi have been the most species population. non-black Aspergillii especially *A.versicolors*, *A.parasiticus*, *Aglaucus*, then finally *A.flavus* often were identifiable. showed an abundance fungal polution in the rice sampels of Mazandaran province although only from 7 semples fungal elements in PDA were isolated and from 100 tested samples and 93% for a while at least one plate from one of samplified grains contamination has been shown of which Aspergillus (43.96%), Cladosporium (13.96%), Alternaria (10.21%), Penicillium (4.79%) were the most reliable pollutants. The most common agents isolated (Aspergillus, Cladosporium, Alternaria and Penicillium) including toxins production ability to food and in case of inaccuracies in terms of maintenance Perhaps it will rise more and more by the time pass. Reporting among isolated Aspergillii *A.flavus* and *A.fumigatus* were the most frequent and some fungal genera isolated as rice polutants in this study as well as in other studies in Iran in other vegetable seeds including Wheat, Sorghum and Maize have also been reported repeatedly. Several studies in other countries in the field of rice fungal contamination indicating in a lot of results the study are consistent to us likely the other Iranian or international researchers. By a survey on Thai's polished rice microflora reported genus Aspergillus, Penicillium and mucoral fungi as the most common have been identified as he same way by [8], Aspergillus and Penicillium for the rice most common storage fungi counts. [9], reviewed Vietnamese rice and showed Aspergillus (43.8%), Fusarium (21.9%) and Penicillium (10.9%) as the most common isolates a above mentioned. In their study on 196 moldy rices sample reported of the genus Aspergillus, Penicillium, Alternaria, Mucor separation. In their study on the stored rice for a year long, from 18 different ecosystem in India, stated that Aspergillus basically causes rice surface fungal contamination reflecting grains surface sterilization influences on lesser and very limited separating them also reviewed imported rice graines and ground rice, 60 fungal species in 30 different genus were separated that *A.candidus*, *A.flavus*, *A.niger*, *Urotium amsteloidami*, *U.rubrum*, *Penicillium citrinum* etc, as the most common isolated agents. in areas of the Mazandaran province west region also found out similarities in the case of above and oue findings for the east areas of the Gilan province especially reasonably due to the rainfall and higher humidity similarities thus genus Aspergillus and Penicillium have been the main causes of pollution like wise in this study also separated and probably indicate of seeds contamination to dust and also inappropriate storage conditions thus increasing influence of these elements in rice and products food consumption, despite the importance of fresh stored and ready to use rice pollution because that almost all harvested and stored rice in Iran purchase and sale of small batches in country as well as and local consumers perform and used, state rice fungal contamination study is very

little are more about toxicogen set fungi and mycotoxins is focused. Based on existing standards the maximum allowed rice moisture is 14%. In study indicating the average samples analyzed moisture content were in the standard range this amount can be used as a key factor effective to the reduction of fungal contamination and mycotoxins produced in product and the results of our recent study showed that *A.parasiticus* prevalence is more than *A.flavus* or *A.niger* perhaps this disorder is attributed to high moisture samples, in other words ideal conditions for *A.parasiticus* have been met while more Aspergillus contamination during rice storage, have been reported [9], proved the relationship between insect invasion and especially rice infection increase to *A.flavus*, this type of pollution can because of long time improper rice storage have been shown. In stored rice conditions of high humidity Aspergillii can cause the most contamination however, a major share of contaminatins are as surface colonization introduced Rice deep tissue infection can be evidence of surface colonization, rice well cooking before consumption,risks caused by the colonization will be be resolved, but if proper storage conditions were be optimum for fungal invasiveness provide rice inner tissue mycotoxin production Possibility and increases the risk of complications.However, a lot of studies have been yeald comprehensive study on the various components of rice fungal flora and ecosystems of infection has not been done, findings show that the fungal polutions cases, contamination with mycotoxins in all samples is zero or close to zero. These results confirm the national standards country reports on the lack of mycotoxin contamination of imported rice complies given that some species are able to produce mycotoxins, these studies in examining the quality of the food in the country is necessary, to determine the types of fungi that infect and the ability to produce toxins by the fungi and measuring mycotoxins in food is too much important while rice production quantity is important but health and maintain quality, especially before consumption are a more important issues.Therefore, identification of fungal contamination as a first step to determine the health of the consume prepared rice is recommended and must also be assessed as a grain health standard.

CONFLICT OF INTEREST

None declared.

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FINANCIAL DISCLOSURE

None.

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DETERMINATION OF CUT-OFF POINT FOR ULTRASONOGRAPHIC FINDINGS IN NASAL BONE FRACTURE: COMPARISON OF THEIR CONSISTENCY WITH PHYSICAL EXAMINATION AND CONVENTIONAL RADIOGRAPHIC FINDINGS

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ABSTRACT

The aim of this study was to demonstrate the cut-off point of ultrasonographic findings in nasal bone fracture and determining their consistency. A longitudinal, descriptive-analytic study was done on 90 patients (more than 15 years old) with clinical manifestations of a fractured nasal bone. Seventy two patients concluded the study successfully. High resolution ultrasonographic investigations were done by an expert consultant without knowledge of the clinical data or examination in five sessions. After collecting and completing information about patients, the data was analyzed using SPSS software. Ninety consecutive patients were enrolled in the study; Seventy two patients (44 men and 28 women) with the mean age of 26.56 ± 12.3 years concluded the study successfully. In comparing the suggestive criteria for nasal bone fracture in physical examination, nasal bone depression had the most sensitivity (61.29%), epistaxis had the highest specificity (80%) and echymosis had the highest score of positive likelihood ratio (1.37) and the most PPV (89.47%). During the first three weeks after nasal trauma, subperiosteal hematoma and lucency in the lateral nasal bone had the highest diagnostic value which were statistically meaningful ($P < 0.001$). The predictive value of lateral nasal bone lucency and subperiosteal hematoma for estimating the elapsed time from the onset of nasal trauma in sonographic investigation were 67.7% and 86.3% (95% CI) respectively which were statistically meaningful ($P = 0.003$, $P = 0.0001$). HRUS is a reliable diagnostic tool for determining the chronicity of nasal bone fracture.

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INTRODUCTION

Nasal fracture is the most common mid-facial fracture [1]. The nasal pyramid is a complex structure composed of the paired nasal bones and the frontal processes of the maxillary bone. A thorough attention must be paid to the caudal and cranial part of the nasal bones, as well as the nasal septum when evaluating a nasal trauma. Nasal bone fracture is responsible for about 40% of maxillofacial bone fractures and is twice more common in male than in female [2]. High Resolution Ultra-Sonography (HRUS) is superior to Conventional Radiography (CR) in comparison and that is because of its low cost, availability and lack of radiation risk to the lenses [3]. For comparing the diagnostic value of HRUS with other radiographic investigations such as plain radiography in diagnosing nasal fractures, several studies have been done [4, 5]. Although the gold standard for the diagnosis of nasal fracture is physical examination but occasionally swelling of the surrounding tissues hinder the physician in fracture diagnosis [5, 6]. Most of nasal fractures are located between the middle and inferior third of the nose [7]. Many studies have shown that plain radiography has some short comings in detecting these fracture lines [8]. In some studies plain radiographic investigations were falsely negative in 25% of patients with nasal bone fractures [9]. The purpose of this study was to determine the cut-off point of ultrasonographic findings in nasal bone fractures and comparing their consistency with physical examination and conventional radiologic findings.

MATERIALS AND METHODS

This longitudinal-analytic study, was carried out in Shohada-Ashayer hospital of Lorestan province (southwest of Iran), between April 2012 and April 2014. Sampling method was census. Seventy two patients (44 males and 28 females) concluded the study successfully. All patients with nasal bone manipulation such as closed or open reduction before referral to our hospital and also those with more than five days interval between the trauma and radiologic investigations were excluded from the study.

First of all a thorough rhinologic physical examination was done on all patients by an expert attending of otolaryngology head and neck surgery department in Shohada-Ashayer hospital; Following clinical examination, CR was taken for all the patients. Stepping or lucency in the lateral nasal bone was considered positive for fracture in CR [Figure-1].



Fig: 1. Lucency and stepping in the lateral nasal view.

After performing CR and HRUS, We collected clinical and imaging data of the patients from their clinical data sheets. Clinical examination was considered as the gold standard for the diagnosis of nasal bone fracture, the patients underwent any curative procedures as needed (such as closed or open reduction). All patients were followed for six months regularly (in the first five days, 3rd, 6th, 12th and 24th weeks after trauma). In each session, ultrasonographic examination was done by an expert radiologist who was blinded to the results of CR or physical examinations. A 14 MHz linear probe was used for sonographic evaluation. Patients were examined in the supine position and in right, left and longitudinal views. The transducer was applied directly to the skin without a standoff pad. A single radiologist performed all the ultrasonograms of the nasal bone during the six months study. Cortical disruption of the nasal bone and having lucency or stepping in nasal bones or nasal septum in HRUS was considered as positive finding for fracture [Figures 2-4].

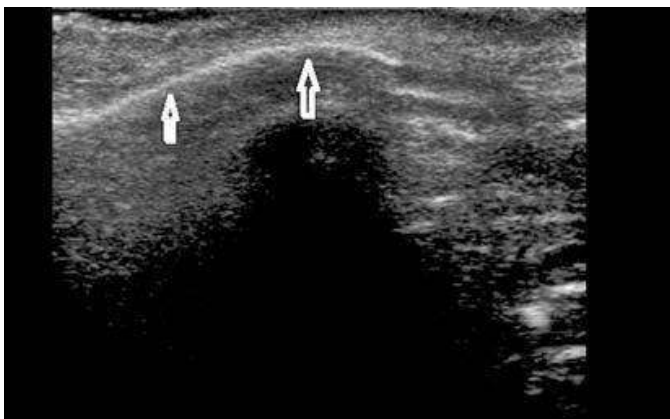


Fig: 2. Oblique longitudinal ultrasonogram shows the normal appearance of the lateral wall of nasal bone as an echogenic line (arrows).

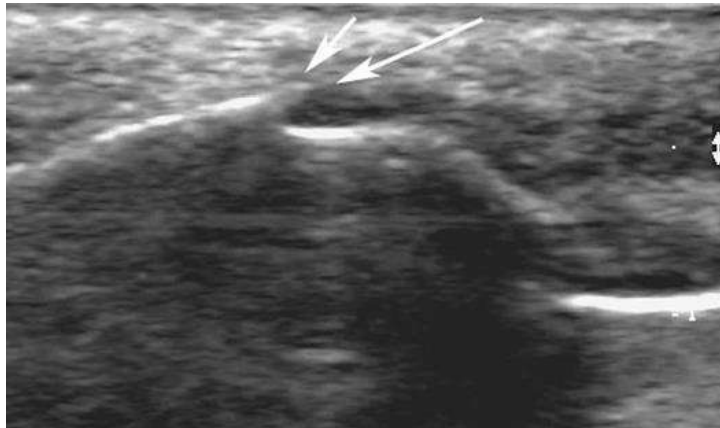


Fig: 3. Sonogram in a patient with a fractured nasal bone (arrows).



Fig: 4. Sonogram shows lucency in the nasal septum (arrow).

Soft tissue swelling and sub-periosteal hematoma were also examined as possible indicators to differentiate an acute from a chronic fracture line. The thickness of periosteal reactions were measured in each sonographic examination and were compared with the thickness of nasal bone periosteum on the opposite side.

After collecting and completing information about patients, the data was analyzed through descriptive statistical techniques (standard mean scores, ratios, frequency percent and Fishers exact test). Moreover, sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), negative likelihood ratio (LR-), positive likelihood ratio (LR+), and their 95% confidence intervals (CI) were calculated and used for determining the diagnostic accuracy of HRUS in nasal bone fracture.

RESULTS

Ninety consecutive patients were enrolled in the study; Seventy two patients (44 men and 28 women) with the mean age of 26.56 ± 12.3 years (range from 17 to 38 years old) concluded the study successfully. After evaluating the diagnostic value of physical examination findings, mobility had the most sensitivity (80.65%) and the highest score of specificity (100%).

In comparing the suggestive criteria for nasal bone fracture in physical examination, nasal bone depression had the most sensitivity (61.29%), epistaxis had the highest specificity (80%) and echymosis had the highest score of LR+ (1.37) and the most PPV (89.47%). The lucency in the lateral nasal bone during sonographic examination in

the first five days had the highest sensitivity (90.32%) which declined to 64.52% in the 5th session of sonographic investigation. The PPV for lucency of lateral nasal bone in ultrasonographic examination was in the range of 84.85% to 88%. In the first sonographic investigation, the sensitivity of stepping of lateral nasal bone was 77.42%. Sub-periosteal hematoma in the first sonographic evaluation had the most sensitivity (100%). On the other hand the specificity and PPV of sub-periosteal hematoma as a diagnostic tool in nasal bone fracture were in the range of 80% to 100%.

During the first three weeks after nasal trauma, sub-periosteal hematoma and lucency in the lateral nasal bone had the highest diagnostic value which were statistically meaningful ($P < 0.001$). The predictive value of lateral nasal bone lucency and sub-periosteal hematoma for estimating the elapsed time from the onset of nasal trauma in sonographic investigation were 67.7% and 86.3% (95% CI) respectively which were statistically meaningful ($P = 0.003$, $P = 0.0001$).

Among sonographic findings, sub-periosteal hematoma and lucency in the lateral nasal bone were consistent for 25 and 85 days respectively (sensitivity: 90.8% & 66.7% respectively) [Figures 5, 6].

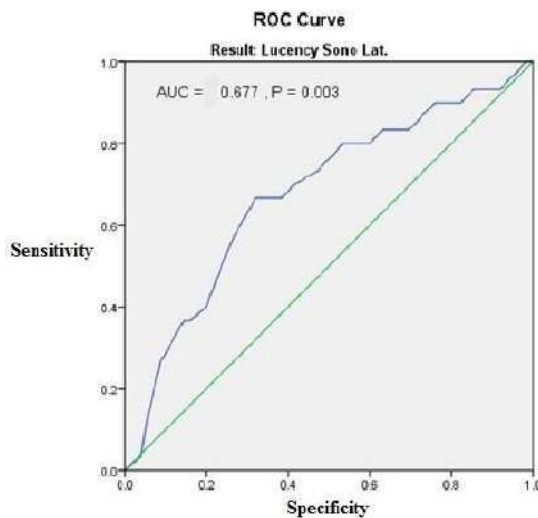


Fig. 5. AUC shows predictive value of lucency in the lateral nasal bone according to the elapsed time from trauma.

DISCUSSION

In the present research, 90 patients with nasal fracture who had referred to the Shohada-Ashayer hospital of Lorestan province (southwest of Iran) during two year were studied.

Adequate radiologic assessment of nasal fracture based on the injuries cause is obligatory for the possibility of legal consequences. For years the standard referral modality for nasal fracture diagnosis was considered to be CR, until some studies showed that CR had 25% false negativeness in patients with a documented nasal bone fracture. Although a conventional radiographic examination still remains the initial step for the radiologic assessment of nasal injury in many centers, but its efficacy and sensitivity in clinical decision-making is controversial; For instance sidewall injuries on conventional radiographies are difficult to be diagnosed [4]. The sensitivity of bilateral nasal bone radiographic view for the diagnosis of nasal bone fracture has been mentioned 75% in the literature [8]. Thiede et al. showed that even a 0.1 mm disruption of nasal bone can be detected by ultrasonography [2].

In a study on 63 patients, researchers found that the accuracy of sonography was superior to plain radiography in detecting nasal fracture line [10]. Danter et al. by evaluating 18 patients suffering from nasal trauma found out that the sensitivity and specificity of a 20 MHz ultrasonography comparing with physical examination were 83% and 50% respectively; They also showed that the sensitivity and specificity of HRUS compared to CR were 94% and 83% respectively [11]. Beck et al. studied 21 subjects suspicious for nasal fracture using a 5-7.5 MHz linear probe and found that all the fracture lines shown by CR were simultaneously detected by HRUS [4]. Zagolski and Streck showed that nasal bone fractures could be diagnosed exclusively on the basis of sonographic investigations [12]. A 14 MHz linear probe was used in the present study and the results were consistent with the results of studies carried out by Mohammadi et al. [13] and Thiede et al. [2]. Furthermore, our results were consistent with the results of Danter et al. [11] in which a 20 MHz probe was used to evaluate nasal bone fractures. It seems that a 14 MHz ultrasound head can detect nasal fractures just as well as 20 MHz ultrasound probe.

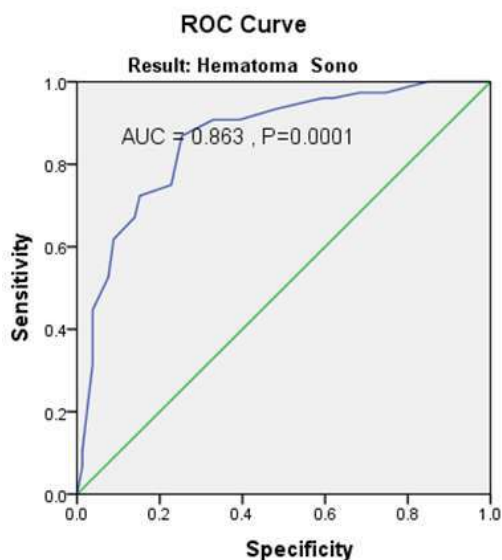


Fig: 6. AUC shows the predictive value of sub-periosteal hematoma of lateral nasal bone according to the elapsed time from trauma.

Danter et al. Study, also showed that sonography can show trauma of the cartilaginous part of the nose more exactly than conventional radiography [11].

Ultrasonography is an easy and accessible method that involves no radiation. Despite the many advantages of ultrasonography including low cost, lack of ionizing radiation and no patient preparation, it has several pitfalls; Above all, operator dependency and the projected external shape of the nose are the commonest limitations of sonography. For over-whelming these limitations, we used a hockey stick probe with a 14 MHz linear array transducer keeping contact between the probe and the nose. We found that during nasal trauma, lucency or stepping in the nasal septum can be detected in sonography which is usually missed in conventional radiographic studies. We also found that the sensitivity and specificity of ultrasonographic evaluations were not only the same but also even higher than CR in detecting lucency or stepping in the lateral nasal bone and this was compatible with previous studies [10]. Physical examination findings in nasal trauma are consistent for 10-14 days and conventional radiographic findings are consistent for years in 85% of patients [5]. In this study we found that among sonographic findings in nasal fracture, sub-periosteal hematoma and lucency in the lateral nasal bone are consistent for 25 and 85 days respectively, which has not been mentioned in previous studies.

CONCLUSION

Due to possible legal matters accurate imaging of nasal fractures in many circumstances is critical. The use of HRUS in the evaluation of fractures has increased. Considering the advantages of ultrasound such as the absence of ionizing radiation and ease of use and given the results of the present study, it is concluded that ultrasound can be an alternative primary technique in the diagnosis of nasal bone fractures, especially in pregnant women. The consistency of ultrasonographic findings in nasal fracture can be detected much more accurately than physical examination and conventional radiologic findings, so it is easier to determine the chronicity of nasal trauma by means of HRUS than Physical examination or CR.

Performing similar studies on larger groups of patients with shorter intervals between ultrasonographic investigations is suggested to confirm the afore-mentioned results.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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PSYCHOLOGICAL AUTOPSY OF SUICIDE COMPLETERS IN LORESTAN PROVINCE: A CROSS-SECTIONAL STUDY

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ABSTRACT

Psychological autopsy is a direct method to investigate the risk factors for suicide and to reconstruct the events leading to death in suicide completers in order to come up with a profile of suicide completers and find ways to deal with it. The present study was conducted on the psychological autopsy of suicide completers in order to investigate the risk factors for suicide among Lorestan societies. This cross-sectional study included all suicide completers (N=34) referred to Shohada Ashayer hospital in Khorramabad over a six months period. The census sampling was applied, and the data collection tools were hospital records, psychological interview, and a self-generated questionnaire on economic and cultural status; method, time, and place of suicide; a history of congenital malformations in childhood, previous suicide attempts, psychosomatic illness, receiving psychiatric and counseling services and dependence on drugs; familial information and smoking. Finally, the collected data were analyzed using the SPSS software via the descriptive statistics, non-parametric tests such as the chi-square test, and normal distributions. In this study, the incidence of suicide was equal in the males and females, and the suicide completers were mostly in the age range of 21-30 years old (64.7%). The most common method of suicide was poisoning (drugs, aluminum phosphide drugs, and opioids), and the most important risk factors were psychological disorders, addiction, and substance dependence. In our study, 29 cases (85.3%) of all subjects were estimated to fit a diagnosis of a mental disorder at the time of the death. Psychological autopsy is a very important tool in assessing the cause of suicide. More studies with larger sample sizes are required in this field to examine the problem of suicide.

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INTRODUCTION

The term *suicide* is derived from two Latin words of *sui* meaning *self*, and *caeder* meaning *killing*. This term was first used by the Frenchman Defonten 1737 [1]. Suicide, meaning self-destruction and elimination, is a phenomenon that has long afflicted human beings in their traditional and tribal as well as their current complex relationships in modern cities, and has bothered the heart and soul of families in the vicissitudes of social changes. Suicide is a major public health problem in all countries. The ratio of completed suicides to attempted suicides in the general population of the world varies from 1:6 to 1:25, with an average of approximately 1:18 [2]. Approximately one million people worldwide lose their lives due to suicide annually. From a global perspective, 60% of the deaths from suicide can be seen among young adults. In most countries, suicide is among the top three causes of death in the age range of 15-34 years old [3].

The causes of mortality are diverse worldwide, but every year there is a group of people who terminate their lives for various reasons. Due to the reprehensible nature of suicide in some communities, access to the real data is difficult, and often the actual number of suicide cases is not mentioned in the official reported statistics. However, according to the available data, suicide is one of the ten leading causes of death worldwide [2]. The high rate of suicide attempters has led different countries of the world to focus on numerous activities on the causes and

influencing factors of attempting suicide. Beyond providing statistics and carrying out epidemiologic studies to prevent suicide, these countries have taken impressive measures within valuable accomplishments. However, in Iran, performing suicide-related studies do not have a long history and the suicide statistics is limited to the epidemiological data in only some provinces of the country [4]. Conducted studies have attributed suicide to chronic causes. This needs a multifactorial model to predict the future cases, and also comprehensive interventions to reduce personal and social stressors. Every preventive effort has to be based on an awareness of the risk factors for suicide [5].

The numerous risk factors for suicide can be examined from different perspectives. In other words, many issues including cultural factors [6], economic factors [7], social factors [8], identity crises, mental illness, depression, bipolar personality disorders, antisocial and borderline personalities [9, 10, 11], addiction and alcoholism, immigration, familial factors (separation of parents, death of relatives, imposed marriages, marital discord, divorce), demographic parameters [2, 11, 12], a history of physical and sexual abuse [11], a familial history of suicide, and many other factors have significant impacts on the incidence of suicide. This wide variety of factors has raised an issue termed “psychological autopsy” that is intended to assess suicide risk factors. Psychological autopsy is, in fact, an evolved psychological profile postmortem. The data include a retrospective analysis of the person’s previous behavioral patterns to the time of death, and are collected through reviewing the writings and letters as well as interviewing with the family, friends, and colleagues [6].

The present study was conducted on the psychological autopsy of suicide completers in order to investigate the risk factors for suicide among Lorestan societies.

MATERIALS AND METHODS

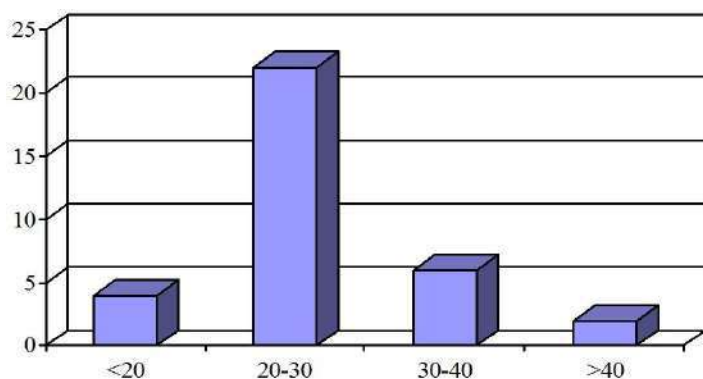
This cross-sectional study, conducted during a six months period (March- September, 2015), included all the suicide completers referred to the Emergency Ward of Shohada Ashayer hospital in Khorramabad, western Iran. The census sampling was applied, which resulted in the inclusion of 34 suicide completers. The data collection tools were hospital files, psychological interview, and a self-generated questionnaire. In the first stage, the data included subjects’ lifetime, gender, marital status, address, telephone number, date of suicide, date of death, suicide method, history of suicide, and history of physical illness was extracted from the hospital files and recorded in the designed checklist. In the second stage, demographic information; economic and cultural status; method, time, and place of suicide; a history of congenital malformations in childhood, receiving psychiatric and counseling services, previous suicide attempts, psychosomatic illness, and dependence on drugs or amphetamines; familial information; occupational status; familial history of mental illness; and history of smoking was extracted using the self-designed questionnaire. The validity of the questionnaire was examined and approved by expert panel.

During the third stage, the data were collected by a psychologist through interviewing with the family members or, in case of their unwillingness to participate in the interviews, through phone calls. Before the data were collected, the family members were provided with the necessary explanations on the purpose of the study and became certain about the confidentiality of the data.

Care was taken to preserve the social dignity of the family members and the foundation of the family, and not to mention the names and the personal characteristics of the deceased. This guaranteed the ethical considerations of the study. In addition, informed consents were taken from the family members of the deceased. The collected data were analyzed using the SPSS software via the descriptive statistics, non-parametric tests such as the chi-square test, and normal distributions.

RESULTS

In this six months study, a total of 34 completed suicides were examined. The incidence rates of suicide in the males and females were equal, showing no significant relationship between gender and suicide ($p > 0.05$). The range of suicide age was 18-62 years, with an average of 28.3 ± 9.73 years. The highest rate of suicide (64.7%) was found in the age range of 20-30 years old, showing a statistically significant difference in terms of age ($p < 0.05$) [Graph-1].



Graph: 1. Distribution of age in the suicide completers referred to Shohada Ashayer hospital of Khorramabad in 6 months (March-September, 2015).

Moreover, the medical history of the subjects showed that 9 cases (26.5%) had a previous history of hospitalization or physical damage, and 12 cases (35.3%) had a history of refractory or chronic illnesses in the family or close relatives. Poisoning (22 cases, 64.7%) was the most common method of suicide, and aluminum phosphide poisoning and drug poisoning were the most common poisoning methods. Self-burning was the second most common suicide method with 9 cases (26.5%). Two cases (5.9%) committed suicide through falling off a height and one case (2.9%) used a firearm.

On psychosocial characteristics, 18 cases (52.9%) were single, 13 cases (38.2%) were married, and 3 cases (8.8%) were divorced. As can be seen, the prevalence of suicide in the single individuals was higher, showing a statistically significant difference in terms of marital status ($p < 0.05$). A significant relationship was found between occupational status and suicide, so that the rates of attempting suicide among the self-employed individuals (13 cases, 38.2%) and housewives (9 cases, 26.5%) were higher than the rates in other occupations ($p < 0.001$). Concerning the educational level of the subjects, 9 cases (26.4%) were at the elementary school, 9 cases (26.4%) at junior high school, and 8 cases (23.5%) at senior high school educational levels. Moreover, 5 (14.7%) and 3 cases (8.8%) were illiterate and had academic degrees, respectively.

The present study also showed a significant relationship between suicide and the rate of familial relations ($p < 0.05$), so that 16 cases (47.1%) had normal social relations and 18 cases (52.9%) had low social relations. Moreover, 6 cases (17.6%) had, and 28 cases (82.4%) did not have a history of legal problems a year before committing suicide. In addition, there was no significant relationship between suicide and family size, abuse history, separation from parents in childhood, or experiences of job change or suspension from work ($P > 0.05$).

A history of previous suicide was found in 11 cases (32.4%). **Table- 1** shows psychiatric diagnoses of suicide completers. The results revealed that 29 cases (85.3%) of all subjects were estimated to fit a diagnosis of a mental disorder at the time of the death. Six cases (16.7%) had a history of recent death of an immediate relative, and out of this number one case had committed suicide.

Table: 1. Association between DSM-IV mental disorders and suicide (n=29 *)

Psychiatric diagnosis (DSM-IV Classification)		Patients N (%)
Disorders usually diagnosed in infancy, childhood, or adolescence	Pervasive developmental disorders	0 (0)
	Mental retardation	0 (0)
Delirium, dementia, and amnesic and other cognitive disorders	Dementia	1 (3.45)
Substance-related disorders		8 (27.59)
Alcohol-related disorders	Alcohol dependence	1 (3.45)
	Alcohol abuse	2 (6.90)
Drug-related disorders	Drug dependence	3 (10.34)
	Nicotine dependence	1 (3.45)
Psychotic disorders	Schizophrenia	5 (35.71)
	Major depressive disorder	9 (31.03)
Mood disorders	Dysthymic disorder	5 (35.71)
	Bipolar I disorder	2 (6.90)
	Bipolar II disorder	1 (3.45)
Anxiety disorders	Generalized anxiety disorder	6 (20.69)
	Obsessive-compulsive disorder	4 (13.79)
	Panic disorder	1 (3.45)
Somatization disorder	Hypochondriasis	0 (0)
Dissociative amnesia		1 (3.45)
Eating disorders	Anorexia nervosa	1 (3.45)
	Bulimia nervosa	0 (0)
Impulse-control disorders not elsewhere classified	Pathological gambling	0 (0)
Adjustment disorders		0 (0)
Personality disorders		2 (6.90)

*A subject may have more than one mental disorder.

DISCUSSION

The risk factors and causes of suicide were investigated in this research, which was a cross-sectional study on the psychological autopsy of the suicide completers referred to Shohada Ashayer hospital of Khorramabad in six months from March to September, 2015. The study attempted to find a solution to prevent suicide and reduce the damage caused by this health problem through comparisons with similar studies.

In this study, the prevalence rates of suicide in the males and females were equal, showing no significant relationship between gender and suicide, whereas maleness has been reported as one of the risk factors for suicide in numerous studies [13, 14]. Moreover, in our study the highest prevalence rate of suicide was found in the age range of 20-30 years old, being consistent with the results of the studies conducted in Kerman and Qazvin [13, 14]. In addition, in another study conducted in Qazvin, the highest prevalence rate of suicide was found in the females under the age of 20, and in the males in the age range of 20-30 years old [15]. Similar results were obtained in the studies conducted by Gururaj et al. and Sharma in India [16, 17].

In our study, more than half of the victims were single (52.9%), and 13 cases (38.2%) were married. This issue can be attributed to lack of support and emotional stability in unmarried and divorced individuals compared to married persons.

Regarding the method of suicide, the most common suicide methods included poisoning (64.7%) and self-burning (26.5%). Poisoning has been reported as the most common cause of suicide in various communities as reported by studies conducted in Qazvin [13, 15], Kerman [14], and India [17, 18]. The only considerable problem in this

regard is the diversity of the materials consumed. For example, the most common poisonous substances in our study were aluminum phosphides (rice tablets), amphetamines, and drugs, while in the studies in Qazvin, the consumption of drugs and benzodiazepines was the most common method of poisoning [13, 15]. Moreover, poisonous organic compounds and organophosphates were reported as the main materials consumed in attempting suicide in the studies by Sharma [17], Anand et al. [18], and in some rural areas of Iran [14].

A history of previous suicide attempts was reported for 32.4% of the cases in our study, and the rate of previous suicide attempts was reported to be 30-60% in a similar study. The rate of expression of suicidal thoughts and informing others of these thoughts in our study was 8.8%, while the rate was reported to be 25% in another study [26]. This difference can be attributed to this issue that the individuals being decisive to commit suicide are reluctant to inform others of their intentions and the details.

In terms of the rate of psychiatric disorders in the studied population in our study, 85.3% showed symptoms of depression and psychiatric problems, while the rate was reported to be 77% by Sheikholeslami et al. [15], 68.75% by Anand et al. [18], and 46.7% by Shafii et al. [19].

Concerning chronic drug addiction, 41.2% of the cases in our study had a history of drug abuse, and this is consistent with the findings of Andru et al.'s study showing drug addiction and dependence among the three major causes of suicide [20]. However, the major issue in this regard is the type of material consumed, so that all the cases of addiction in our study were related to drug addiction, while in the studies conducted outside Iran, the highest rate of dependence leading to suicide has been reported for alcoholism. Religious prohibitions can be considered as a determinant in this regard [20, 21].

CONCLUSION

In order to control, prevent, and reduce the prevalence of this problem in the community, taking the following measures is recommended:

1. Distributing the results of the present study in the health network of the province in order to increase doctors and other medical staff's awareness of the most common local risk factors for suicide.
2. Holding training workshops on therapeutic and psychiatric services for medical staff and promoting psychiatric services.
3. Increasing the number of service-providing centers for mentally-ill patients.
4. Identifying high-risk groups and monitoring them in order to control their risky behaviors.
5. Increasing public awareness through the media and training in prevention methods.
6. Fighting drug addiction and treating addicted cases.
7. Controlling and reducing unemployment as one of the most important risk factors for psychiatric disorders.
8. Training in family-strengthening techniques and having comprehensive interventions to reduce the suicide rate in the community through encouraging the participation of various governmental and non-governmental societies and institutions.
9. Applying many other methods that can effectively and significantly prevent suicide and reduce the rate of suicide attempts in the community.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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None.

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RESPONSE AND COMPATIBILITY OF PLASMA LEVELS OF NESFATIN-1, GLUCOSE AND INSULIN RESISTANCE INDEX TO CIRCUIT RESISTANCE TRAINING IN OBESE DISABLED MEN

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ABSTRACT

Nesfatin-1 is a rather new adipokine that secretes from adipose tissue and has a role in the mechanism of blood glucose regulation, improving insulin sensitivity, appetite regulation and metabolism. Given the importance of resistance exercise in the prevention and treatment of overweight and obesity, the aim of this study is to examine the response and adaptation of plasma levels of nesfatin-1 to circuit resistance training among obese men with disabilities. 22 obese disabled men with aged 25 ± 5 years and with an BMI of 30 ± 2.5 kg/m² were selected and randomly divided into 2 groups: experimental and control. Experimental group included 12 subjects who participated in an eight-week resistance training, 3 sessions per week with intensity of 60-65 % and with the gradual increase to 70-80% with one repetition maximum at the end of each session. And control group included 10 persons who took part in no training or physical activities during the study period. Blood samples to assess the levels of nesfatin-1, glucose and insulin resistance were taken before the study, after one session and after the eight weeks of training. The collected data was analyzed by using K-S test, ANOVA with repeated measures in SPSS and with a significance level of $\alpha < 0.05$. The findings showed that one session immediately after and eight weeks of circuit resistance training didn't affect the plasma levels of nesfatin-1 in obese disabled persons. While the amount of glucose and insulin decreased significantly. Therefore using this type of training can be helpful in decreasing glucose and insulin resistance in obese disabled men. Circuit resistance training has a significant effect on glucose level of blood but has no significant effects on levels of nesfatin-1.

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KEY WORDS

Nesfatin-1, Glucose, Insulin resistance, Resistance exercise, Physically disabled, Obesity

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INTRODUCTION

In the past, adipose tissue was seen as the source of fat storage but in recent years it has been recognized as a major endocrine organ that produces bioactive mediators which have a role in controlling blood pressure, metabolism of glucose, resistance to insulin and inflammation and obstruction of arteries [1]. These bioactive mediators are proteins that are called adipokines. It is thought that adipokines participate in the development of insulin resistance in different tissues, in type II diabetes and cardiovascular diseases [1]. In fact, adipose tissue is not only an inactive source of energy to the body, but also it's known as an active endocrine organ that produces biological materials called adipocytokines [2, 3]. Nesfatin-1 is one of these adipocytokines that is produced in adipose tissue and participates in the regulation of appetite and hemostasis of energy and metabolism and is affected by inflammatory cytokines and insulin [4]. The amount of this adipocytokine in epidermis adipose tissue is more than the amount in visceral adipose tissue and its amount is affected by the diet, inflammatory cytokines and insulin and participates in appetite regulation and energy balance [5]. In 2006, Nesfatin-1 was discovered by Oh et al, as an anti-appetite polypeptide with 82 amino acids derived from the post-translation process of Nucleobindin 2 gene (NUCB2) in hypothalamus of rats; they divided the discovered parts derived from NUCB2 as: number 1 to 82, nesfatin-1; number 85 to 163, nesfatin-2 and number 166 to 396 nesfatin-3. It was found that nesfatin-1 participates in the function of the digestive system and gastric emptying and its plasma concentration changes by feeding, fasting, diabetes and physical activity [7, 6]. Nesfatin-1 is expressed in hypothalamus of rats and humans and its expression reduces in the paraventricular nucleons of hypothalamus while fasting or hungry [8]. Nesfatin-1 is one of neuropeptides that regulates appetite and is produced in hypothalamus and has an

important role in energy balance. Ramanjaneya et al (2006) has also shown that the amount of nesfatin-1 has a positive correlation with body mass index in blood circulation [9]. Activity and movement are human beings essential needs and disabled people especially need more movement to be healthy and succulent for they are faced with more movement limitations than others. Until half a century ago it was thought that the disabled people have no place in sports competitions and could only watch sports events. But today due to significant developments in disabled sports, its precious role is becoming more obvious in the lives of the disabled people, in a way that today it has become an inseparable part of their lives but diversity of exercises suitable for the disabled has made them needless of all complicated sports equipment. Different factors affect the production of adipocytokines such as physical activities; and the response of each adipocytokine depends separately on the length and intensity of the exercise. However, enough research hasn't been done with respect to the effects of exercise on nesfatin-1. Haghshenas et al (2011) found that twelve weeks of resistance training increases the level of nesfatin-1 and reduces the level of glucose and insulin significantly [10]. Ramanjaneya et al (2010) showed that fasting for 24 hours before killing the mice will reduce expression of nesfatin in epidermis adipose tissue significantly [9]. Taji Tabas et al (2016) showed that ten weeks of resistance training had an effect on the level of nesfatin in women with type II diabetes, this training brought about a significant increase in nesfatin-1 and a reduced glucose and insulin significantly [11].

Since little research has been conducted on the effects of exercise and physical activities on this adipocytokine and because these research have conflicting results, and since there has been no research on the response and compatibility of circuit resistance trainings on the amount of nesfatin-1, glucose and body resistance to insulin in obese men, the present research aims to analyze the response and compatibility of an eight-week circuit resistance training program to the plasma levels of nesfatin-1, glucose and resistance to insulin in obese disabled men.

MATERIALS AND METHODS

Subjects

This semi-empirical research was done with two groups: experimental and control. The population includes all the obese disabled men in Zahedan (city in east of Iran). Subjects were selected in a targeted way, after filling out the consent form they were divided into two groups randomly, 12 of them in the experimental group and 10 of them in the control group. In this research it wasn't possible to control the affecting factors precisely, but to control the unwanted influence and confounding variables the subjects were asked to abstain from smoking, using alcoholic beverages or doing any strenuous physical activities during the study period. To omit the influence of circadian rhythm on hormonal changes, all the tests and sampling were done in a particular time for all the groups.

Methods of measuring anthropometric indices

Measurement of the anthropometric indices including weight, height, BMI (body mass index), percentage of body fat and WHR (waist-hip ratio) was done with the least amount of clothes on and without shoes.

Measurement of height and weight was done while the subjects were standing and by using height gauge installed on the wall (with an accuracy of one millimeter) and digital weighing scale (with an accuracy of 0.1 kilogram) respectively. BMI was calculated through the body weight (kilogram) divided by the squared height (square meter). Thickness of the skinfold on the chest, stomach and thigh was measured by Harpenden Skinfold Caliper. Percentage of body fat was estimated by Jackson and Pollock 3-site Skinfold Equation [12, 13, 14].

Jackson and Pollock 3-Site Skinfold Equation [12]:

$$Db = 1.1093880 - (0.0008267 \times \text{sum of skinfolds}) + (0.0000016 \times \text{square of the sum of skinfolds}) - (0.0002574 \times \text{age})$$

Db is the body density of men, skinfold sites are abdominal, triceps and suprailiac and age refers to the age of the subjects.

Then the percentage of body fat was measured by Siri formula [12]:

$$\text{Siri formula is: } \%F = (4.95/Db - 4.50) \quad | \quad \%F = \text{body fat percentage}$$

WHR was measured by inelastic tape measure and without putting any pressure on the body of the person (with an accuracy of 0.1) and by measuring the least waist circumference in the area between the lowest rib and iliac crest, and if the thinnest waist circumference was not recognizable, the measurement was done in the smallest horizontal circumference of this site, and the waist measurement divided by hip measurement calculated the WHR. Hip

circumference was measured in a relaxed and loose state [15]. All the above mentioned indices were measured again after the eight-week circular resistance training.

Methods of measuring biochemical variables

To measure the biochemical variables of both groups, 10 ml blood was taken of the right brachial vein of each subject in a 12-hour fasting condition 48 hours before the first session of the training and immediately after the training and 48 hours after the last session of training.

The subjects were asked to refrain from any intense exercise 48 hours before the first session and 48 hours after the last session of the training. The blood samples after centrifugation and plasma separation were kept in a -30 degrees centigrade condition to be analyzed with the blood samples after training sessions and the one at the end of the study. The level of fasting blood sugar was measured by the colorimetric method and based on the enzymatic method and by using the glucose laboratory kit of Pars Azmoon company made in Iran and by Hitachi device made in Germany. The insulin resistance index was calculated by using HOMA-IR index [15]:

$$HOMA - IR = (FPL(\mu u/ml) \cdot FPG(mmol/l)) / 22.5$$

FPL= fasting insulin (microU/L)

FPG= fasting glucose (mmol/L)

The plasma level of nesfatin-1 was measured by laboratory kit of human nesfatin-1 made by Phoenix Pharma Company in the USA.

Training Plan

In the experimental group, the circuit resistance training was performed for eight weeks, three sessions each week. The subjects attended two orientation sessions to get familiar with safety precautions related to exercising with weights and systematic use of bodybuilding equipment; then one more repetition for all the six activities was determined, with repletion to the tiring limitation. To use this method the subject moved the weight under the maximum until exhaustion, in a way that the number of movements won't be less than 10 before exhaustion. Then according to Brzycki equation [16] the maximum amount of force (one-repetition maximum) of each subject was calculated for that exercise [17]:

$$1RM = \frac{w}{[1.0278 - (0.0278r)]}$$

1RM= the maximum amount of force, w=the size of the weight, r= the number of receptions before resistance overload

The resistance training included exercising with six devices (bench press, standing cable curl with rope, rope press down, military press, military press behind and arm row cable) and free weights that was six movements all in all. After teaching the subjects how to work with the devices, on each device three sets were performed with 12 repetitions, and after the first four weeks of training, one more repetition maximum was also assigned. The resistance training program started with an intensity of 60 to 65 percent and with one more repetition maximum and gradually increased to 70 to 80 percent with one repetition maximum at the end of each session. The time allocated to perform each movement in any of the stations was 60 seconds and the resting time between two stations were two minutes and the resting time between each two sets was 1 minute. Each training session included warm up (6 minutes of slow running and 4 minutes of stretching exercises), resistance training with devices and free weights (40 minutes) and cool down (5 minutes of slow running or walking and 5 minutes of stretching exercises). During the study period the persons in the control group didn't participate in any training programs.

Data Analysis

Kolmogorov–Smirnov test was used to analyze the normal distribution of the data. For the intragroup and intergroup changes ANOVA with repeated measures was used. In all the tests the significance level was set at $\alpha < 0.05$. The analysis of data was done by using SPSS version 22.

Findings

The average of age of subjects in control and experimental groups was 24.66 and 25.33 respectively. And the average of height in the control group was 171.20 cm and in the experimental group it was 169.66 cm. The normal distribution of the data was confirmed by Kolmogorov–Smirnov test.

Table: 1. statistics results of anthropometric subjects in control and experimental groups in pretest and posttest

Variable	Group Stage	Control group (10 persons)	Experimental group (12 persons)
	Weight (kg)	Pretest	87.98±6.89
Posttest		88.45±6.41	82.12±4.56
BMI (kg/m ²)	Pretest	29.75±2.55	29.67±2.68
	Posttest	30.24±2.44	28.75±2.54
WHR	Pretest	0.88±0.83	0.91±0.17
	Posttest	0.89±0.85	0.89±0.23
Percentage of body fat	Pretest	32.15±6.22	33.44±6.88
	Posttest	32.48±6.78	32.03±6.43
Age (years)	24.66±3.22		25.33±3.02
Height (cm)	171.20±4.56		169.66±5.34

The values are shown in standard deviation ± average.

According to table 1, the average of body weight, BMI, waist-hip ratio and percentage of body fat in control group has increased during the time between the pretest and the posttest; while the average of these factors in the experimental group has decreased.

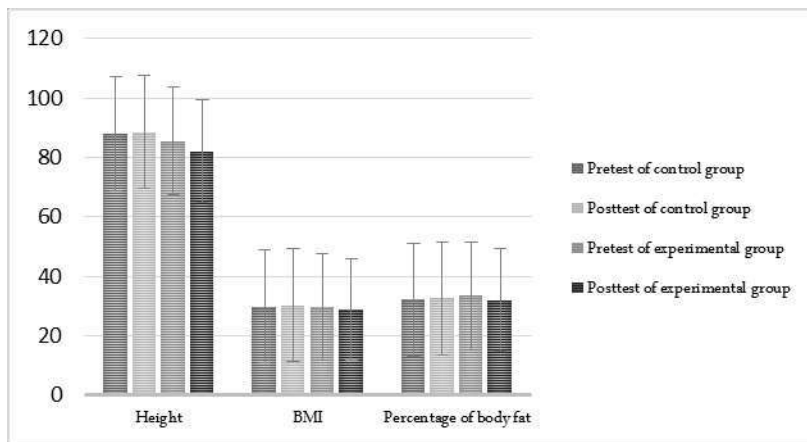


Fig: 1. The comparison of weight, BMI and percentage of body fat in pretest and posttest in control and experimental group

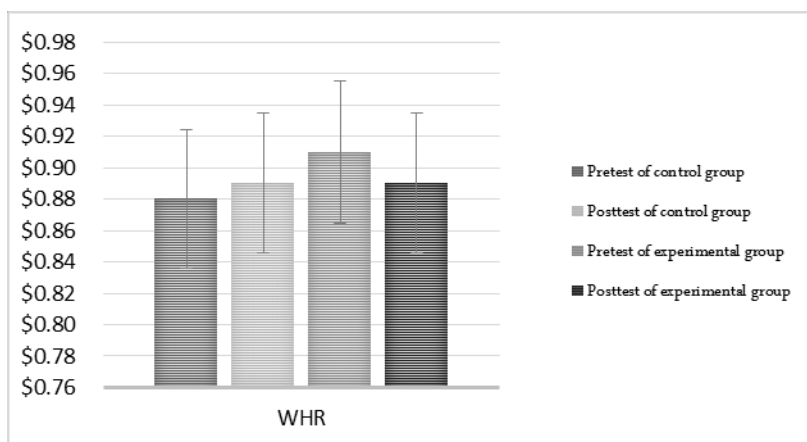


Fig: 2. Comparison of WHR variable in pretest and posttest of control and experimental groups

Table: 2. Intragroup test of ANOVA with repeated measure in biochemical variables

Variable	Sum of squares of type 3	Degree of freedom	Average of squares	F	P
Nesfatin-1 (ng/ml)	77454.15	2	32278.49	2.50	0.087
Glucose (mol/l)	497.03	2	248.51	4.76	0.015*
Insulin (μU/ml)	5.52	2	2.76	2.36	0.10
Resistance to Insulin	6.59	2	3.30	8.11	0.001*

Difference at the level of 0.05 is meaningful (P<0.05).

By using ANOVA with repeated measures in the amounts of glucose (p=0.015)(figure 3) and body resistance to insulin (p=0.001) (char 4) a significant decrease is seen. While in the amounts of insulin (p=0.10) and nesfatin-1 (p=0.087) a significant change was not seen [Table- 2].

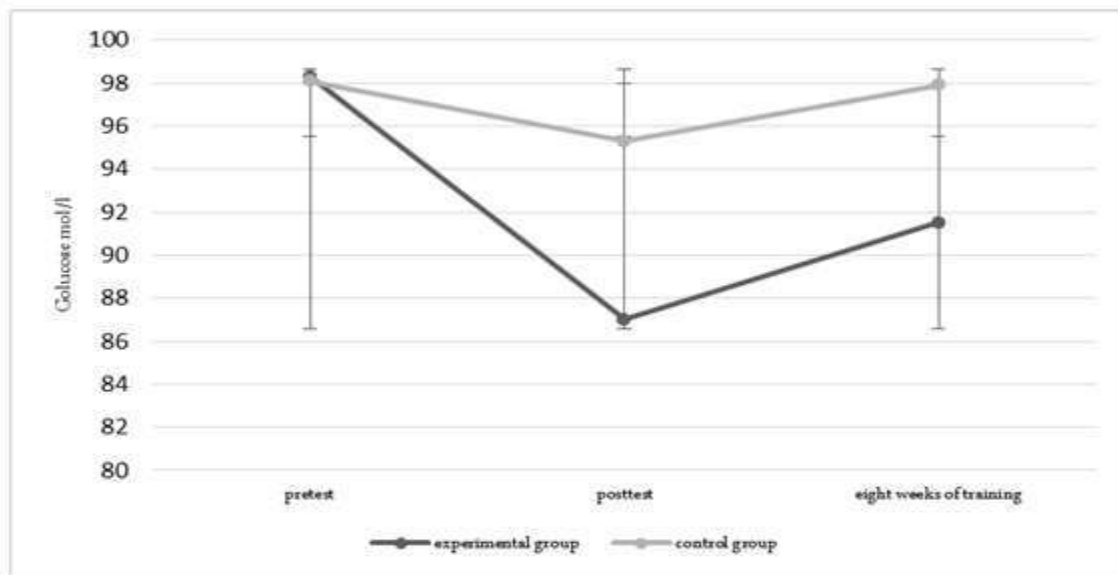


Fig: 3. Glucoselevel in pretest, immediately after one session and posttest

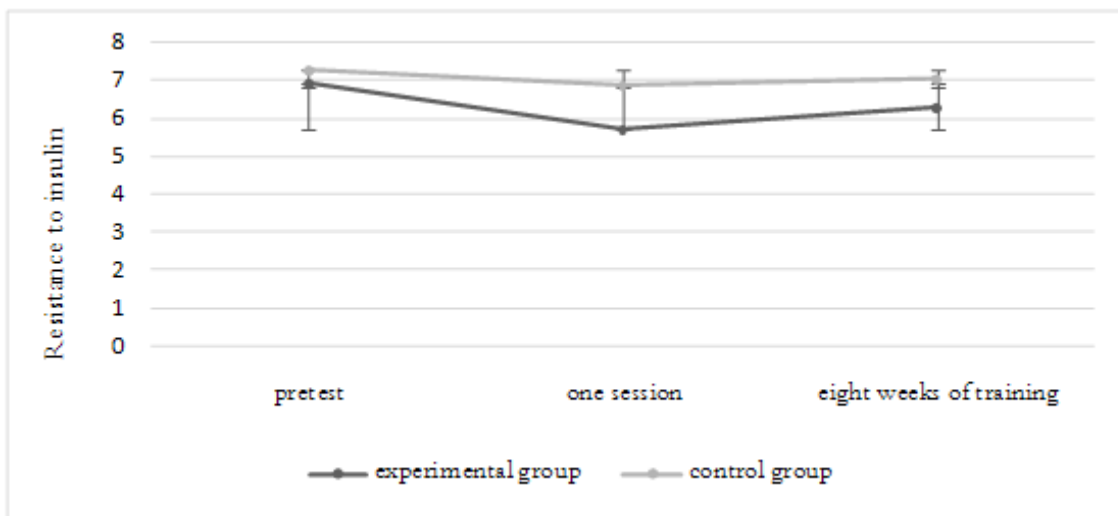


Fig: 4. Resistance to insulin in pretest, immediately after one session and posttest

Table: 3. Intergroup test of ANOVA with repeated measure in biochemical variables

Variable	Sum of squares of type 3	Degree of freedom	Average of squares	F	P
Nesfatin-1 (ng/ml)	1329241.90	1	1349041.90	1.94	0.18
Glucose (mol/l)	350.41	1	350.41	0.35	0.56
Insulin (μ U/ml)	13.55	1	13.55	0.49	0.49
Resistance to Insulin	8.16	1	8.16	1.14	0.29

By using the intergroup ANOVA with repeated measures a meaningful change in the amount of nesfatin-1 ($p=0.18$), glucose ($p=0.56$), insulin ($p=0.49$) and resistance to insulin ($p=0.29$) was not seen.

Table: 4. The statistical results of biochemical variables in pretest, after one session of training and posttest

Variable	Group	Control group (10 persons)	Experimental group (12 persons)
	Stage		
Nesfatin-1 (ng/ml)	Pretest	10.11 \pm 40.34	10.05 \pm 40.31
	After on session	10.06 \pm 40.30	10.01 \pm 40.16
	Posttest	10.01 \pm 40.46	9.26 \pm 36.45
Glucose (mol/l)	Pretest	98.10 \pm 25.54	98.30 \pm 11.43
	After on session	95.30 \pm 23.11	87.00 \pm 15.27
	Posttest	97.90 \pm 24.19	91.50 \pm 7.70
Insulin (μ U/ml)	Pretest	16.80 \pm 3.59	15.93 \pm 2.81
	After on session	16.26 \pm 3.16	14.99 \pm 3.25
	Posttest	16.30 \pm 3.27	15.58 \pm 2.81
Resistance to insulin	Pretest	7.27 \pm 2.09	6.92 \pm 1.27
	After on session	6.86 \pm 1.95	5.71 \pm 1.81
	Posttest	7.02 \pm 1.88	6.30 \pm 1.01

The values are shown in standard deviation \pm average

The major findings of the present research showed that immediately after one session of training and also after eight weeks of circuit resistance training, there was no significant change in the plasma levels of nesfatin-1 and these findings are in line with the works of Ghanbari-Nikai et al (2011) that showed nesfatin-1 hadn't change after one session of RAST and NCKB exercises among young kickboxers [18], and also the work of NazarAli et al that showed no change in nesfatin-1 among overweight women after eight weeks of resistance training [19]. However, the findings aren't in line with the work of Tavassoli et al (2014) that showed a significant change in the level of nesfatin-1 after 12 weeks of circuit resistance training among overweight young adults [20] and the work of Taji Tabas et al that showed after ten weeks of resistance training among women with type II diabetes the amount of nesfatin-1 increases significantly [11]. And with respect to the effects of resistance training on nesfatin-1 little research has conducted. The discrepancy seen in these results is due to the difference in the age and gender of subjects and difference in the length and intensity of training.

One of the limitations in the research that might have caused no change in nesfatin-1 is the diet of subjects in both control and experimental groups. For the expression and secretion of nesfatin-1 is related to nutrition status [21]. And the amount of secreted nesfatin-1 is related to the diet [9]. Therefore, it is suggested to the subjects to control their diet during the study period and refrain from changing their diet. But since this research is semi-experimental controlling the subjects' diet was not possible. Generally, research has shown that the amount of nesfatin-1 is affected by different factors. Ramanjaneya et al (2010), has also shown in their work that TNF and interleukin-6 and insulin increased the inner cell expression of nesfatin-1 genes in cultured fat cells, the findings show that expression and secretion of nesfatin-1 is regulated via different ways [9]. Zhang et al (2012) showed the direct effect of central nesfatin-1 on peripheral insulin sensitivity or improvement of insulin activity from changes related to signaling pathways and that nesfatin-1 of hypothalamus through neural pathways can help increasing sensitivity to peripheral insulin by decreasing gluconeogenesis and increasing glucose absorption of glucose [22, 23]. Abaci et al (2012) in a research showed that the amount of oral glucose in fat children might not be enough for nesfatin-1 response and that nesfatin-1 might be an effect of short-term regulators caused by foods [24]. Other findings of this research include significant decrease in glucose level and insulin resistance index in experimental group that is in line with the works of Cauza et al [25], Sharjerd et al [26], Togighi et al [27], Taji Tabas et al [11] and El-Kadar [28] who argue that resistance training can decrease the level of glucose and insulin resistance. The

findings show that insulin remained unchanged after resistance training and it's in line with the works of Tifighi et al and Taji Tabas et al and it doesn't approve the works of Shahrjerd et al and NazarAli et al who showed significant decrease in insulin. The conflicting results may be due to the difference in the time of blood taking, difference in training protocols and difference in subjects' societies.

According to table 1 the average of weight, BMI, waist-hip ratio and percentage of body fat in control group has increased during the time between pretest and posttest; while the average of these factors in the experimental group decreased. Therefore according to this research weight loss and decrease of body fat can be the reason of glucose decrease and insulin resistance.

CONCLUSION

Generally we can say that eight weeks of circuit resistance training and one session immediately led to significant decrease in glucose level and resistance to insulin; however no change was seen in plasma levels of insulin and nesfatin-1. The results of present research showed that resistance training has an important role in controlling and improving harmful or risk factors in diseases related to glucose (diseases related to metabolism of carbohydrates) and insulin resistance (diseases such as hyperlipidemia, high blood pressure and arteriosclerosis); and by controlling weight, daily exercise and healthy diet (rich in fibers and low fat) lots of these diseases can be prevented. Therefore, it is recommended to the obese disabled persons to use resistance trainings as a preventive measure to lose weight and decrease the probability of obesity disorders and resistance to insulin. Since this research is one of the first studies on the effects of circuit resistance training on plasma levels of nesfatin-1 among obese men, more research is needed to analyze more profoundly the mechanisms affecting nutrition and changes of nesfatin-1 related to the action of glucose and insulin after trainings with more intensity and longer period.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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FINANCIAL DISCLOSURE

None.

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ARTICLE

EFFECTS OF ORGANIC AND CHEMICAL FERTILIZERS ON YIELD COMPONENTS OF COMMON WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

This experiment was laid out in order to study on effect of organic fertilizer and P fertilizer on yield and yield components of common wheat (*triticumaestivum* L.) cv. Chemran Islamic Azad University, Khorramabad branch, Iran in 2015. The experiment was laid out in a split-plot design based on randomized block design with three replications. Treatments were organic fertilizer (0, 10, 20 ton/ha manure and 10, 20 ton/ha compost) in main plots and phosphate fertilizer (0, 50, 75 and 100 percent based on proposed soil analyzing) in sub plot. Analysis of variance results showed that effect of organic and chemical fertilizers and interaction effect of them were significant on some yield components of common wheat. Based on the results, combined application of organic manure or vermicompost with chemical fertilizer has a better effect on yield and yield components of common wheat rather than single application of them and control treatments. Higher grain yield was obtained in 20ton/ha organic matter and 75% phosphate fertilizer recommendation treatment with the average of 5472 kg/ha as compared with control treatment (without consuming organic matter and phosphate about 3 ton/ha). Therefore, we can conclude that combined application of organic and chemical fertilizers had more efficiency because of some positive interaction between their microorganisms in soil that result to synergistic effect and laid to increase in yield components and in final grain yield. In final our results indicated that higher grain yield observed in combined application 20ton/ha organic matter and 75% chemical P fertilizer and application of combined fertilizer is better for farmer in wheat field of Khorramabad region for high yield and economic benefits in common wheat.

INTRODUCTION

Wheat (*triticumaestivum* L.) is the national staple food in forty-three countries. According to the statistics of the food and agriculture organization (FAO), during 2008-2009 growing season 682 million tons of wheat were produced and it is estimated that up to 690 million tons will be produced in 2012- 2013 growing season. The experts contend that the amount of the annual wheat production must be 2% higher than the annual demand. The world does not have enough potential for increasing the soil level cultivated with wheat; therefore in order to increase the wheat production, we have to increase the productivity of the fields which have been cultivated with wheat. Grain yield of small grain cereals is determined by two main components, grain number per unit area (grains perm²) and mean grain weight. Environmental conditions around 20 days pre- and 10 days post-anthesis are considered critical for grain yield determination [1]. During pre-anthesis, the potential grain number per unit area [2] and potential grain weight [3] are defined. The final grain number per unit area is set immediately after anthesis, while grain filling occurs during the remaining post-anthesis period [4].

Grain yield of small grain cereals is determined by two main components, grain number per unit area (grains perm²) and mean grain weight. During pre-anthesis, the potential grain number per unit area [2] and potential grain weight [3] are defined. The final grain number per unit area is set immediately after anthesis, while grain filling occurs during the remaining post-anthesis period [4]. Grain yield is usually strongly associated with the number of grains per unit area [1, 2]. While this association has been extensively reported for a relatively wide range of environments and nutrition.

The use of organic fertilizers, such as manure, vermicompost and crop residues in crop production has been a known activity for hundreds, even thousands of years, thanks to new varieties, changing climatic conditions, new aims in cultivation (higher yields, better quality, maximum efficiency of production), new technologies of food processing and efforts for nature protection and sustainable agriculture, these „old“ materials and new technologies are nowadays a target of refinement and globally focused attention [5, 6]. Organic fertilizers are the products of the degradation of organic matter through interactions between earthworms and microorganisms. Organic fertilizers are finely divided peat-like materials with high porosity, aeration, drainage, and water-holding capacity and usually contain most nutrients in the available forms such as nitrates, phosphates, exchangeable calcium and soluble potassium [7]. The preparation and use of organic manures as a nutrient management may provide a hygiene and useful way of disposal and utilization of waste which would otherwise have created a healthy environment [8]. They told that, French bean was most responsive to vermicompost treatment on growth and yield in comparison to farmyard manure, chemical fertilizer and mixed treatments under irrigated condition of Srinagar valley. They also concluded that vermicompost is particularly good for farmers, consumers and ultimately for soil as it can be used as a resource for maximum crop productivity with more financial output in comparison to those chemical fertilisers. [9] found better yield in vermicompost treatment. Same observation was also reported [10]. [11] reported that yield increased with fertilizer and manure application.

KEY WORDS

Common wheat, Grain yield, Manure and straw

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Therefore the aim of this study is evaluation of effects of some organic fertilizer such as manure and vermicompost and chemical fertilizers on yield and yield components of common wheat in Lorestan province, Iran.

MATERIAL AND METHODS

Field material and experimental design

A field experiment was laid out in the Faculty of agronomy and plant breeding, Islamic Azad University, Khorramabad Branch, Iran during the growing seasons 2014- 2015. The experiment was laid out in order to evaluate the effects of manure and vermicompost and chemical fertilizers on yield and yield components of common wheat. The Khorramabad region has a continental semi-arid climate with annual precipitation of 500 mm. About 50% of this falls during the wheat and barley growing period.

Treatments

The experiment was laid out in a split-plot design based on randomized block design with three replications. Treatments were organic fertilizer (0, 10, 20 ton/ha manure and 10, 20 ton/ha compost) in main plots and phosphate fertilizer (0, 50, 75 and 100 percent based on proposed soil analyzing) in sub plot.

Yield and yield components determination

There were 15 rows in each from plot; rows were 6 m long with 0.2 m row spacing. At maturity, two outer rows for each plot, 50 cm from each end of the plots, were left as borders and the middle 1 m² of the three central rows were harvested. Then yield components were calculated as standard methods with using 10 plants. To determine grain yield, biomass yield and harvest index, we removed and cleaned all the seeds produced within two central rows in the field. Then grain yield and biomass yield recorded on a dry weight basis. Yield was defined in terms of grams per square meter and quintals per hectare. Replicated samples of clean seed (broken grain and foreign material removed) were sampled randomly and 1000-grain were counted and weighed. The straw yield was accounted with follow:

$$\text{Straw yield} = \text{biological yield} - \text{grain yield}$$

Statistical analysis

The statistical analysis to determine the individual and interactive effects of treatments was conducted using MSTAT-C software [12,13]. Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.

RESULTS

Spike length: The results showed that, the effect of organic fertilizer, P fertilizers and interaction between them on spike length was not significant [Table 1]. The comparison of the mean values of the spike weight for interaction effect of organic fertilizer and P fertilizers showed that application of 20 ton/ha vermicompost and 100% P fertilizer treatment had the highest (8.99 cm) and control treatment had the lowest spike length (7.6 cm) and difference between them were significant [Table 2].

Table 1: Analysis of variance (mean squares) for yield and yield components of common wheat under application of organic manure and P fertilizer

S.O.V	df	Spike length	Spike weight	Number of grain per spike	1000 grain weight	Grain yield	Straw yield	Biomass yield
R	2	1.82	0.24	0.95	0.14	0.58	0.44	1.57
Organic fertilizer	4	1.78	0.48	0.36	5.24*	3.64*	0.54	2.4
P fertilizer	3	0.18	4.69**	4.47**	1.12	5.08**	5.61**	5.43**
O*P	12	1.33	1.69*	2.53**	0.52	0.83	5.6**	0.75
CV	-	24	7.07	16.8	5.95	15	16	14.5

ns: Non-significant, * and **: Significant at 5 and 1% probability levels, respectively

Spike weight: The results showed that, the effect of P fertilizers and interaction between organic fertilizer and P fertilizer on spike weight was significant only [Table 1]. The comparison of the mean values of the spike weight for interaction effect of organic fertilizer and P fertilizers showed that application of 20 ton/ha manure and 100% P fertilizer treatment had the highest (1.85 g) and application of 20 ton/ha vermicompost and 0% P fertilizer treatment had the lowest spike weight (1.01 g) and difference between them were significant [Table 2].

Number of grain per spike: The effect of P fertilizers and interaction between organic fertilizer and P fertilizer on number of grain per spike was significant and the effect of organic fertilizer was not significant on it [Table 1]. The comparison of the mean values for interaction of organic fertilizer and P fertilizers on number of grain per spike of wheat showed that combined application of application of 20 ton/ha manure and 50% P fertilizer treatment had the highest (33) and combined application of 10 and 20 ton/ha vermicompost and 0% P fertilizer treatment had the lowest number of grains per spike (19) and difference between them was significant [Table 2].

1000 grain weight: The effect of organic fertilizer on 1000 grain weight was significant and the effect of P fertilizers and interaction between organic fertilizer and P fertilizer was not significant on it [Table 1]. The comparison of the mean values for interaction of organic fertilizer and P fertilizers on 1000 grain weight of wheat showed that combined application of application of 10 ton/ha vermicompost and 75% P fertilizer treatment had the highest (43.5 g) and control treatment had the lowest 1000 grain weight (36 g) and difference between them was significant [Table 2].

Table 2: Mean comparison interaction effect organic fertilizer and P fertilizer on yield components of common wheat

Treatments	Spike length (cm)	Spike weight (g)	Number of grain per spike	1000 grain weight (g)	Grain yield	Straw yield	Biomass yield (kg/ha)
M ₀ P ₀	7.6c	1.33b-e	21.7j	36d	2475f	5031c	7736d
M ₀ P ₁	8.6abc	1.59a-e	29c	38cd	3313ef	6573abc	9708a-d
M ₀ P ₂	8.2abc	1.45a-e	27g	38.3cd	3503ef	5176c	9617a-d
M ₀ P ₃	7.7c	1.28b-e	29.3c	37.3cd	3896cde	6483abc	9167bcd
M ₁₀ P ₀	7.7c	1.48a-e	26h	40abc	4125b-e	5479bc	10570a-d
M ₁₀ P ₁	8.8abc	1.47a-e	26h	40.4abc	5129abc	6681abc	11331abc
M ₁₀ P ₂	7.9abc	1.18c-e	26h	41.5abc	5267ab	6980abc	12147a
M ₁₀ P ₃	7.7c	1.37b-e	28de	40.2a-d	4652a-d	6248abc	10250a-d
M ₂₀ P ₀	7.7c	1.15d-e	22j	41.2abc	4134b-e	6987abc	11781ab
M ₂₀ P ₁	8.2abc	1.85a	30b	41.2abc	4712a-d	6979abc	11093a
M ₂₀ P ₂	8.8abc	1.65a-c	28ef	41.2abc	5462a	6704abc	12200a
M ₂₀ P ₃	7.7c	1.71ab	33a	42abc	4567a-e	5683bc	9500a-d
C ₁₀ P ₀	8.4abc	1.12de	19k	40.2a-d	4000b-e	5570bc	10136a-d
C ₁₀ P ₁	8.2abc	1.44a-e	27g	42.5ab	4912abc	7463ab	12292a
C ₁₀ P ₂	8.3abc	1.75a-b	23i	43.5a	4242a-e	6333abc	10836abc
C ₁₀ P ₃	8.5abc	1.48a-e	28d	39.4a-d	4754a-d	5004c	9650a-d
C ₂₀ P ₀	7.7c	1.01e	19k	41abc	4133b-e	5271c	9417a-d
C ₂₀ P ₁	8.99a	1.3b-e	27fg	40.63a-d	4718a-d	7671a	11436abc
C ₂₀ P ₂	8.3abc	1.2c-e	29c	39.9a-d	4418a-e	5248c	11327abc
C ₂₀ P ₃	8.9ab	1.54a-d	27g	39.5a-d	4215a-e	5689abc	8833cd

Means by the uncommon letter in each column are significantly different ($p < 0.05$). (M₀=Nom application of organic fertilizer, M₁₀= application of 10 ton/ha manure, M₂₀= application of 20 ton/ha manure, C₁₀= application of 10 ton/ha vermicompost, C₂₀= application of 20 ton/ha vermicompost, P₀= 0% phosphor fertilizer, P₁= 100% phosphor fertilizer, P₂=75% phosphor fertilizer and P₃=50% phosphor fertilizer)

Grain yield: The results showed that, the effect of organic and P fertilizers on grain yield were significant [Table 1]. The comparison of the mean values of grain yield for interaction effect of organic fertilizer and P fertilizer showed that application of 20 ton/ha manure and 75% P fertilizer treatment had the highest (5462 kg/ha) and control treatment had the lowest grain yield (2475 kg/ha) and difference between them were significant [Table 2].

Straw yield: The effect of P fertilizer and interaction effect of organic and p fertilizers on straw yield were significant only [Table 1]. The comparison of the mean values of straw yield for interaction effect of organic and P fertilizer showed that combined application of 20 ton/ha vermicompost and 100% P fertilizer treatment had the highest (7671 kg/ha) and combined application of 10 ton/ha vermicompost and 0% P fertilizer treatment had the lowest straw yield (5004 kg/ha) and difference between them were significant [Table 2].

Biomass yield: The effect of P fertilizer was significant on biomass yield [Table 1]. The comparison of the mean values of biomass yield for interaction effect of organic and P fertilizer showed that combined application of 10 ton/ha vermicompost and 100% P fertilizer treatment had the highest (12292 kg/ha) and control treatment had the lowest biomass yield (7736 kg/ha) and difference between them were significant [Table 2].

DISCUSSION

These results of study showed that application of organic fertilizers such as manure and vermicompost had the positive effect on yield components of common wheat, but combined application of them with P fertilizer had the synergistic effect of common wheat yield components. The positive effect of organic and bio-fertilizer on yield and yield components of many crops were revealed by many authors [16, 17]. This may result from its ability to increase the availability of phosphorus and other nutrients especially under the

specialty of the calcareous nature of the soil, which cause decreasing on the nutrients availability, results agree with [16].

The results of this study showed that effect of organic and chemical P fertilizer was significant on some wheat yield components such as 1000 grain weight, number of grain per spike and grain yield [Table 1]. Grain yield for treatments was mainly determined by grain number per unit area. Grain yield variability was the result of the potential growing conditions in each treatment generated by differences in different amounts of application of organic and chemical fertilizers during the vegetative and reproductive stages. This was also reported in other studies [17]. In the present study application of organic and chemical P fertilizer increased number of grain per spike that results to increase in high grain yield and productivity. The results correspond to Kato and Yamagishi (2011) finding that spikes density of wheat varieties were higher in organically managed field than conventional field due to higher pre-anthesis dry matter production.

However, organic manure and vermicompost had a significant effect on grain yield and biomass [Table 1]. The grain yield was significantly higher in combined application of 20 ton/ha organic manure and 100% P fertilizer treatment. With increased in application of these organic and chemical fertilizers grain yield increased significantly [Table 2]. Using organic manure and the application of biofertilizers such as vermicompost and nitrogen fixing bacteria has led to a decrease in the use of chemical fertilizers and has provided high quality products free of harmful agrochemicals for human safety [18]. According to the present study, vermicompost with high water-holding capacity and proper supply of macro and micro-nutrients [7], has a positive effect on production of barley and subsequently enhanced grain yield. Improved growth, development and yield of plants have previously been reported in the presence of optimal amounts of vermicompost [19]. These findings are in accordance with the observations of [18, 20].

In the present study, significant differences were observed among organic and P fertilizers regarding the average number of grain per spike, 1000 grain weight, biomass and grain yield. For application of organic fertilizer increased average number of grain per spike and biomass yield in simple mean comparison. This means that we can apply these fertilizer with together for achieved to maximum of some yield components for increase of grain yield as well. Higher grain yield in common wheat achieved in combined application of 20 ton/ha manure and 75% P chemical fertilizer. Application of different organic and biological fertilizers increase the yield of many crops. For example, some biological fertilizer consist the most effective species of nitrogen stabilizing bacteria for increase efficiency of crop production [21]. [14] revealed that application nitrogen and phosphate biofertilizers increased yield and yield components of maize under Boroujerd environmental condition.

In present study biomass increased with application of organic and chemical fertilizers. Many field studies showed a significant contribution of organic and biological fertilizers for the yield and biomass increase of the field crops, which vary in range from 8-30% of control value depending on crop and soil fertility. Biomass yield was increased under application of some biological fertilizers, which positively influenced the plant photosynthesis and dry matter accumulation more actively that agree with [22]. [23] founded that this fertilizers had significant effects on main yield components, seed yield, essential oil in Chamomile. They concluded that this fertilizer can be considered as a replacement for chemical fertilizers in Chamomile medicinal plant production. A significant difference was observed among manure and vermicompost regarding the average yield and yield components, but in many treats both application of these fertilizers had a better effect on above treats. In the present study 1000 grain weight increase may under the effect of combined application of organic and chemical fertilizers which induced the uptake ability of the roots to nutrients and positive increase in the yield parameters because of improving the root system as a source-sink relationship to the reproductive part (shoot). [24] suggested that photosynthetic material exchange activity is stimulated through symbiosis with microorganisms in plants with using of organic biofertilizers that increases the efficiency of photosynthetic phosphorus. They also told that may be photosynthetic capacity of plants treated with phosphorus-solving microorganisms increases due to increased supply of phosphorus nutrition. Combined application of organic and chemical fertilizers increased number of grain per spike, 1000 grain weight, biomass, straw and grain yield in interaction mean comparison. Therefore, this is a synergic effect of these fertilizers on yield components of common wheat too. This means that we can apply nutrient with together for achieved to maximum yield and its components as well. Given the importance of these elements by improving growth conditions and they can increase yield and its components somewhat. Meanwhile, plant type, variety and concentration of macro-nutrients in the root environment and stages of plant growth can be effective on how to use and how these elements impact on seed quality and quantity [15].

According to the present analysis, organic fertilizers such as manure and vermicompost had increased weight of 1000 seeds by enhancing the rate of photosynthesis and the biomass production [25] and increase in yield of barley was more than nitrogen fertilizer [Table 2]. The present result is in agreement

with the report of [26] on *F. vulgare*. 20 ton. ha⁻¹ application of organic fertilizers such as manure and vermicompost was more efficient rather than P fertilizer on grain yield and harvest index [Table 2]. However the results clearly demonstrate the effectiveness of organic fertilizers in increasing the biomass yield rather than chemical fertilizer [Table 2]. Vermicompost increases the growth rate because of the water and mineral uptake, such as; nitrogen and phosphorus [27], which leads to the biomass and grain yield improvement. In final we concluded that application of organic fertilizers increased yield and yield

components of wheat rather than chemical P fertilizer. It is clear from the present study that vermicompost and manure fertilizer successfully manipulate the growth of wheat, resulting in beneficial changes in yield and yield components of wheat.

CONCLUSION

Organic and chemical fertilizers had a positive effect on grain yield of wheat due to synergistic effect of them. Based on the results, combined application of manure and vermicompost with chemical fertilizer has a better effect on yield and yield components of common wheat rather than single application of them. Therefore, we can conclude that combined application of these fertilizers had more efficiency because of some positive interaction between some microorganisms with plant root in soil that result to synergistic effect and laid to increase in yield components and in final grain yield. In final the result of the present study showed that for achieve to highest wheat yield in Lorestan province of Iran we can apply 20 ton/ha manure and 75% P fertilizer with together.

CONFLICT OF INTEREST

There is no conflict of interest

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None

FINANCIAL DISCLOSURE

None

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ARTICLE

THE EFFECT OF EXERCISE AND DRUGS ON COGNITIVE FUNCTION AND BDNF PROTEIN

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ABSTRACT

Exercise is an effective factor that can promote the cognition function of brain like spatial and object recognition tasks. Thus, exercise can act in different part of the nervous system, such as stimulating neurons augmentation and increasing cell survival. The effect of exercise can change the dentate gyrus function that it causes cognitive improvement and has effect on object recognition, specific neurogenic and neurotrophic. In this manner, exercise influences cognition, BDNF and cell proliferation in particular. Results have revealed that nerve revival is enhanced by treadmill training which leads to enhancement of BDNF mRNA expression in motoneurons. Brain-derived neurotrophic factor (BDNF) which is a member of the neurotrophic factors has a critical role in the observance and survival of neurons, synaptic integrity and synaptic plasticity. Stress also modulates BDNF expression. Drug addiction is a disorder that has been defined as the need to search and take drugs. Absence of drugs causes the addicted drug-users lose self-control, and negative emotional (called withdrawal syndrome) appears. The purpose of this review is to show exercise effects on the nervous system and BDNF which is an important protein. It also reveals the influences of drugs on BDNF and the effects of exercise, addiction and BDNF on the process of learning and memory.

INTRODUCTION

Exercise is physical activity that is programmed, repetitive and purposefully in the sense that leads to amelioration or fortification of one or more parts of physical fitness is an objective [1]. Exercise increases the neurogenesis, learning and memory enhancement. There are many evidences that suggest BDNF as the variable which improves learning and memory through exercise [2]. Stress elevates the level of glucocorticoids that causes them to have an effect on BDNF expression. For instance, corticosterone (CORT) treatment of rodents decreases the expression of BDNF in the hippocampus [3, 4]. BDNF level in limbic structure and serum is enhanced by administration of anti depressions [5, 6]. Drugs have a moderating role in BDNF level. while drugs can increase the measure of BDNF in some parts of the brain, they can decrease the level of this neurotrophine in other parts [7, 8].

Exercise increases the level of endorphins and can have euphoria effects. It also observed that exercise can reduce the tendency to addiction [9]. It has been revealed that the increase in the level of exercise in rats is accompanied by decrease in self-administration [10]. Similarly, the teens who exercise have less tendency to smoke and use drugs [11]. In this review, we consider the corresponding effects of exercise and addiction on cognition function of brain. Also we talk about BDNF (an important protein), has a critical role in cognitive acts like learning and memory. In this manner, we focus on cellular changes that cause increase in the level of BDNF via exercise, addiction pathway and its effect on learning and memory. Physical activity in our daily life can improve health. A positive relation between exercise and health has been reported by the department of health and human services (HHS). Exercise is divided in two general types: 1) aerobic exercise, 2) anaerobic exercise, and each of them has an effect on different types of muscles. Evidences have revealed that the effects of exercise are depended on intensity, species, sex and the method of learning.

Exercise can change nervous system operation

Exercise can increase skeletal muscle activity; however, the physiological effects of exercise are only on muscles but also they influence all body systems as well as nerves system. Exercise is also necessary for activities of daily living, and one of the most serious consequences of disease is the limitation of exercise capacity. The brain receives almost the same absolute blood flow at rest and at exercise [12]. But How Exercise influences brain? It is clear that physical activity can increase brain health and plasticity of neurons [13, 14]. Exercise can improve cognition functions [15, 16], function of hippocampus such as learning and memory [17, 18], and increase natural operation of hippocampal in rodents like long term potentiation, neurogenesis, the level of growth factor and dendritic spine [18, 19]. Also exercise can increase the number of cholinergic neurons [20] and the stabilization of information as long-term memory [15, 21-23]. Wheel running as a voluntary exercise has shown that it can increase the firing rate of hippocampal cells and synaptic plasticity [24].

KEY WORDS

Exercise, BDNF, Addiction, Cognition.

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Exercise promotes operation of spatial learning and memory tasks [16, 17, 25], and thus it can modify LTP in brain [26]. In this regard, the voluntary exercise increases LTP in medial PP to dentate gyrus synapses [27]. Exercise instigates cell proliferation, enhances neuronal survival, and has a strong effect on neurogenesis [28]. The effects of exercise can fundamentally improve neuronal survival [29, 30]. So exercise has potent effect on cognition, BDNF, and cell proliferation [31]. Running increases neurogenesis fourfold and enhances operation in cognition tasks like Morris water maze [32, 33]. Also Physical activity is accompanied with increase in glutamate activity by enhance in expression of NMDA receptors [26, 34]. How can exercise improve neurogenesis and cell survive? During exercise, Glutamate, as an excitatory neurotransmitter, is released from presynaptic terminal axon and can activate the receptors in postsynaptic terminals. After that, Ca^{++} is released and the signaling begins. Firstly, transcription of gene that is related to synaptic plasticity and neuronal survival like neurotrophin factors, chaperon proteins and antioxidant enzyme are increased. Then, they reform the energy metabolism of mitochondria and correction of free radical production. Finally, Ca^{++} is released from endoplasmic reticulum. Exercise can increase the functional ability of the brain via actions in synapses and neural stem cells [33]. Also, evidence shows that exercise can increase the expression of BDNF mRNA in hippocampus [21, 35-39]

BDNF plays an effective role in mediating the beneficial effects of exercise on cognitive functions like learning and memory as well as long term potentiation [17, 40]. Exercise, as an external factor can change the operation of nervous system. This alteration is accompanied by an increase in cognition function and behavior. It is repeatedly distinguished that exercise has influence on brain especially hippocampus and can increase the amount of neurogenesis, neurotrophins and LTP. Also it has been shown that increase in glutamate via exercise can activate the cellular cascade that causes improvement in synaptic plasticity. Exercise can enhance level of BDNF that has critical role in learning and memory.

Influence of exercise on learning and memory

Physical activity has an effect on brain function. It is distinguished that exercise can improve learning and memory through increase in neuronal plasticity, and change the level of large numbers of genes that are important in memory. These processes are associated with changes in neuronal activity, synaptic structure and the concentration of neurotransmitters [41]. Physical activity has revealed increase in the cognition in both object recognition [42, 43] and spatial tasks [44], improvement of neurogenesis, and neurotrophic changes in the dentate gyrus of rats [31, 45-47]. Aerobic exercise can improve spatial memory and can invert age-related decline in hippocampal volumes [48]. Physical activity also results in physiological changes and increases cell proliferation in hippocampal [17, 18, 24]. Hippocampal synaptic function is important in learning and memory [49]. Hippocampus is the brain region where cell proliferation continues throughout life in the adult mammals like human [50, 51]. Exercise improves neurogenesis and increases dendritic spine density in the Hippocampus [52, 53].

BDNF is thought to mediate changes in hippocampal synaptic plasticity and so it seems that voluntary exercise induced BDNF may affect learning and memory by mediating changes in neuronal plasticity [37, 54]. Exercise through changes in brain function can affect cognition and behavioral function. Just as defined in researches, physical activity improves neuronal plasticity and synaptic function. Hippocampus (an important area for learning and memory) function is influenced by exercise. Possibly positive effect of exercise on hippocampus is caused by the release of brain derived neurotrophic factor (BDNF).

How can exercise increase the level of BDNF?

Physical activity can increase brain function like learning and memory, and has an effect on hippocampus via changes in BDNF concentration. BDNF is one member of Neurotrophins (NTs) - are an important signaling molecules which play a critical role in the growth and development of central and peripheral nervous systems [55, 56]. This molecules contains nerve growth factor (NGF1) [57] and brain derived neurotrophic factor (BDNF) which are synthesized in central and peripheral nervous system, vascular endothelium, immune cells [58-60] as well as neurotrophins 3 (NT-3) and neurotrophins 4 and 5 (NT-4/5) [61]. They promote the growth, proliferation, migration and survival of neurons; regulate neurotransmitters like synthesis and secretion and modulate the development of synaptic plasticity. Also they modulate immune cells [62-64].

BDNF is the most abundant neurotrophin in the central nervous system and has major role in neuroplasticity [65-67] in pathology of psychiatric diseases [68]. The concentration in brain BDNF is associated with change in serum BDNF [69]. Pre-pro-BDNF is a precursor of BDNF. Pro-BDNF then is division into mature BDNF [70]. Mature BDNF is connected with two trans-membrane receptors that are located in dendrites. One of them is tropomyosin-related kinase B (TrkB) receptor which dose the majority of the known functions of BDNF and has high-affinity with this neurotrophin. The other receptor is pan neurotrophin p75^{NTR} which interacts with the precursor pro-BDNF [71]. After their contiguity, the intracellular signaling cascades are activated by autophosphorylated tyrosine kinase and N-methyl-D-aspartate (NMDA) receptor currents [67]. When BDNF and TrkB rebounded to each other, three intracellular pathway will be activated: phospholipase C (PLC), phosphatidylinositol 3-kinase (PI3K), and mitogen-activated protein kinase [MAPK, or extracellular signal related kinase (ERK)] [72-74]. Exercise can increase expression of BDNF. But how does it happen?

One possible way that voluntary exercise can increase the level of BDNF is via increasing histamine acetylation and diminish DNA methylation in promoter IV and means increase in BDNF expression and this enhance of BDNF is clear in different region of brain especially hippocampus [9, 21]. Another way that exercise can increase BDNF is via PGC1 α . PGC1 α is produced through exercise in skeletal muscles and make some major beneficial metabolic of physical activity [75]. PGC1 α has an important role in brain that accompanied by neurodegeneration [76, 77]. Voluntary exercise increases the concentration of PGC1 α in different area of brain. It is distinguished that , the PGC1 α - depend myokine, FNDC5, is release from muscles through exercise and cause positive operation of exercise [78, 79] FNDC5 is a glycosylated type I membrane protein and is delivered into the circulation after proteolytic cleavage. Irisin is the secreted form of FNDC5 that contain 112 amino acids [79, 80] and there is positive relation between physical activity and Irisin [81, 82]. Also FNDC5 is expressed in the brain [83-85]. The level of FNDC5 in brain region like hippocampus is increased via endurance exercise. It is observed that PGC-1 α and FNDC5 compose BDNF expression in the brain. The other way that exercise can change the level of BDNF is through norepinephrine levels[86], and IGF-1 measure.they can crossing the blood-brain barrier, have also been discussed as exerciserelated inducers of BDNF [87].

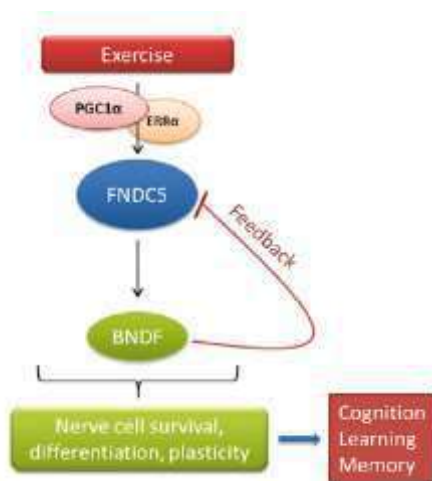


Fig. 1: The hippocampal PGC-1 α /FNDC5/ BDNF pathway in exercise [88]

Exercise and brain neurotransmitter

Exercise can increase the concentration of dopamine and via this way can activate the reward pathway[89-91]. Also exercise can decrease the level of glutamate in striatum [92] forced exercise like running on treadmill can improve the calcium in serum. Calcium can penetrate the blood brain barrier and activate synthesis of dopamine [93]. Physical activitylike force running enhance the concentration of tyrosine hydroxylase that is the rate limiting enzyme in dopamine synthesis [89, 93-96]. Voluntary exercise in rodents is also accompanied bydopaminergic neurons activation in the ventral tegmental area (VTA) [97]. Also distinguished that, voluntary exercise (wheel running) in rats can increase the level of dopamine in nucleus accumbens (NA) [98] and can improve the levels of Fos-B and tyrosine hydroxylase mRNA in the NAc, and decreased gathering of dopamine D2-receptors [99-101]. Chronic high levels of exercise may lead to an upregulation of dopamine D1 receptor-signaling [102-104]. It is probable the reason for euphoria after exercise is due dopamine release. Also exercise can decrease the concentration of glutamate.

Addiction is one of the major problems of societies, and can cause decrease the level of health and active people in countries. Exercise via increasing the rate of self-reliance in young person and also via activating the pathways that increase the euphoria can decrease the tendency of youth to use abuse drug.

Could exercise decrease the tendency for using drug?

Exercise like abuse drugs can enhance the amount of euphoria [105, 106]. It was distinguished that physical activity decrease drug self-administration [107]. Also voluntary exercise (wheel running) diminished the oral consumption of a liquid amphetamine solution [108]. In other studies have shown running wheel reduce intravenous self-administration of cocaine and decreased responding maintained by methamphetamine [109], it was interesting that, even the physical activity was not exist, the diminish of using drug was continuously happen [110].

Norepinephrine release in the frontal cortexwas decreased during exercise [111]. The norepinephrine is important for both cocaine-primed rein- statement and stress-induced in rodents [112, 113]. The catecholamines, can decrease the amount of glutamate, but promote the reply of this amino acid to drug administration [114]. Changing in glutamate signaling pathway has an important role in mediating drug searching and relapse after chronic drug exposure [115]. Exercise increases the concentration of exogenous opioid peptides in plasma. Opioid plasma can bind with opioid receptors [116-119]. Exercise

through increasing the amount of endogenous opioids can decrease the sensitivity to exogenous opioids [120, 121]. Also physical activity can decrease self-administration of morphine and heroin via changing the central opioid receptor populations. Exercise has effect on cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling. This signaling mediated cocaine administration upregulates D1 and reinforcing effects of dopamine agonists are certainly correlated with their ability to stimulate cAMP production. When the PKA is active directly, self-administration of cocaine was increased and inhibition of PKA decreases cocaine self-administration and subsequent drug-seeking behavior during reinstatement [122, 123]. The mice which have regular treadmill in daily planned have lower levels of transcripts encoding adenylate cyclase subtypes and activating polypeptides in the striatum relative to controls. Exercise modulate dopamine cAMP-regulated neuronal phosphoprotein [124], A target of PKA that is essential for drug reinforcement [125]. Exercise palliate the positive reinforcing effects of different classes of abuse drugs [9] and may function as an alternative non-drug reinforce that competes with the drug and decrease vulnerability. Miller et al, showed that mice that received both exercise and methamphetamine are less interested in self-administration compare the sedentary rats [126]. Also the mice with running wheel have been reduced the use of alcohol consumption under 2-bottle free access conditions [127]. It has shown wheel running reduced rates of utilization of cocaine self-administration where under non-concurrent conditions [128]. In human study, teens who have regular exercise in the daily schedule are less likely to use cigarettes and illicit drugs than less active groups, and regular exercise from childhood to adulthood reduces the tendency towards smoking and use of illicit drugs like marijuana [131-129, 11, 10]. Exercise be an appropriate substitute for drugs. Because exercise, as well as drug, increases the activity of signaling pathways of dopamine. This increase will reduce damage caused by drug use [9]. Thoren et al, have shown that physical activity and exercise could be remedy for patients with addiction disorder because rhythmic exercise could activate the central opioid systems [132]. Generally, exercise with different way, can act as an efficacious way for treatment of using drugs. Firstly, exercise can decrease the tendency of using drugs, and it was observation in many researches that athlete teenagers have less interest in using drugs. Secondary, physical activity can active the signaling pathways to cause euphoria.

Addiction and neurocirculation of drug abuse

Generally, there are three explanations for drug abuse: (1) Forced to search and consumption of drugs (2) Loss of control by limiting drugs, and (3) After drug discontinuation and lack of access to drugs is intense emotional states, like anxiety, dysphoria and irritability [133]. Addiction is now understood as a pathological of natural rewards. The reward center is the mesocorticolimbic system, including the ventral tegmental area, nucleus accumbens, amygdala, and hippocampus [134].

The euphoria through using drug is due to a dopamine surge in the mesolimbic pathways. For example using or injecting Opioids produce a dramatic euphoric. When addictive drugs are injected, they can decrease the thresholds of reward stimulation in brain [135]. All drugs of abuse activate the dopamine system in brain, like mesolimbic, ventral tegmental and ventral striatum area neurons on nucleus accumbens [136-139]. Also the dopamine projections in Ventral tegmental area such as the prefrontal cortex and amygdala are other forebrain areas play a critical role in shaping drug-taking behaviors [140]. And also the central nucleus of the amygdala (CeA) has akey function in the acute reinforcing actions of drugs of abuse [141].

Table 1: Drugs and their mechanism

Drugs	Effects in brain	Mechanism
Cocaine and amphetamines	Active the dopaminergic pathways in NA and amygdala and enhance the level of dopamine [142]	Have direct actions on dopamine terminals [142]
Opioids	1) In VTA (ventral tegmentom area) and amygdala with direct or indirect actions through interneurons can active the opioids receptors 2) They can increase the amount of release DA with act on VTA and NA 3) Activate elements independent of the dopamine system [142] 3) Reduce the inhibitory GABA release on dopamine [143] 4) Via increase the level of norepinephrine, play a role in rewarding effects [144]	1) Increase the release of dopamine 2) Has a effects on intern-eurons [142]

Alcohol	1) Has a direct or indirect effect on GABA _A (g-aminobutyric acid) in VTA, NA and amygdala 2) Enhance the release of dopamine and opioids peptides in VTA, NA and central nucleus of amygdala [142]	1) Activated the GABA receptors 2) Increase the release of dopamine
Nicotine	1) Activates nicotinic acetylcholine receptors in the VTA, nucleus accumbens, and amygdala, either directly or indirectly, through actions on interneurons [142] 2) Excitatory glutamate transmission in the VTA [145]	Increase the activity of nicotinic acetylcholine receptors [142]
Cannabinoids	1) Active the CB1 receptor in VTA, NA and amygdala 2) Increase the release of dopamine in VTA and NA [142]	1) Activated the CB1 receptors 2) Increase the release Dopamin with unknown mechanism [142]

Evidences have shown that two major neurotransmitter that have a critical role in reward pathway are dopamine and peptides of opioid. Also the corticotropin releasing factor, norepinephrine, and dynorphin are the major neurotransmitters in amygdala that is associated with negative reinforcement.

Addiction and learning and memory

In learning pathway, the dopaminergic cells in ventral tegmental area (VTA) release dopamine into prefrontal cortex (PFC), amygdala and nucleus accumbens. The addictive drugs use these cells to affect the learning pathway [146-148]. It has been observed that, the addicted animals learn better due to glutamatergic projections from PFC to the Nucleus accumbens emerges [149-151]. Addictive drugs operate the release of dopamine in the nucleus accumbens (NAC) [152], but different drugs can increase the level of dopamine in different ways. Addictive drugs can increase the strength of excitatory synapses on dopaminergic neurons in the VTA in rodents [153]. Also addictive drugs can increase the level of dopamine release with different mechanism [137, 147, 154]. And as mentioned earlier, dopamine plays an important role in learning process [146]. Research has shown that addictive drugs can release dopamine every time the drug is used but in normal condition there is a tolerance development of released dopamine in biological. But, in chronic users, this tolerance occurs and increases the dose. Dopamine is an important influence on the synthesis of proteins that cause physiological changes. Further, it was found that dopamine plays a role in gene expression of BDNF. The evidences have shown that normal expression of the dopamine D3 receptor in nucleus accumbens can increase BDNF from dopamine neurons in both during development and in adulthood [155].

On the other hand, when the periods of abstinence of drugs is increased the level of BDNF changes [156-158], the stimulating BDNF receptors in the amygdala, VTA or NAC promotes [159-161], and microinjection of BDNF into the PFC can reduce of drug demand [162]. On the other hand, it was found in additional research that BDNF and TrkB signaling are enhanced in the nucleus accumbens after short and long term of cocaine administration. This increase is not observed in the core region of NAC, VTA and hippocampus [163]. It is interesting that the level of BDNF in the VTA, NA and amygdala, progressively are increased during drug consumption [156]. Also the evidences have shown that chronic exposure to opiates addictive drugs can decrease neurogenesis and alter synaptic transmission in the hippocampus and decrease long-term potentiation (LTP) in hippocampus- a form of synaptic plasticity that be a mechanism for learning and memory [164-167]. On another hand, research showed chronic exposure to morphine increases the level of BDNF protein in the hippocampus and serum in the sedentary morphine-dependent rodents and human [168-171]. Chu, Zuo et al. Found that morphine reduces the amount of BDNF in the VTA, and BDNF levels in ventral tegmental area will increase the morphine withdrawal [172] it was observed that, after long-term withdrawal from self-administered of heroin the expression of BDNF mRNA is enhanced [173].

CONCLUSION

According to the recent studies, we can conclude that exercise can change the brain BDNF level by cellular and molecular pathways in different areas of the brain. BDNF has a significant role in learning and memory. Exercise also can elevate learning and memory activities. Addiction that is defined as a pathological behavior or repeated use of a substance, forces the addict to use drug or do something by affecting on reward pathway. Evidences suggested that the brain neurotransmitters are released by drugs which effect on learning and memory pathways. Recent studies investigate the effect of drugs on BDNF,

and this review has shown that drugs have different impression on the level of BDNF in various region of brain. Exercise can be considered as a factor for the prevention of addiction. It is mentioned in many articles that when exercise is accessible, the level of self-administration is less than non-exercise group. As well as, Adults who do exercise regularly have a less tendency to use cigarette and illicit drugs. Due to the growing trend of drug abuse in today's society, it is suggested that the exercise should be institutionalized in young people and teenagers.

CONFLICT OF INTEREST

There is no conflict of interest

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None

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ARTICLE

ESTIMATION OF GENETIC PARAMETERS FOR MILK YIELD, SOMATIC CELL SCORE, AND FERTILITY TRAITS IN IRANIAN HOLSTEIN DAIRY CATTLE

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ABSTRACT

This study was conducted to estimate genetic parameters for the lactation curve parameters, milk yield, somatic cell score (SCS), age at first calving (AFC), calving interval (CI) and lactation length (LL) in Iranian Holstein cows. The data originated from the national Animal Breeding Center of Iran, belonged to the first lactation dairy cows from 2001 to 20104. The genetic parameters were estimated using REML method by applying random regression model (RRM). To compare the models, different criterion LogL, AIC and BIC values were used for considered traits. Based on obtained results, RRM with legend repolynomial (3,3) were chosen as better model for milk yield and SCS traits. Based on obtained results, for milk yield and SCS traits the additive genetic variance was highest in the beginning and end lactation and permanent environmental variance was highest in beginning of lactation than other lactation period. Heritabilities estimate for milk yield and SCS traits, were found to be lowest during early lactation (0.48, and 0.04 respectively). Heritabilities estimated 0.02 ± 0.01 , 0.04 ± 0.01 and 0.10 ± 0.01 for AFC, CI and LL, respectively. Additive genetic correlation between adjacent test days was more than between distant test days. The was estimated negative genetic correlations between fertility traits and positive phenotypic correlations between them. The genetic trends of milk yield and SCS was showed an increasing phase during previous years.

INTRODUCTION

Milk production is the most economically important trait in the dairy cattle breeding industry. However, in addition to milk yield, reproductive and health traits are among the major traits that should be improved genetically in dairy cattle. In recent years intensive selection for milk yield has depressed reproductive performance of cows [1]. One of the important breeding program processes is the estimation of genetic parameters using appropriate models. The TD Model has been described using various models, such as repeatability, fixed regression, multiple-trait and random regression model (RRM). Among the models that consider TD production, RRM has been widely observed to increase the accuracy of breeding value predictions [2]. Among these advantages are more precise adjustment for temporary environmental effects on the TD, avoidance of the use of extended records for culled cows and for records in progress, and the possibility of genetic evaluation for any part of lactation curve. Therefore, using TD measurements in an RRM could increase the accuracy of genetic evaluations. Schaeffer and Dekkers (1994) introduced the concept of the RRM for the analysis of TD records in dairy cattle as a means of accounting for the covariance structure of repeated records over time or age [2].

Deficient reproductive performance exhibited as longer calving intervals and increased involuntary culling, may result in less milk and fewer calves per cow per year, lower voluntary culling and consequently increased replacement costs and finally, reduced returns [3]. Before the 1990 most attention of dairy cattle breeding programs were focused on milk production. Since the negative genetic relationship exist between milk production and fertility this caused decline in fertility performance of Holstein dairy cow [4]. Therefore, in current decades in many breeding programs fertility traits have been included. But the heritabilities of fertility traits are low, ranging from 0.01 to 0.1, which leads to slow improvement in fertility performance [5,6]. Genetic analysis of fertility traits for various breed is studied in different countries, such as: Zebu cow in Mexico [7]; South African Holstein cows [8]; Spanish dairy cattle [9]. Although, some investigations have been carried out in Iranian Holstein dairy cows in regard to the estimation of genetic parameters of milk yield traits [10,11,12,13] but compare different order Legendre polynomial (LP) of fertility traits still needs further work.

Thus, the objectives of this study were 1) to compare different order LP of the RRM to determine the best fitting model to TD milk yield and SCS, and 2) to estimate genetic parameters milk yield, SCS, age at first calving (AFC), calving interval (CI) and lactation length (LL) in Iranian Holstein cows.

MATERIALS AND METHODS

The test day (TD) milk yield records obtained from national Animal Breeding Center of Iran, belonged to the first lactation dairy cow from 2001 to 2014. Edited data included the following: The TD data were excluded before 5th day and after the 305th day of lactation. In addition, irregular data for milk yield (<2 and >70 kg) were excluded. Cows had also, only cows with more than 5 TD records, and herds with more than 5 cows per herd in year of calving and cows with at least one known parent and age at first calving between 18 to 45 months were kept. The data of cows with the first TD at least 60 days after parturition and TD intervals less than 15 days were discarded. Finally edited data included 795724 and 278230 for milk yield and SCS traits, respectively. The fertility records were: AFC, CI and LL. The RRM fitted for the genetic analysis was used as following:

KEY WORDS

Genetic evaluation, Dairy cow, Random regression model, Fertility traits

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$$y_{ijltmno} = F_{ijl} + \sum b_{ijnp} z_{tp} + \sum a_{ijmp} z_{tp} + \sum p_{ijmp} z_{tp} + e_{ijltmno}$$

Where $y_{ijltmno}$ is the o^{th} TD record (Milk yield and SCS) of m^{th} cow in t^{th} DIM, in l^{th} herd-test-date (HTD) effect; F_{ijl} is fixed effects in the model (including herd-test date, interaction between year-season of calving and DIM and dam age (linear and quadratic). Z_{tp} is p^{th} LP for t^{th} DIM. b_{ijnp} is the n^{th} fixed regression coefficients p^{th} class of cows calving age-season. a_{ijmp} and p_{ijmp} are regression coefficients for additive genetic and permanent environment effects respectively; $e_{ijltmno}$ random residual effect associated with $y_{ijltmno}$. The traits descriptive statistics are summarized in Table 1 and 2.

Table 1: Descriptive statistics of data sets for milk yield (kg) and SCS

	Milk (kg)	SCS
Number of TD records	795724	278230
Number of total animals	277400	278230
Number of animals with record	65100	65320
Number of dam with progeny	2100	2210
Number of sire with progeny	8840	8922
Number of HTD	16973	16989

Table 2: Descriptive statistics of data sets

DIM	5	35	65	95	125	155	185	215	245	275	305
Mean of Milk yield (kg)	29.24	34.80	33.76	33.41	34.08	33.5	32.75	31.96	31.00	29.89	28.91
Maximum Milk yield	59.43	70.35	65.21	60.27	56.86	56.65	59.51	58.70	57.54	56.67	54.76
Minimum Milk yield	9.13	13.45	11.51	9.17	8.09	8.41	9.17	9.20	8.81	7.75	7.42
Mean of SCS	4.33	4.31	4.03	4.03	4.06	4.11	4.14	4.19	4.23	4.27	4.30
Maximum SCS	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30
Minimum SCS	9.9	9.9	9.8	9.9	9.8	9.8	9.8	9.7	9.6	9.8	9.7

Mean of AFC, CI and LL was 840.2 days (28 month), 494.70 and 335.45 respectively. To calculate the standard DIM (d^*), the following equation was used:

$$d_i^* = -1 + 2 \left(\frac{d_i - d_{min}}{d_{max} - d_{min}} \right)$$

Where d_{min} and d_{max} are minimum and maximum, and d_i , t^{th} DIM. For the t^{th} standardized DIM, the n^{th} polynomial is given as [14].

$$P_n(d_i^*) = \frac{1}{\sqrt{\pi}} \sum_{i=0}^n (-1)^i \binom{n}{i} \left(\frac{2i-2n+1}{2} \right) (d_i^*)^{2i-n}$$

Where d_i^* , is the i^{th} DIM; and i , is order LP function; m , index number needed to determine the k^{th} polynomial.

The matrices notation of the model can be written as: $y = Xb + Q\alpha + Zpe + e$; where y is the a vector of observations, b is the a vector of fixed effects, a and pe were vectors of additive genetic and permanent environment effects respectively, e is the vector of residual effects and X , Q and Z are the incidence matrices. The (co)variance structure for random parts of the model was defined as:

$$\text{Var} \begin{bmatrix} a \\ pe \\ e \end{bmatrix} = \begin{bmatrix} G \otimes A & 0 & 0 \\ 0 & I \sigma_p^2 & 0 \\ 0 & 0 & R \end{bmatrix}$$

G is the genetic covariance matrix of the random regression coefficients, \otimes is the kronecker product function, A is the additive genetic relationship matrix coefficients among animals, σ_p^2 is the variance of the permanent environment effects, I is the identity matrix, and R is the diagonal matrices of residual variance. For estimated heritability for i^{th} DIM was calculated as:

$$h_i^2 = \frac{\sigma_{a(i)}^2}{\sigma_{a(i)}^2 + \sigma_{pe(i)}^2 + \sigma_e^2}$$

Where $\sigma_{a(i)}^2 = qGq'$, $\sigma_{pe(i)}^2 = qPq'$, where q is the vector of the associated polynomial function; G and P , are the (co)variance matrices for additive genetic and permanent environmental RR coefficients, respectively; and $\sigma_{a(i)}^2$, $\sigma_{pe(i)}^2$ and σ_e^2 are additive genetic, permanent environmental and residual variances for i^{th} days in milk, respectively.

Additive genetic correlation for 305-days production between LP were calculated as:

$$r_{g(i,j)} = \frac{\text{Cov}_{g(i,j)}}{\sqrt{\text{Var}_{g(i,i)} \times \text{Var}_{g(j,j)}}$$

Where $\text{Cov}_{g(i,j)}$, is genetic covariance between i and j day, $\text{Var}_{g(i,i)}$ and $\text{Var}_{g(j,j)}$ are additive genetic variance i and j day, respectively. Goodness of fit for the models was examined using likelihood based criteria as LogL, AIC (Akaike's information criterion.) and BIC (Bayesian information criterion). AIC and BIC criteria are:

$$\begin{aligned} AIC &= -2 \log L + 2 \times k \\ BIC &= -2 \log L + k \times \log(N - r(x)) \end{aligned}$$

Where, k is the number of parameters estimated, N is the sample size and $r(x)$ is the rank of the coefficient matrices for fixed effects in the model. The model giving the lowest $-LogL$, AIC and BIC values is chosen as

the better approximating model. Residual variance were considered homogeneous along the lactations, since the use of homogeneous residual variance in the literature is cited as a good assumption for use in data analysis of dairy cattle [15]. Estimation of genetic parameters with REML methodology was done by WOMBAT program [16]. The significance of fixed effects was examined using GLM procedure of SAS software [17].

RESULTS

Fig. 1 shows changes of the milk yield and SCS traits along lactation month and indicates that the high amount of milk yield in 2 and 3 month (Peak of lactation), however, low amount of SCS During this time.

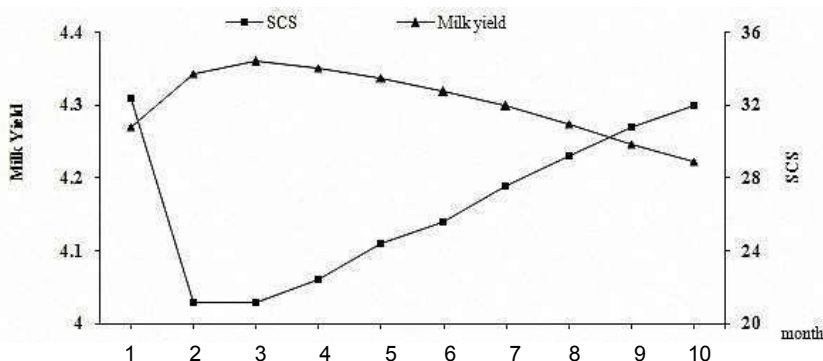


Fig. 1: Average of milk yield, and SCS along months

The values of comparison criteria (logL, AIC, BIC) for the different LP of milk yield, SCS traits were given in Tables 3, and 4 respectively. Selection of a bestmodel depends partly on the criteria that were used. For milk yield and SCS the LP (3,3) model had the lowestLogL, AIC and BIC values. Therefore, among models, for milk yield and SCS traits the LP (3,3) model was selected as the best model (LP (3,3) is 3 and 3 order for additive genetic and permanent environmental effects respectively).

Table 3: Criteria used for comparison of the models and their levels for the milk yield (best model in bold)

Model	K	logL	AIC	BIC
LP (2,2)	7	-915.327	-922.327	-940.059
LP (3,3)	13	-906.304	-919.304	-952.225
LP (4,4)	21	-1007.456	-1028.456	-1081.618
LP (5,5)	31	-960.737	-991.737	-1070.188
LP (6,6)	43	-963.850	-1006.850	-1115.631

LP (i,j) is i and j order for additive genetic and permanent environmental effects respectively;

BIC: Bayesian information criterion and AIC: Akaike’s information criterion

Table 4: Criteria used for comparison of the models and their levels for the SCS (best model in bold)

Model	K	logL	AIC	BIC
LP (2,2)	7	-2998.460	-3005.460	-3023.724
LP (3,3)	13	-2985.576	-2998.576	-3032.485
LP (4,4)	21	-3068.138	-3089.138	-3143.898
LP (5,5)	31	-3074.508	-3105.508	-3186.321
LP (6,6)	43	-3088.152	-3131.152	-3243.216

The additive genetic (AG), permanent environmental (PE) and phenotypic variances of milk yield and SCS traits as a function of DIM are shown in Fig 2 and 3 respectively. Estimated genetic parameters based on the best model. AG, PE and phenotypic variances of milk yield was higher at the beginning and end of lactation. AG variance of SCS was higher at the beginning of lactation (0.8) and then decreased until about 75 days of lactation also AG highest (0.95) in 160 DIM. AG and phenotypic variances was higher at the beginning of lactation (25.7 and 26.7 respectively) and then decreased until about 95 days of lactation and this trend continued up to 245 DIM and then increased at the end of lactation.

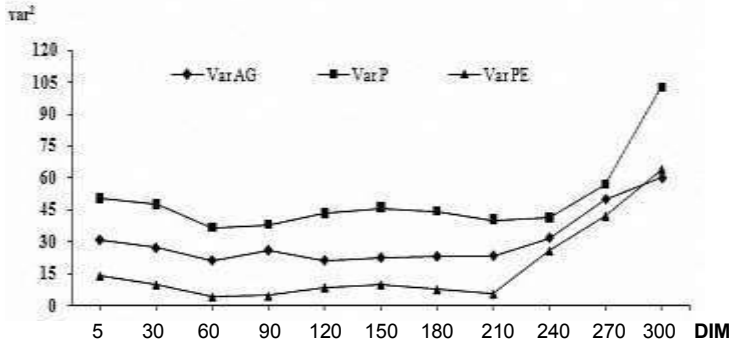


Fig. 2: Trajectory of additive genetic (AG), permanent environmental (PE) and phenotypic (P) variances of milk yield as a function of DIM

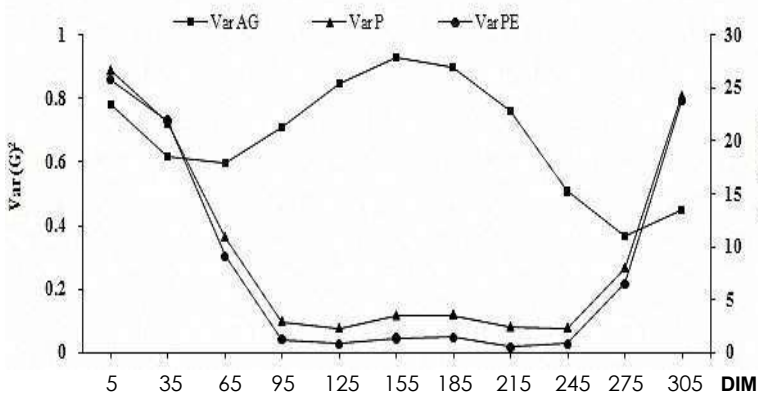


Fig. 3: Trajectory of additive genetic (AG), permanent environmental (PE) and phenotypic (P) variances of SCS as a function of DIM

Heritabilities of milk yield and SCS traits as a function of DIM are shown in Fig 4 and 5, respectively. The estimate of heritabilities for different DIM obtained ranged from 0.45 ± 0.04 to 0.60 ± 0.05 and 0.04 ± 0.01 to 0.31 ± 0.03 , for milk yield and SCS traits, respectively. The changes in heritability estimates for TD milk yield was high in the early lactation and then sudden drop in 90th DIM and then trend increased up to 210th DIM and the end of lactation was lower observed. The heritability of SCS in the early lactation was obtained lower than other periods. The estimates of repeatability obtained in the current study were ranged from 0.62 to 0.86 and 0.54 to 0.96 by milk yield and SCS traits, respectively [Fig. 5].

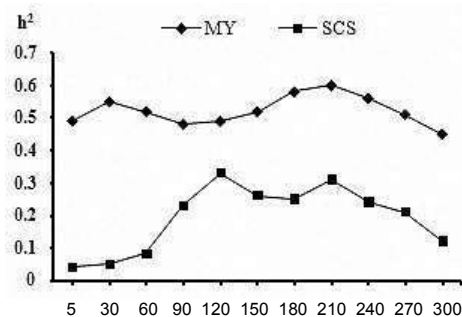


Fig. 4: Estimated heritability (h^2) of milk yield (MY) and SCS as a function of DIM

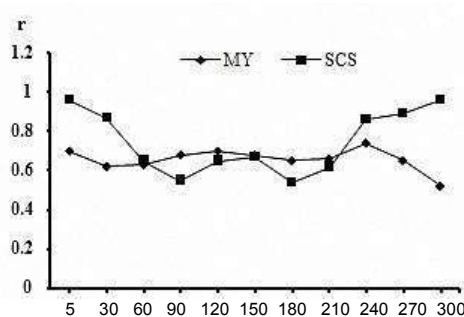


Fig. 5: Estimated repeatability (r) of milk yield (MY) and SCS as a function of DIM

The estimated heritabilities for AFC, CI and LL were 0.02, 0.04 and 0.10, respectively [Table 5]. The genetic correlation coefficients ranged from -0.31 (the lowest) between AFC and LL to -0.15 (the highest) between AFC and CI. The phenotypic correlations ranged from 0.62 (the lowest) between LL and AFC to 0.93 (the highest) between CI and AFC [Table 4].

Table 5: Heritability (diagonal), Genetic (above diagonal) and phenotypic (below diagonal) correlations between age at first calving (AFC), interval between first and second calving (CI) and lactation length (LL) traits

Traits	AFC (day)	CI (day)	LL (day)
AFC (day)	0.02±0.01	-0.15	-0.31
CI (day)	0.93	0.04±0.01	-0.28
LL, (day)	0.62	0.68	0.10±0.01

Estimates of additive genetic correlation between TD milk yield, and TD SCS at different stages of lactation estimated in RRM are shown in Fig. 6. As it is shown, the (co)variance structure of TD data during trajectory was considering RRM, therefore, with this method separate (co)variance components for different days of lactation are estimating that by using them genetic correlation between different days can be calculated. The values of genetic correlations ranged between TD from 0.40 to 0.99 and 0.21 to 0.99 for milk yield and SCS traits respectively.

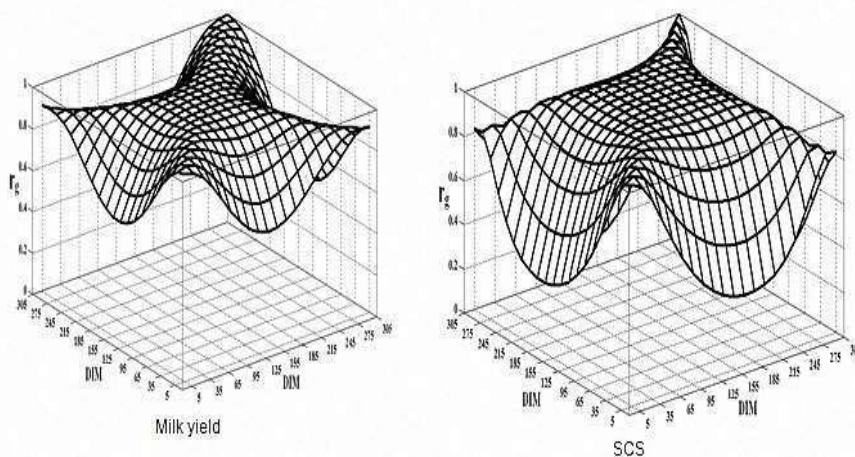


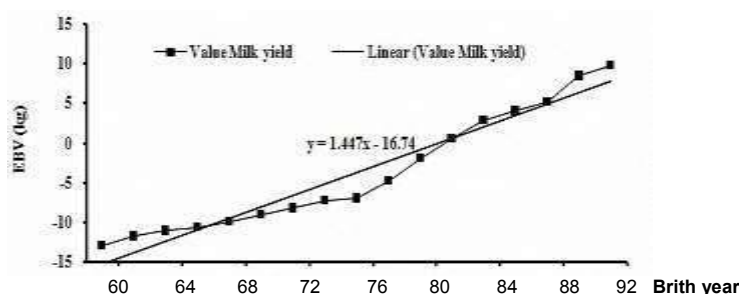
Fig. 6: Additive Genetic correlation (rg) milk yield and SCS traits as a function of DIM

The phenotypic correlations of milk yield and SCS traits of between different TD were given in Tables 6. The estimates of phenotypic correlations obtained in this study were ranged from 0.09 to 0.95 and 0.02 to 0.24 by milk yield and SCS traits, respectively.

Table 6: Phenotypic correlations of milk yield (diagonal) and SCS (below diagonal)

305	275	245	215	185	155	125	95	65	35	5	TD
0.45	0.44	0.47	0.51	0.63	0.68	0.71	0.80	0.84	0.87	-	5
0.45	0.44	0.47	0.50	0.63	0.69	0.71	0.81	0.84	-	0.24	35
0.31	0.28	0.4	0.42	0.60	0.64	0.68	0.91	-	0.20	0.22	65
0.09	0.21	0.32	0.51	0.55	0.78	0.84	-	0.21	0.15	0.18	95
0.15	0.30	0.64	0.76	0.87	0.92	-	0.10	0.16	0.10	0.12	125
0.16	0.20	0.61	0.71	0.88	-	0.11	0.10	0.15	0.11	0.14	155
0.22	0.32	0.71	0.77	-	0.14	0.09	0.08	0.11	0.09	0.11	185
0.51	0.71	0.95	-	0.15	0.07	0.06	0.07	0.08	0.05	0.08	215
0.79	0.90	-	0.18	0.10	0.07	0.03	0.05	0.09	0.04	0.07	245
0.93	-	0.14	0.15	0.10	0.04	0.02	0.03	0.04	0.02	0.04	275
-	0.12	0.09	0.08	0.06	0.02	0.02	0.04	0.03	0.03	0.05	305

Regression coefficients for estimated animal breeding value on animal birth year as the indicator of genetic trend were estimated for milk yield and SCS traits [Fig. 7]. The results showed positive genetic trend for both traits during previous years.



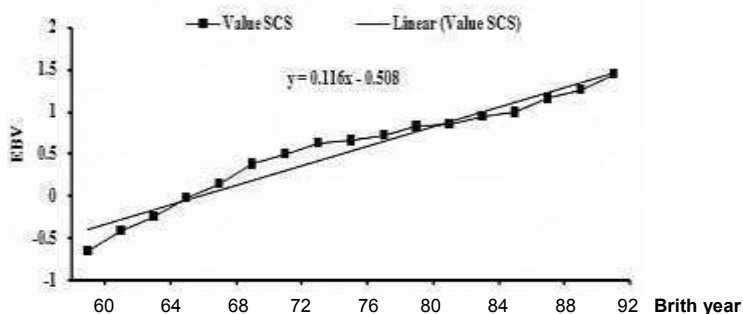


Fig. 7: Genetic trends of milk yield and SCS traits

DISCUSSION

In this study, LP with different orders was compared for better fitting performance of TD milk yield and SCS. The results showed that the criteria of values changed when the order of fit the effects (AG and PE variances) considered to be various, agreeing with the results presented by Mohammadi et al. (2014a); Bignardi et al. (2009); Albuquerque et al. (2005) [10,18,19].

The trends in the AG and PE variances in this study in the first lactation are consistent with other studies [17,18,20]. On the other hand, inconsistent the results achieved in this study, AG and PE variances of milk yield was increased as lactation progressed and was highest in middle lactation and subsequently decreased at the end of lactation for Iranian Holsteins [21] and Turkish Holstein-Friesian [22]. We speculated that the differences between results may be due to the effects of some parameters such as environmental effects, number of data, software, the method and the breeds. The results of this current study showed that estimates of heritability of SCS were lowest than milk yield. The trend of heritabilities of yield traits in this study, were similar to results obtained in the Iranian Holsteins, by Mohammadi et al. (2014b); Shadparvar and Yazdanshenas (2005) [11,21].

The heritability estimates of first lactation milk yield reported from 0.28 Iranian Holsteins [10] to 0.51 in Holstein-Friesian cows [23]. Some authors reported higher heritabilities at the beginning and at the end of lactation [23]. This increase in heritabilities estimate is associated not only with the increases on the values of AG components but also with the reductions in values of PE components between models. Because heritabilities is low in early lactation for both traits, is obtained PE at this stage of lactation high and given that AG variance was higher in late lactation. The small differences in heritabilities estimate between models do not indicate a preferred order of the LP[10,11]. Estimates for additive genetic variance of fertility traits were less than residual variance; therefore, estimates for heritability for these traits were low. It was very close to most of estimates reported in other studies [8,24,25].

The results indicating that AFC has a relatively low heritability alike other reproductive traits. Thus, under the current conditions, changes in environmental factors would likely have a higher impact than the selection for these traits. The estimate of heritability for AFC in the present study is within the range (0.086 to 0.15) for those estimated for Angus-Blanco Orejinegro Zebu multi-breed cattle population in Colombia [26] and Iranian Holstein [24,27,28]. The estimate of heritability for AFC was lower than Vergara et al. (2009) for Angus Blanco Orejinegro Zebu cattle in Colombia (0.15±0.13); Wasike et al. (2009) for Boran cattle in Kenya (0.04±0.06); Chegini et al. (2015) for Iranian Holstein (0.133) and Suarez et al., 2006 [24,26,29,30] but close to Farhangfar and Naeemipour Younesi (2007), Chookani et al. (2010) for Iranian Holstein (0.014±0.005) [27,31]. Estimates of heritability for CI is similar to that in the reported in the South African cattle by Makgahlela et al. (2008), in the Iranian Holstein Cows by Faraji- Arough et al. (2011); [8,25]. On the other hand, inconsistent the results achieved in this study with results Ghiasi et al. (2011); Vergara et al. (2009); Toghiani Pozveh and Shadparvar (2009) [5,26,32] showed that heritability for CI was between 0.11 and 0.18. The results obtained heritability of LL (0.10±0.01) are supported by previous research, such as Berry and Cromie (2009) in Irish Cattle; Froidmont et al. (2012) [33,34] in Holstein cows but inconsistent with by results Chegini et al. (2015) and Haile-Mariam et al. (2003) [24,35] Different values estimated for heritability of fertility traits in this study could be due to several factors such as: breed of animal, management system, environmental factors, size and structure of data, model of analyses, nutrition or health care and statistical methods employed. The AFC had negative genetic correlations with CI and LL. This suggested that selection for smaller AFC would improve the lactation curve traits and also adversely lengthen CI (Chegini et al. 2015) [24]. The estimated negative genetic correlations of fertility traits Ghiasi et al. (2011) and zero genetic correlations for Iranian Holstein dairy cattle by Farhangfar and Naeemipour (2007); Pozveh and Shadparvar (2009); for Nellore cattle in Brazil by Gressler et al. (2005) [2,27,32,36]. Conversely, Vergara et al. (2009) reported a moderate and positive genetic correlation between AFC and CI (0.33) [26]. Suggested that the differences in sign and value of genetic correlations may be due to differences in breed of animal, environmental conditions, methods of estimation, and accuracies of variance and covariance components [25,26]. The results showed that the

phenotypic correlations between fertility traits (AFC, CI and LL) were positive, agreeing with the results presented by Faraji-Arough et al. (2011) [25]. Generally, an genetic correlations between TD yields was highest when periods closer to each other and the lowest was observed between distant TD. These results are in agreement with previous studies which have reported the effect of parity on the estimation of genetic parameters in Holstein-Friesian [11,15,20,37]. According to the results it was observed that phenotypic correlation decreased with increasing distance TD. Phenotypic correlation of SCS was estimated to be lower than of milk yield. These results are in agreement with those obtained by (Shadparvar and Yazdanshenas 2005) [21].

Estimating genetic and environmental trends in a population allows the assessment of the effectiveness of the selection procedure and gives the opportunity for monitoring management conditions. It also supplies the animal breeder with essential information to develop more successful programs in the future [38]. The results obtained of genetic trends, similar trends were reported by Sahebbonar (2010) [39] using the 305 day measures of the traits and Abdollahpour and Moradi Sahrbabak (2010) [17] for milk yield and Cheginiet al. (2015) [24] for SCS. The indicated that Iranian Holstein dairy cattle population genetically improved for milk yield and SCS traits. The interest of farmers to use sperms from genetically superior bulls could be the main factor which caused these changes.

CONCLUSION

It is assumed that all mates are of similar genetic merit and this can result in bias in the predicted breeding values if there is preferential mating. Among the different models in this research, it seems that the LP (3,3) might be sufficient to capture most of the genetic and permanent environmental variability observed in the shape of daily milk production. The results obtained in this study (Heritabilities, genetic and phenotypic correlation and Etc. parameters of traits) provided population specific parameters with a higher accuracy that could be used in order to develop the national selection index of Holstein dairy cows. Although favourable positive genetic trends obtained for milk yield and SCS traits in Iranian Holstein cows, this could not lead to improvement in the fertility performance. The low heritability for fertility traits suggested that their genetic improvement would be slow. For improving these traits, improvements in nutrition and reproductive management could be useful. The genetic trend was positive for milk yield and SCS traits during previous years.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

There is no conflict of interest

FINANCIAL DISCLOSURE

None

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ARTICLE

INFANTILE-ONSET ACUTE PRIMARY ADRENAL INSUFFICIENCY: X-LINKED ADRENAL HYPOPLASIA CONGENITAL - A MOLECULAR ANALYSIS OF DAX1

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ABSTRACT

X-linked Adrenal Hypoplasia Congenital (X-linked AHC) occurred because of some abnormality for instance mutation or deletion in DAX1 on the X chromosome. This disorder, mostly emerges in male who have delayed or arrested in puberty as well as infertility. All these properties called hypogonadotropichypogonadism. Adrenal failure, which is the most significant phenotype of AHC resulting in glucocorticoid and mineralocorticoid deficiency subsequently influences the developmental transition of adult from fetus. DAX1 (dosage sensitive sex reversal-DSS) an important protein encoded by the gene NROB1 (orphan nuclear receptor) located in a specific region on the X chromosome. It is expressed throughout the hypothalamic-pituitary-adrenal-gonadal (HPAG) axis suggesting its pivotal role in early human sexual development. It potentially interacts with many important cellular receptors like androgen receptor (AR), estrogen receptor (ER) and progesterone receptor (PR). But, each of them are regulated by different mechanisms. Recent research reports on experimental animals highlight that the Dax1 can be alternatively spliced suggesting that functional role of Dax1 is more diverse and complex. Familial mutations in DAX1 are often associated with the pathological conditions like Adrenal hypoplasia congenital (AHC), hypogonadotropichypogonadism (HH). Unlike the mutations, the duplication of this gene resulting in dosage sensitive sex reversal (DSS). In this review, we summarize the molecular background of DAX1, biological function, impact of its mutation in AHC formation and clinical significance of the protein expression in HH.

INTRODUCTION

Genetic analysis of male patients with primary adrenal insufficiency revealed 13 novel mutations within the coding region of the NROB1 gene, which are predicted to inactivate the DAX1 function that were three nonsense mutations (c.312C>A, p.Cys104X, c.670C>T, p.Gln224X; and c.873G>A, p.Trp291X), five duplications (c.269_270dup, c.421_422dup, c.895_896dup, c.989dup, c.999_1000dup), and five deletions (c.483del, c.745_746del, c.734_740del, c.1092del, and c.1346del) [1].

The PREVALENCE of X-linked AHC is unknown. It has been widely estimated at 1:12,500 live births [2]. Some ratio express at less than 1:70,000 males [3]. Majority of the affected cases are newborns, aged >8 weeks, where some of them may exhibit the disease in later stage of childhood [4].

DAX1 (dosage sensitive sex reversal-DSS) is an important protein encoded by the gene NROB1 (orphan nuclear receptor) located in a specific region on the X chromosome. This protein is a well-studied molecular candidate involved in adrenal gland development and also responsible for steroidogenesis in adults. It is expressed throughout the hypothalamic-pituitary-adrenal-gonadal (HPAG) axis suggesting its pivotal role in early human sexual development. It potentially interacts with many important cellular receptors like androgen receptor (AR), estrogen receptor (ER) and progesterone receptor (PR). But, each of them are regulated by different mechanisms. However, the molecular mechanism of DAX1 in multiple stages of development is inadequately understood. Recent research reports on experimental animals highlight that the Dax1 can be alternatively spliced suggesting that functional role of Dax1 is more diverse and complex.

Clinical description

In the onset interval of one week to 3 years. Several cases is recognized between three weeks within 60% to nine years old with 40% [5]. The age of onset is fluctuated, they may have fertility problems or late puberty or delayed Onsted failure [6- 8]. In these individuals, residual glucocorticoid and mineralocorticoid activity present in the hypoplastic adrenal cortex may explain the late onset [9]. The failure of pubertal development may be caused by either abnormal hypothalamic or pituitary regulation of gonadotropin secretion, although the testicular steroidogenesis is largely intact, the functional maturity of Sertoli cells and also spermatogenesis are impaired. The type of mutation doesnot predict clinical phenotype [10]. The first symptom Adrenal insufficiency could be Hypoglycemia, which company with seizures, and then there are some common symptoms as it mentions before like: vomiting, feeding difficulty, dehydration, and shock caused by a salt-wasting episode. Mineralocorticoid deficiency may be the presenting feature of X-linked AHC in some cases [11, 12]. The most important role of Mineralocorticoids are the maintenance of fluid and electrolyte balance. Sometimes hyperpigmentation is appear in X-linked AHC, which caused by increased pituitary production of POMC (proopiomelanocortin), these pigmentation is gradually disappear with appropriate steroid therapy [9].

KEY WORDS

DAX1, NROB1, Adrenal hypoplasia congenital (AHC), Hypogonadotropichypogonadism (HH)

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Hypogonadotropichypogonadism (HH) is commonly associated with the expression of NROB1 in the hypothalamus and the pituitary. Its prospect the mutation DAX1-gene NROB1 responsible for HHG phenotypes. Which means the DAX1 is essential for development of function of hypothalamus- pituitary [13]. Azoospermia has been reported in individuals with classic X-linked AHC and severe oligospermia has been found in some individuals with partial forms of this condition [14]. There has been shortage of source and research about Fertility of individuals with AHC.

GENETICS OF AHC

The actual causes of AHC were unknown, prior to the identification of the DAX1 gene (*Dosage-sensitive sex-reversal, AHC, on the X chromosome, gene 1*). Initially, X-linked AHC was observed as contiguous genetic syndrome in some patients who already suffer with other genetic disorders like Duchene muscular dystrophy, glycerol kinase deficiency, ornithine transcarbamoyltransferase deficiency and mental retardation restricted to the locus Xp21.3-p21.2 [15]. In 1994, duplication of this genomic region was found to be linked with dosage-sensitive XY sex-reversal (DSS) in humans [16]. In the same year, DAX1 gene was successfully cloned and also experimental studies proved that loss-of-function mutations in the coding sequences of DAX1 could cause X-linked AHC and HHG [17]. In addition to that, DAX1 over expression were found to gain an induced sex-reversal in male mice highlighting that DAX1 gene is sole responsible for DSS [18]. DAX1 gene belongs to an orphan member of the nuclear receptor superfamily.

At present, the gene has been renamed as “NROB1” as per the uniform nomenclature system specific to the nuclear receptors where the protein product is alternatively referred as “DAX1”. NROB1 gene dosage is very crucial in normal human developmental process [19]. Functionally, the DAX1 interacts with wide range of molecular players of early development. On comparing with other nuclear receptors, the DAX1 protein has been found to be deficient of a normal DNA-binding domain [20]. The DAX1 protein also serves as a dominant-negative regulator to regulate the transcription of other nuclear receptors like steroidogenic factor 1(SF1). It also acts as an antagonist to SRY to establish that it is a potential anti-testis gene. DAX1 plays an essential role in the development of several hormone-producing tissues which include the hypothalamus, adrenal, pituitary glands, and reproductive structures like testes and ovaries. DAX1 serves a potential transcriptional regulator which controls the activity of various genes during embryonic development of hormone producing tissues. There are numerous reports which have highlighted that NROB1 gene mutations could result in both X-linked congenital adrenal hypoplasia (AHC) and hypogonadotropichypogonadism (HH) [21].

Mapping of Dax1 gene and protein structure

The genomic arrangement of NROB1 is simple with just two exons which have been partitioned with a single intron in between them [17, 22]. Exon 1 is quite larger than exon 2 where it spans around 1168 base pairs in length. A 3385 bp intron is preceded before the exon 2 which is 245 bp in size. The NROB1 gene is transcribed to produce an mRNA made up of 1413 nucleotides which encodes a functional DAX1 protein consist of 470 amino acids [23]. Exon 1 encodes the DNA binding domain (DBD) and partial ligand binding domain (LBD) where Exon 2 is responsible for the additional region of the LBD. The DAX1 protein has been classified as an orphan member under nuclear receptor superfamily. The members of nuclear receptor superfamily have an organized structure consisting of sub regions from A to E which makes a unique characteristic for each domain. Among these sub regions, region “A-B” is known as a modulator domain which varies in size and also exhibits evolutionary dissimilarity. These region constitutes a hormone independent transactivation domain called as “activation function 1(AF-1)”. The most highly conserved “C” region represents a DNA-binding domain (DBD) that contains two zinc fingers. More essentially, it aids to recognize hormone response elements and binding to promoters of target genes. This region also helps for receptor dimerization. Apart from A-B, C domain, the DAX1 also has two more notable domains namely “D” and “E”. The D region serves a potential docking site for corepressors where it also acts as a hinge between DBD and the ligand-binding domain (LBD). The E region is considered as second most highly conserved region that acts as LBD to attain various events like ligand binding, receptor dimerization and nuclear localization [21]. Among the nuclear receptor superfamily, the DAX1 is unusual and holds quite salient features [24]. The amino-terminal domain (NTD) has a novel structure consisting of 3.5 alanine/glycine rich repeats of a 65–70 amino acid motif that has no known homology to any other proteins [21]. The repeats show 33–70% identity to each other, and also contain cysteine residues in conserved positions that could potentially form zinc fingers [25]. DAX1 lacks the modulator domain (Region A/B), conventional DBD (Region C) and hinge region (Region D) but contains an AF-2 transactivation domain [21]. The carboxyl-terminal domain (CTD) of DAX1 shows peptide homology with strongest amino acid similarity to the LBD of other nuclear receptors complex. The CTD of DAX1 is very similar to the LBD of the testis receptor, COUP-TF, and retinoid X receptor (RXR) [24- 26].

MOLECULAR MECHANISMS OF DAX1 ACTIVITY

In general, nuclear receptors (NRs) play an inevitable role in steroidogenesis as well as maintenance of corresponding tissue functions. These NRs widely act as efficient transcription factors which could control and alter the core genetic network that is crucial for major processes like reproduction, development and

homeostasis. The NR activation is limited to various extracellular and intracellular signals [26]. Specifically, these receptors can be activated via the binding of hydrophobic compounds, i.e., steroids, retinoid, and thyroid hormones [26]. As an important member of NR, Dax1 also regulate expression of some novel genes which are critical for embryonic as well as postnatal development of vital hormone producing organs like hypothalamus, pituitary and adrenal glands. This significant role makes the DAX1 is key transcriptional repressor of the HPAG axis. It has been well known that DAX1 activity could modulate transcriptional repression of various other steroidogenic receptors like androgen (AR; NR3C4), estrogen (ER; NR3A1-2) and progesterone receptors (PR; NR3C3) and also liver receptor homologue-1 (LRH-1; NR5A2) [27, 28].

In addition to that, DAX1 could also act as a shuttling RNA binding protein between the nucleus and cytoplasm by it regulates mRNA levels of many genes [29]. Major in vitro experiments have highlighted that DAX1 could auto-regulate itself by recognizing the presence of a single stranded loop of its own promoter and also interacting with DNA hairpins. As an example, DAX1 inhibits a necessary step in steroid biosynthesis by blocking steroidogenic acute regulatory protein (StAR), an essential protein helps the cholesterol transfer to the inner mitochondrial membrane [29, 30]. DAX1 has also been reported to regulate genes that do not have hairpin structures by executing an inhibitory protein-protein interactions. Dax1 mediated inhibition of Steroidogenic factor 1 (SF1) is a well-known example inhibitory protein-protein interaction [31]. SF1 is an essential regulator of steroid hydroxylase gene expression which is abundantly expressed at the reproductive system which includes the HPAG axis [32]. The entire class of steroidogenic hydroxylases holds the SF1 responsive elements in their promoter regions [33]. In connection to this, promoter region of NROB1 also has a functional SF1 response element. However SF1 is not the single regulator for Dax1 expression since the feedback mechanism of Dax1 mediated SF1 regulation has been well established with animal models.

FUNCTIONAL ROLE OF DAX1 AND AHC

Familial mutations of NROB1 gene could result in the production of a defective DAX1 protein with impaired stability and altered activity that eventually exhibit a reduced transcriptional silencing ability in a disease condition known as congenital adrenal hypoplasia (AHC) [11]. These genetic mutations are believed to be the causative factor for direct transcriptional effect with a reduced capacity and also DAX1 mutants poorly interact with their target nuclear receptors and corepressor proteins [34]. Clinically, the AHC subjects show signs of developmental failure in the adult zone of the adrenal gland emphasizing the regulatory role of DAX1 in steroidogenesis. DAX1 protein is normally expressed during the entire development of adrenal cortex, but its exact role in adrenal morphogenesis is poorly understood [35]. Recent studies have elucidated that expression of DAX1 has been observed in all regions of hypothalamic-pituitary-adrenal-gonadal (HPAG) axis, which highlights that DAX1 could be functionally linked with maintaining pluripotency in the early development of adrenal gland. Collective evidences from experimental animal models suggest that DAX1 may have pleiotropic roles, with complex and distinct functions in development and adult function throughout the HPAG axis. Additionally, post transcriptional functions and other disruptive mechanisms of DAX1 in each of these tissues are known very less, especially in pathological conditions like AHC and HH [21].

PHENOTYPIC RELATIONSHIP WITH DAX1 EXPRESSION

Clinical subjects with AHC are phenotypically heterogeneous in relevant to the expression of DAX1 protein which has been identified in variety of tissues from the developing adrenal cortex, gonad, anterior pituitary, and hypothalamus, and also in adult adrenal cortex, Sertoli and Leydig cells in the testis, theca, granulosa, and interstitial cells in the ovary, anterior pituitary gonadotropes, and the ventromedial nucleus of the hypothalamus [36]. This pattern of expression suggests that DAX1 is involved in the development and function of the HPAG axis. Apart from these tissues the protein also present in intermediate level at cerebral cortex, spinal cord, thymus, heart, lung, and kidney [19]. In general, mutational spectrum of DAX1 contributes a distinguished phenotypic heterogeneity which may be varying between the same mutation as well as different mutations in an affected family. This could be an imperative phenomenon which suggests that environmental effects or modifier genes could alter the clinical manifestations of the disease. AHC is commonly diagnosed during infancy, contradictorily some patients may not exhibit the disease until later stage of their life. These abnormal mutations and downstream phenotypic expression have been reported in females. Occasionally, the mutation type or location may not be sufficient to predict disease severity and the early onset of adrenal insufficiency in affected individuals [37].

MUTATIONS OF DAX1

In the recent years, now it has been clearly demonstrated that AHC is absolutely caused by DAX1 gene mutations and other innumerable mutations including deletions, alterations of splice-sites, missense mutations, nonsense mutations and frameshift mutations [5]. These mutations cause a change in one of the building blocks (amino acids) of DAX1 which is tend to be basis for the production of an abnormally short protein that could be even a nonfunctional protein. Loss of function mutations of DAX1 leads to

severe adrenal insufficiency and hypogonadotrophic hypogonadism, which are the major characteristics of AHC. DAX1 mutations occurring naturally at specific location provide insights into the structural and functional relationships. In evidence of this, a little truncation of 9 amino acids from the carboxyl-terminus is sufficient to eliminate complete transcriptional repression by DAX1 [38]. The gene also harbors a large number of missense mutations which account for about one-quarter of other mutations and tend to cluster in the ligand binding-like domain at carboxyl-terminal [3]. It is also noted that amino-terminal region of the protein also yields few missense mutations possibly because of redundant function of the repeated LXXLL protein interaction domains [11]. Genomic deletion of genetic material in the X chromosome, specifically at NROB1 region may result in a condition called adrenal hypoplasia congenital with complex glycerol kinase deficiency. In addition to that affected individuals may show elevated levels of lipids in their blood, urine and also they may have complications in regulating blood sugar levels. In rare cases, the amount of genetic material may be deleted extensively which is common like as Duchenne muscular dystrophy (DMD). Like the deletions, the genomic duplication on the X chromosome, especially the region contains the NROB1 gene can cause a condition called dosage-sensitive sex reversal. The extra copy of NROB1 gene prevents the formation of male reproductive tissues where people identified with this duplication usually appear as a female though they are genetically male with both an X and a Y chromosome.

DIAGNOSIS

Clinical features of Adrenal hypoplasia congenital

The incidence of AHC is very rare, which accounts approximately 1 in 12,500 live births worldwide [39]. Many affected children exhibit the clinical symptoms like vomiting, poor feeding, hyperpigmentation, convulsions, vascular collapse that leads to unexpected mortality. This disorder also emerges with several biochemical abnormalities including hypoglycemia, hyponatremia, hyperkalemia, increased plasma ACTH, low level cortisol and aldosterone in serum [40]. Along with adrenal failure, hypogonadotropic hypogonadism (HH) is also an additional feature of X-linked AHC. "HH" is has been reported for the cause of delayed puberty in some cases already diagnosed with AHC [41]. But this can be successfully treated with testosterone replacement therapy. However, males identified with classic X-linked AHC are found infertile where the exogenous gonadotropin therapy is still unsuccessful [37].

AHC diagnosis

NROB1 is the only gene, which its mutation causes the x-linked AHC. Almost everyone with X-linked AHC has positive family background including a remarkable mutation in the NROB1 gene. AHC can be diagnosed with reliable physical and biochemical tests. Physical diagnosis of the disorder relies on the reports from anatomical observations and other clinical examinations. For the biochemical confirmation of the disorder, a routine hormone profiling in body fluids like blood and urine can be performed to measure levels adrenal hormones including cortisol, aldosterone and androgens. Identification of low cortisol and elevated ACTH levels after stimulation could be applied for early diagnosis of adrenal insufficiency [42].

There are genetically tests have been done for X-Linked AHC diagnosis, which the first step is sequencing of NROB1. If there is not any specific pathogenic variant, the next phase is deletion/duplication analysis. With sequencing analysis, we are able to diagnose the pathogenic and harmless variants. The pathogenic variant includes small intragenic deletions/insertions, missense, nonsense, and splice variant which are not identifiable as an exon or whole-gene deletions/duplications. It is proposed to take PCR before sequence analysis in order to investigate the epidemic deletions of one or more exons. Several methods used for diagnosis of exon or whole- gene deletion/duplications including: quantitative PCR, long- range PCR, multiplex ligation- dependent probe amplification (MLPA), and chromosomal microarray (CMA) [43]. Occasionally, X-linked AHC could cause unexplained deaths in male infants before recognition of the disorder in the family. To overcome this, rapid diagnostic methods can give hope to prevent sudden death after an early start of mineralocorticoid and glucocorticoid treatment. In recent days, advanced molecular diagnostic methods of AHC have been applied towards initiating rapid treatment schedules. Now it is clear that all affected individuals show the 100% positivity for family history with X-linked inheritance mode. Current molecular genetic approaches have opened up promising ways to identify disease causing mutation in DAX1 (also known as NROB1) which is directly associated to cause X-linked AHC [44].

Evaluation for the contiguous gene deletion syndrome

X-LINKED AHC may include the glycerol kinase deficiency (GKD) and in some ones the Duchenne muscular dystrophy (DMD), as a part of contiguous gene deletion syndrome. GKD diagnosed by the measurement of serum concentration of triglycerides and urine glycerol. If the serum concentration of creatinekinase (CK) is over normalized, DMD is suspicious and then diagnosis of diseases is done with the molecular genetic tests. If there is not any diagnosis of pathogenic variant the immunohistochemically staining of dystrophin on muscle biopsy will be running. If, on the base of clinical symptoms and levels of plasma creatine kinase and urine glycerol, there is any suspicion of complex glycerol kinase deficiency, then we propose FISH for NROB1 or a microarray. If there is not any identifiable deletion for NROB1 by FISH or microarray analysis, then the study proposes deletion/duplication analysis [43].

Prenatal diagnosis

X-Linked AHC: if we couldn't identify pathogenic variant in family members, the DNA analysis extracted from fetal cells obtained by amniocentesis (from the fifteenth up to eighteenth week) or chorionic villus sampling (between the tenth and twelfth week) is necessary. Complex glycerol kinase deficiency: for diagnosis, we can use the FISH or deletion/duplication analysis. The DNA analysis extracted from fetal cells obtained by amniocentesis (from the fifteenth up to eighteenth week) or chorionic villus sampling (between the tenth and twelfth week) is necessary [43]. In the following section of the research, there are some recommendations for evaluating the extremeness of disease and the need of a person given with the x-linked adrenal hypoplasia:

- ✓ The monitoring and checking of glucose, cortisol, and ACTH serum density
- ✓ Checking the performance of kidneys, including the measuring of serum BUN and creatinine
- ✓ The checking of serum and urinal density of electrolytes
- ✓ The search of vascular blood gases
- ✓ The check of aldosterone and plasma renin activity

TREATMENT OF AHC

Children who are involved, treated with special care. And considering the physiological processes observed in them, some patient need to hyperkalemia correction. Maintenance hormone treatment is promising because of replacement doses of glucocorticoids and mineralocorticoids allow patients to live the early ages. If steroid replacement therapy isn't sufficient the person maybe dies. Children treated with relevant hormonal therapy may show Hypogonadotrophic hypogonadism (HH) after maturity that can be easily distinguished from other causes of adrenal insufficiency [45]. In rare cases, the affected individuals display a deficiency of pituitary hormones like LH and FSH when the production of other hormones (ACTH, GH, TSH, and PRL) is normal. Hence the relevance of hypogonadotrophic hypogonadism to hypothalamic or pituitary dysfunction or both is unclear [46]. Clinical care of the affected children is more challenging and effective disease management is also very important. The patients may require intensive administration with appropriate genetic counseling and complete clinical course [47]. Acute Adrenal insufficiency attacks, can treat with closely monitor blood pressure, clinical status, blood sugar and electrolytes. In these cases, you may need correction of hypokalemia. These people have treated with management of saline, glucose, and hydrocortisone. If serum electrolytes do not improve, a mineralocorticoid added or solucortf will be increased. Enough sodium should also be provided.

After treating the initial acute attack, the patients need to begin replacement doses of glucocorticoids and mineralocorticoids, and nutritional supplements of sodium chloride, in younger children. Steroid doses should be increased during stress times, at this time may be required to prescribed sodium and glucose as well. Dying cause of acute adrenal insufficiency in people with x-linked adrenal hypoplasia congenital.

CONCLUSION

Recent advances in molecular diagnosis and clinical treatments have advanced our understanding of the AHC as well as HH disease at the earliest stage and also have extended patient life expectancy. Most of the X-linked AHC patients are infants and diagnosed with adrenal insufficiency during the first few months of life. In the later stage of the life, males are generally presented with idiopathic primary adrenal insufficiency where more than 50 % of them may have DAX1 mutations. Hence the NROB1/Nr0b1 is marked as a central player in the molecular network of steroid hormone pathway and an effective modifier of HPAG axis development. Besides, pleiotropic role of DAX1 makes it a key molecule for the regulation of ER, AR, PR, LRH-1, and SF1 target genes throughout HPAG axis development.

However, the biological role of HPAG axis formation through the molecular mechanisms of DAX1 action is not explained clearly so far. The unanswered questions around DAX1 suggest that still there is a molecular mystery to be resolved by identifying an undiscovered function that is so crucial for the development of sexually dimorphic system like human.

CONFLICT OF INTEREST

There is no conflict of interest

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ARTICLE

ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS

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ABSTRACT

Stem cells are unspecialized cells in living bodies which have high potential of division, and can be differentiated under special physiological circumstances or in the presence of special factors to yield a variety of matured cells or specialized tissues. Mesenchymal stem cells (abbreviated as MSC) possess the property of heterogeneities and fibroblastosis, and are self-renewal and can be differentiated. Over the years, many initial and most flourished studies were performed on MSc which were separated from bone marrow; but in the recent years the use of adipose tissues have become common due to few special characteristics such as easy access, great texture and high reproduction power. The mesodermal cells have certain surface markers namely CD73, CD44, and CD90 but lack hypotonic surface markers such as CD11c, CD31, CD34, CD45, CD80 and CD86. In addition, mesodermal cells have the power to differentiate into a variety of tissues; either into other mesodermal cell types or into non-mesodermal tissues. These cells can also be used in tissue engineering and cell therapy to repair or replace damaged tissues with healthy tissues, as well as in the manufacture of vital drugs. Thus, this study aims to express few specific characteristics of MSCs isolated from adipose tissues.

INTRODUCTION

A few cells in the body are the responsible for survival and a classic example of such cells are the stem cells. The stem cells can be differentiated and transformed to adult stem cells under specific circumstances. The evolution of 'Stem cell' studies and research in the biological sciences domain drives to a major academic field that focuses on technologies used for emerging a complete organism from a single cell. The substitution of damaged cells with the healthy cells is primarily studied. This improvises knowledge on embryology, developmental biology, grafting mechanisms and transplantation and can be used to treat cancer. The stem cells are self-renewal and can be transformed into osteoblasts, chondrocytes and adipocytes with high competence of division, which can create a regenerative cell population. One of the most important stem cell that attracts the attention of scholars is the MSCs that are categorized in the adult stem cells.

The MSc are known as pluripotent and fibroblastic cells, which can be separated from bone marrow, adipose tissue, umbilical cord blood, lungs [1-3], skin [4] and spleen [5]. The MSc count a valuable resource with the high productivity for repairing the tissues due their immunological characters, and high potency to proliferate and differentiate. Having this prominent specialty, the stem cells are used as a critical treatment tool to cure several diseases. According to several research results, treatment with MSCs exhibit greater immunity in a short term.

The application of such cells in surgeries, stress, and different infectious conditions, decrease the probability of a transplant rejection and the usage of sub receptor drugs. The unique potential of stem cells, including the ability to differentiate and transform into specific cells in vivo, gives evidence that these cells can be used in transplantation to treat tissue-damaging diseases as a faithful method in the future. Considering the feasibility of cell isolation, the adipose tissues remain as good and suitable resource as umbilical cord stem cell and bone marrow cells are more complicated. Moreover, many stem cells can be separated from a single adipose tissue. Based on the recent investigation, the adipose tissue stem cells are able to cure the liver injuries, muscular dystrophy, allergy, and myocardial infarction.

STEM CELLS

These are self-renewable cells that possess varying potency to differentiate into multilineages and the ability to form clones (clonogenic) [6]. Ideally, the stem cell used for regenerative medicinal applications should meet the following criteria [7]:

- Found in abundant quantities (millions to billions of cells).
- Harvested by a minimally invasive procedure.
- Differentiate along multiple cell lineage pathways in a regulative and reproducible manner.
- Safely and effectively transplant into either an autologous or allogeneic host.
- Manufactured in accordance with current Good Manufacturing Practice guidelines [2, 8].

Two kinds of stem cells can be defined based on tissue source: embryonic stem cells and adult stem cells. Embryonic stem cells are pluripotent and can give rise to various cell types present in the body. Generally, adult stem cells are limited by number and the type of cell into which it can differentiate. For cell-based tissue regeneration, a potential advantage of using stem cells from an adult is that the patient's own cells could be expanded in culture and then re-introduced into the patient without the problem of tissue

KEY WORDS

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rejection by the immune system. The biology of adult stem cells and their potential use in gene therapy have provided opportunities for therapeutic use in tissue regeneration [9].

There are some characteristics for adult stem cells, which make them suitable for clinical uses: like ease of harvest, high expansion rate in vitro and multilineage differentiation capacity [10]. Though the application of cellular therapy and regenerative medicine is rapidly growing, for regenerative medicinal purposes, stem cells should meet the above mentioned criteria. Cell therapy using stem cells and their progeny is a promising approach that is capable of addressing many unmet medical needs. Recently, stem cell research has quickly progressed, allowing researchers to isolate and purify stem/progenitor cell populations from various tissues (i.e. hematopoietic, vascular endothelial and neural stem cells, as well as hepatic oval cells) [11]. In addition, several studies have shown that stem cells also benefit from immune modulatory capabilities [12].

MESENCHYMAL STEM CELLS

The self-renewable multipotent MSCs are found in many adult tissues, including the bone marrow, trabecular bone, adipose tissues, and muscles. According to some specific culture conditions, these cells can give rise to multiple mesenchyme-derived cell types, such as osteoblasts, chondrocytes, adipocytes, and myoblasts [13]. In addition to phenotypic characterization, the above mentioned are other minimal criteria that were proposed by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy to define MSCs [14]. Cells which exhibit characteristics of MSCs were isolated from adipose tissue, amniotic fluid, amniotic membrane, dental tissues, endometrium, limb bud, menstrual blood, peripheral blood, placenta and fetal membrane, salivary gland, skin and foreskin, sub-amniotic umbilical cord lining membrane, synovial fluid and Wharton's jelly [6, 15]. These days the MSCs have gained some attractiveness for clinical applications; example transplantation of bone, liver, cardiac, skeletal muscle and CNS at various stages, including clinical trials. The cause of this attention is the relative ease of expansion by well-described protocols, and the ability for induced-differentiation into a host of cell lines in vitro without ethical concerns attributed to embryonic stem cells [16].

It is known that progenitor cells from different sources exhibit similar lineage differentiation properties in the same environment. There are some evidences which say that the precise location of stem cells within native tissue is of significant interest that aids in processing and culture within the laboratory. MSCs may be derived from a common perivascular origin. Culture of perivascular cells from multiple tissues results in products expressing CD surface markers typically like MSCs (CD44, CD73, CD90 and CD105) that exhibit anticipated clonal proliferation and multi-lineage potential in suitable inductive conditions. These cells (a) should exhibit plastic adherence (b) possess specific set of cell surface markers, i.e. cluster of differentiation (CD) 73, D90, CD105 and lack expression of CD14, CD34, CD45 and human leucocyte antigen-DR (HLA-DR) and (c) have the ability to differentiate in vitro into adipocyte, chondrocyte and osteoblast. These properties can be traced in all MSCs, although few differences exist in MSCs isolated from various tissue origins [6, 15, 16].

Moreover, MSCs being able to become mesodermal lineage can also be able to become into a variety of cell lines which is originating from the ectoderm and endoderm for instance hepatocytes, neurons, and cardiomyocytes. The multi-lineage differential potential of MSCs is investigated in vitro culture functional assays using specific differentiation media. This feature makes MSCs to be considered as a suitable source for tissue repair [17]. Although in most cases the isolated MSCs are heterogeneous in proliferation and differentiation, the expression of the characteristic MSC markers stand prominent. Cultivation of MSCs in vitro has three biological properties that qualify them for use in cellular therapy: (a) broad potential of differentiation, (b) secretion of trophic factors that favor tissue remodeling, and (c) immune regulatory properties [15]. Performance of MSCs is depended on a series of mechanisms in vivo including: (A) differentiation potential, (B) release of paracrine factors influencing the microenvironment, (C) scavenging of reactive oxygen species, (D) immune modulatory function and (E) fusion and rejuvenation of resident committed progenitor cells [5, 18, 19]. MSCs are an excellent candidate for cell therapy because (a) human MSCs are easily accessible; (b) the isolation of MSCs is straightforward and the cells can expand to clinical scales in a relatively short period of time; (c) MSCs can be bio-preserved with minimal loss of potency and stored for point of care delivery; and (d) human trials of MSCs thus far have shown no adverse reactions to allogeneic versus autologous MSC transplants, enabling creation of an inventory of third-party donor MSCs to widen the number of patients treated by a single isolation.

MSC transplantation is considered safe and has been widely tested in clinical trials of cardiovascular, neurological, and immunological disease with encouraging results [20]. Due of their great ability to treat many hazardous diseases in animal, recently MSCs are being explored for use in humans. Although the primary mechanisms of action have not been fully elucidated, studies indicate that MSCs can act on several levels of endogenous repair to bring resolution of diseases. MSCs have been shown to protect cells from injury and directly promote tissue repair when administered to treat animals undergoing acute renal failure, MSCs prevent apoptosis and elicit proliferation of renal-tubule epithelial cells in a differentiation-independent manner. When injected into the myocardium after an infarction, MSCs can reduce the incidence of scar formation. When administered to prevent the onset of IDDM, MSCs protect β -islets from autoimmune attack; and when administered after onset of the disease, they promote temporary restoration of glucose regulation, suggesting protection and repair of damaged islet tissues.

Many *in vivo* transplantation studies recently illustrated that adult MSCs have the ability to differentiate into mesoderm-derived cell types as well as into cells with neuro-ectodermal and endodermal characteristics, proposing that trans-differentiation occurs in mammalian systems [13]. MSCs have also been shown to modulate the immune system and attenuate tissue damage caused by excessive inflammation moreover they are able to promote tissue repair directly [20]. Gene delivery by non-viral methods, including native DNA, liposomes, cationic polymers, and electroporation, is less efficient than virus-mediated DNA delivery. Typically, transfection efficiency by non-viral methods is limited to 20–25%. Furthermore, adult MSCs tend to resist trans-gene delivery by classic non-viral methods, as primary cultured cells do [18,21]. Despite these features, non-viral methods have several advantages, such as lower manufacturing costs, no (or weak) immunogenic responses with repeated administration, and are generally safe. Therefore, improving the transfection efficiency of non-viral methods for adult MSCs would prove to be beneficial in cell therapy [9].

ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS (ADSCS)

Adipose tissue

Bone Marrow (BM) was the original reference source for MSCs isolation, but now they are being isolated from a multitude of adult tissues, including muscle, adipose tissue, connective tissue, trabecular bone, synovial fluid, and perinatal tissues, such as umbilical cord, amniotic fluid, and placenta. In particular, ubiquity, ease of retrieval and the minimally invasive procedure required for harvesting the adipose tissue (AT), makes it an ideal source for high yield MSCs isolation. Moreover, adipose tissue-derived MSCs (ADSCs) can be maintained longer in culture and possess a higher proliferation capacity compared to BM-derived MSCs. Though they are beneficiary, low number of harvested cells, limited amount of harvested tissues and donor site morbidity or patient discomfort are included the limitations with BM-MSCs in providing a sample. Hence, there was a need for *ex vivo* expansion or further manipulation of these cells before their preclinical or clinical use to satisfy the safety and efficacy requirements [8]. Therefore, AT was considered an attractive and alternative source which can be provided in large quantities from AT fragments [22].

Adipose tissue, similar to BM, is a mesodermal derived organ which includes a population of stem cells. These can be enzymatically derived from AT and a homogenous population can be made in culture under suitable conditions, in order to express mesenchymal growth and exhibit stable growth and proliferation kinetics [10]. Adipose contains more multipotent cells per cc than BM, so it is apparently a good source of stem cells i.e. one gram of adipose tissue yields $\sim 5 \times 10^3$ stem cells, which is 100-fold higher than the number of MSCs in one gram of BM [14]. Adipose is a highly complex tissue and organ with a big role in energy metabolism, endocrinology, immunity, comprising mature adipocytes (>90%) and a stromal vascular fraction (SVF), which includes pre-adipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, monocytes/macrophages, lymphocytes, and ASCs (14–16). The density of the AT stem cell reservoir varies as a function of location, type, and species [21]. It is now appreciated that AT contains a heterogeneous cell population that can change a function of obesity and diabetes [23].

The adipose-derived SVF cells and ASCs provide a special and influential advantage for tissue engineering and regenerative medical applications [19]. ADSCs are ubiquitous and easily obtained in large quantities with little donor site morbidity or patient discomfort making the use of autologous ADSCs an appropriate research tool and cellular therapy [22]. At a cellular level, AT consists of mature adipocytes surrounded by fibroblasts, nerves, endothelial cells, and immune cells and pre-adipocytes cells contained within a stromal-vascular cell network [24]. Enzymatic digestion of AT, specifically lipoaspirate, generates a heterogeneous population of adipocyte precursors within a pellet of cells termed the stromal vascular fraction (SVF). Recently the capacity of such adipose-resident cells rise increasing attention in order to undergo multi-lineage differentiation in a manner we have learned to recognize as typical of stem cells [16].

Today, a customary method of AT aspiration is a surgical procedure which is relatively easy to harvest a large volume of tissue, obtaining an abundance of isolated stem and therapeutically active cells without the requirement of cell expansion in tissue culture facilities [14]. It is believed that a new era in regenerative medicine and clinical reconstruction has begun. Investigation and clinical use of BMSC is now standard; however, concerns over acceptability of harvesting techniques and potentially low cell yields (1 in 10⁵ MSCs in culture adhere after initial plating) have driven the search for alternative autologous MSC sources. The identification of multipotent precursor cells within processed lip aspirates (PLAs) from human AT, building from well-established lessons in stem cell biology, has offered an alternative source. Characterization of such populations has revealed remarkable phenotypic similarities to BMSCs, while being accessed by a considerably more tolerated harvesting procedure. Other features include expression of specific markers of MSCs and un-expression of some markers such as hematopoietic markers for instance CD106, which make them unique.

BM derived MSCs have been the subject of many academic researches, but a big problem in this way was that the donor site morbidity that limited the amount of marrow that could be obtained [10]. Morphologically, ADSC are fibroblast-like cells and preserve their shape after *in vitro* expansion. Average doubling time of tissue cultured ADSC is between 4 to 5 days, and are similar to BMSC [14]. Stromal cells that have pre-adipocyte characteristics can be isolated from AT of adult subjects, propagated *in vitro* and induced to differentiate *in vitro* towards the osteogenic, adipogenic, myogenic and chondrogenic lineages

when treated with established lineage specific factors. A variety of names have been used to describe the plastic adherent cell population isolated from collagenase digests of AT. Among them, the international Fat Applied Technology Society reached consensus to adopt the term “adipose- derived stem cells” (ADSCs) to identify the isolated, plastic adherent, multipotent cell population [10].

The ADSCs have many advantages in tissue repair. For example, considering its mesodermal origin, ADSCs can be a valuable tool for repairing bone and cartilage defects. However, the use of ADSCs is expanding to both ectodermal and endodermal lineages such as: simulation of peripheral nerve repair, functional recovery in spinal cord damage, and liver injury repair. Besides, in the field of surgery, ADSCs can be used widely as fillers in plastic and cosmetic surgery.

In conclusion, it is evident that, besides other sources of MSCs, adipose derived MSCs are one of the best and influential sources being easily accessible and available through non-invasive method. It can be easily expanded to millions of cells without significant changes in phenotype and genotype, as well as the potential for being used in autologous transplantation in a wide variety of disorders from nerve to cardiac injuries and musculoskeletal problem [10].

As ADSCs have mesodermal origin, they have the potential to differentiate into several lineages of osteogenic, chondrogenic, adipogenic, cardiomyogenic, myogenic, and neurogenic cells [16]. They can differentiate into tissues of endodermal and ectodermal lineages such as hepatocytes, pancreatic islet cells, endothelial cells, neural cells, and epithelial cells [22]. There are so many factors such as donor's age, donor's BMI, type of AT (white or brown) and localization of AT (subcutaneous or visceral), type of surgical procedure, culturing conditions, exposure to plastic, plating density, and media formulations that influence both proliferation rate and differentiation capacity of ADSCs. A detailed comparison of five different subcutaneous depots was determined. For example, ADSCs isolated from the arm and thigh maintained best adipogenic potential as a function of advancing age [14]. ADSC therapy in regenerative laboratories and clinical settings was used in treatment of wound beds with poor blood supply and for healing of radiation injuries. The safety and efficacy of ADSCs in reconstructive medicine was evaluated in many clinical trials [22].

History

The initial methods to isolate cells from AT were pioneered by Rodbell and colleagues in the 1960s [25]. In 2009, Sun and colleagues generated induced pluripotent stem (iPS) cells (see Glossary) by viral transduction of human ADSCs under feeder-free conditions, finding this to be fast and more efficient than induction of pluripotency in human fibroblasts [26]. In 2008, Yoshimura et al. expanded on their earlier work characterizing surface markers in lipoaspirate samples and adherent-ADSCs to release a 40-patient series of fat transfer procedures for breast augmentation, incorporating fat grafts supplemented with simultaneously extracted SVF cells in a process termed cell-assisted lipotransfer (CAL) [16].

Surgical strategies for tissue loss replacement initially laid on the historical maxim “replace tissue with like-tissue”: fatty tissue has been transplanted since 1893, but literature has always shown only controversial results in the degree of lasting of corrections, due to fat reabsorption. SVF provides a rich source of pluripotent ADSCs, which were first identified by Zuk and named processed lipoaspirate (PLA) cells [14]. In 2007, Kucerova et al. showed that ADSCs can indeed facilitate anti-cancer therapy through expression of prodrug converting enzymes. In 2008, Yu et al. found that ADSCs promote tumor growth by enhancing tumor cell proliferation and suppressing apoptosis [26]. Yoshimura et al. used adipose-derived SVF cells in augmentation of soft tissues by cell- assisted lipotransfer (CAL) [51] in treatment of breast augmentation and facial lipoatrophy. In facial lipoatrophy, no complications or adverse side effects were noticed [22].

In 2001, for the first time, there was a great scientific effort for the discovery of accessible resources in order to separate stem cells from the fat tissues, and the efforts were completely successful [27]. Zak et al. (2002) separated the MSCs from the fatty tissues [28]. The research investigations by the Ivone Perce proved that separating stem cells from a patient's fat tissue change these cells by the molecular methods is feasible and can be used in improving the tissue [29]. Min showed that the synchronic linkage between fat tissue and undiscovered stem cells obtained from animal fat tissue lead to stability. Following this process, the density of the veins increased six times than normal and the increase in size of tissue and its stability was observed, as a result of secretion of vein-producing factor from the stem cells which were derived from the fat tissue [30].

Differentiation of adipose derived stem cells

The stem cells have three main specialties:

- Differentiation [31]
- Auto renewal capacity [32]
- Plasticity [33]

Differentiation is a process where unspecific cells under given conditions can be transformed to specific cells. ADSCs have this ability to differentiate into mesenchymal lineages and non- mesenchymal lineages.

Adipogenesis

Adipogenesis can be used in human soft tissue reconstruction as fat grafting is not limited to mature adipocytes transplant. However, in the case of using the ADSCs they have the ability to differentiate and support new blood vessel growth [34]. Fat tissue isolation and re-injection of cells in accordance with adipogenesis and angiogenesis can improve the long-term survival of fat grafts. In reconstructive plastic surgery, the fat tissue is the main damaged tissue, which can be amended by overcoming problems that are related to angiogenesis and long-term survival [35]. On the other hand, the diagnosis of the molecules which were expressed in the differentiation of the fat tissue-derived stem cells (ADSCs) can be feasible to use them as target drugs in order to prevent adipogenesis in obesity, diabetes mellitus and cardiovascular disorders [25]. Adipocyte differentiation takes about 14 days' time and after this period the intracellular lipid can be observed through staining with oil red, however, the gene of factor of adipocyte differentiation signaling in vivo remains unknown. It is presumed that most important stimuli could be insulin and glucocorticoid. In vitro the first stage of adipogenesis is stimulated by the IGF-1, which is the quasi-insulin growth factor. Glucocorticoids, insulin, fatty acids and growth hormones have equal leading roles in adipocyte differentiation [26].

When cultured in an adipogenic medium, ADSCs express several adipocytic genes, including lipoprotein lipase, Ap2, PPAR(γ), leptin, GLUT4, and develop lipid-laden intracellular vacuoles, which stand as the definitive markers of adipogenesis [36, 37].

Chondrogenesis

ADSCs can be used in curing arthritic joints or in joint reconstruction. Scientists always look for suitable treatment of osteochondral defects. Meanwhile, in this process cell therapy with mesenchymal cells has been considered very special. These cells have high potential of reproduction and can easily differentiate to the osteochondral tissue, which is a cell resource [38]. In laboratory, the chondrogenic differentiation results when TGF- β , insulin and ascorbate are added to the medium culturing environment. The increase of extra cellular matrix proteins of cartilage will happen if suspension of cells is in the 3-d calcium alginate gel. In this condition, collagen type II and aggrecan type IV can develop in a cell for several weeks. High-density cell culture techniques can ease the phenotypic changing of cells to dense chondrogenic nodules under the specific conditions. If the cells are monolayered then the chondrogenesis is not potential enough to culture ADSCs [39].

Researches show that smooth and skeletal muscle can be created in the absolute culture environment including 5% horse serum, glucocorticoid like hydrocortisone or dexamethasone, during ADSCs differentiation. The culturing of the ADSCs in presence of the 5-azacytidine, hydrocortisone, and dexamethasone, causes the expression of the genes which are related to the muscle in accordance with normal myogenesis. This process is accompanied with the early expression of the master regulatory factors like myf-5, myoD, myf-6, and myogenin followed by myosin heavy chain expression. This process can be done during two weeks, including increasing length of tissue and multi-nucleating the cells [37].

Osteogenesis

In the recent decades, several cell groups separated fat tissues of human and other species have the potential to differentiate into osteoblast in vitro. Under the osteogenic conditions, ADSCs make stimulate expression of genes and proteins related to the osteoblast including alkaline phosphatase, osteonectin, osteopontin, osteocalcin, bone sialoprotein, Runx2, BMP-2, BMP-4, PMP receptor and PTH receptor. The ADSC differentiation can help in bone grafting and joint break reconstruction. Primarily, Lee et al. (2003) showed that in vitro the differentiation of ADSC to osteoblast lineages was similar to bone forming in vivo. Through their work, ADSC were separated from epididymal adipose tissue in rat, and then were moved into the Sub-Q; and after 8 weeks reasonable evidences of bone forming was observed [40]. Many works showed that dexamethasone was required for stimulating osteogenesis in vitro; however, its exact mechanism remains unclear. The experiment also showed that β -glycerophosphate is necessary for calcification and mineralization. The study explained that for stimulating osteogenesis, the environment should contain ascorbate and β -glycerophosphate derivations with vitamin D or dexamethasone [37].

CONCLUSION

Adipose-derived stem cells are able to differentiate to the several types of cells and thus are very profitable in reconstructive medicine. In reconstructive medicine, researches and preclinical investigations are trying to overcome the problems faced with the usage of mesenchymal stem cells in order to cure diseases. Mesenchymal stem cells can be separated from fat tissue and bone marrow. For many years, the bone marrow was an important resource for providing the mesenchymal stem cells using in tissue engineering, but in recent years, fat tissue has replaced bone marrow tissues as the separation of mesenchymal stem cells from fat tissue is easier and has minimal side effects. It can also differentiate into several types of tissues. This research aims to investigate adipose-derived stem cells, have better privilege when compared with other stem cells.

CONFLICT OF INTEREST
 There is no conflict of interest

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ARTICLE

FACTORS AFFECTING HOSPITAL INFORMATION SYSTEM ACCEPTANCE BY CAREGIVERS OF EDUCATIONAL HOSPITALS BASED ON TECHNOLOGY ACCEPTANCE MODEL (TAM): A STUDY IN IRAN

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ABSTRACT

Technology acceptance model (TAM) is a valid model for predicting the technology acceptance by the users. This study aimed to identify factors affecting hospital information system acceptance by caregivers (physicians and nurses). This descriptive-analytical study was conducted in 2015. The research population was the physicians and nurses working in public hospitals of the city of Bandar-Abbas in Iran. The convenient sampling method was used to select 172 persons. Data collection tool was a questionnaire which its validity and reliability was measured. Data were entered within SPSS software and analyzed using descriptive and inferential statistics and the Spearman's rho test. Direct and positive relationship exist between the variables of image in using IT applications and perceived usefulness ($R=0.26$, $P<0.01$). The level of computer skills had a direct positive effect on perceived ease of use ($R=0.40$, $P<0.01$) and behavioral intention ($R=0.30$, $P<0.01$). Furthermore, subjective norms only had a positive direct effect on perceived ease of use ($R=0.65$, $P<0.001$). Perceived ease of use had no effect on behavioral intention, but had a direct positive effect on PU ($R=0.46$, $P<0.001$). Also, perceived usefulness had a positive direct effect on behavioral intention ($R=0.61$, $P<0.001$). Image in using IT application, the level of computer skills, subjective norms, Perceived ease of use, and perceived usefulness regarding of the use of IT application had a more prominent role in the acceptance of hospital information system by the users. Therefore, it is necessary to consider these variables while designing, purchasing, or revising hospital information systems.

INTRODUCTION

Information technology has a great impact on different professions. It improves the quality and reduces the executive costs. Healthcare facilities are not an exception in this regard. These institutes produce a great deal of data that should be collected, distributed, registered, retrieved, and summarized. The use of IT in health care services as electronic medical records and electronic health records is fundamental to improve the quality of health and treatment services [1, 2]. The use of technology has made changes in data processing which have led the hospitals to rely on information systems that are tools to receive, retrieve, maintain, process, and display the data. With the advances in technology and the use of the computer, contexts like radiology information system and hospital information system (HIS) were developed in 1950s and late 1960s, respectively. Hospital information system (HIS) is one of the information technology tools that includes all the functions and operations related to the process of providing care to patients in different parts of the hospital [1-5].

In recent years, IT has affected the health sector similar to other sectors and made significant changes in the field of health care and treatment. Many processes in this sector became systematic [2, 3]. In hospital settings, IT in the form of clinical information systems has considerable effects on the improvement of clinical processes and patient satisfaction [4, 6]. It is also interesting that IT may be considered effective by some employees and useless by some others. It is important to pay attention to the facilitators of the acceptance of an information system in the clinical setting in order to achieve the objectives of the health system [6, 7]. The acceptance of IT in health care is markedly different among medical specialists [8] in different institutes and countries [9, 10]. Different factors affect the acceptance of a certain technology in a given hospital including the size of the hospital, dependence on the system, and whether the hospital serves as a teaching hospital [9, 11-15].

There are many publications that focused on the study of acceptance of technology, specifically based on the Technology Acceptance Model (TAM) [16]. This model was developed by Davis et al. it is a valid model

KEY WORDS

Technology acceptance model, Information technology, Hospital information system, Physicians, Nurses

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for predicting the technology by the end users. The model has been used in different areas of health to assess the tendency of the users to accept the technology [17]. Nowadays, many organizations and institutions including the health sector are investing in different technologies; therefore, they should make attempts to accept and use new systems and technologies. In this regard, the use of the verified model based on the TAM for the evaluation of acceptance technology by users is very important [18].

The TAM is one of the technology acceptance analysis models. This model analyzes the user's behavior based on the conditions and the way new technologies are used. The model has been implemented in many field studies and analyzes the necessary explanations on a technology that are required by the user and all the limitations that result in lack of technology acceptance by the user's; these analyses are proportionate by the user's need. This model has constantly developed. The main objective of the TAM is to provide a basis for predicting the effect of the external factors on internal factors [19]. A study by Wu et al (2007) on the utilization of the mobile health technology indicated that self-efficacy was an important and effective factor on the idea of the healthcare staff to use the mobile health program. Computer skills also had a positive effect on the comprehension of the simplicity of the use of HIT applications. They also found out that learning and using the new IT application was easier for care givers with computer skills [20].

Since physicians and nurses are the largest and yet most important users of HISs. Thus, they have central role in the acceptance and success of those systems [21, 22]. Sharifian et al. suggested that nurses' acceptance of HISs was influenced by factors such as performance expectancy, effort expectancy, social influence and facilitating conditions. Furthermore, they declared that performance expectancy had the strongest effect on user intention [23]. Whether the users in the area of health accept HISs indicates its success or failure in that area and organization. On the other hand, with regards to the heavy costs of purchasing and implementing these technologies, they are considered a failure if they are not employed by users, even if they are good and beneficial technologies. As a result, the acceptance of HISs by the users, especially by physicians and nurses is of utmost importance. Therefore, the aim of this study was to evaluate the factors affecting the acceptance of HIS by the physicians and nurses in the teaching hospitals of Hormozgan University of Medical Sciences based on the TAM.

MATERIALS AND METHODS

This descriptive-analytical study was conducted in 2015. The research population was the physicians and nurses working in Shariati, Shahid Mohammadi, and Children's Hospitals of the city of Bandar-Abbas in Iran. The convenient sampling method was used to select 172 persons. The data collection tool was a questionnaire that was designed for the evaluation of IT acceptance (hospital information system) based on the TAM. In this study, we use of the questionnaire that designed by Ping Yu et al. [17] study after obtaining the author permission. This questionnaire included demographic information in addition to 18 questions in 6 domains of behavioral intention (BI) to use IT applications (2 questions), perceived usefulness (PU) of IT applications (4 questions), perceived ease of use (PEOU) of IT applications (4 questions), social norms (SN) regarding of the use of IT applications (2 questions), image in using IT applications (3 questions), and voluntary use of IT applications (3 questions). The questions related to the domains were scored according to a 5-point Likert scale (0: totally disagree, 5 totally agree). In addition, the questionnaire contained one question to determine the total capability of using the computer.

The questionnaire was translated into Persian language. The test-retest method with an interval of 15 days was employed to evaluate the reliability of the questionnaire using the Pearson correlation coefficient ($r=87\%$). The validity of the questionnaire was corroborated by a panel of experts. The researcher attended the aforementioned hospitals in person for data collection and gave the questionnaire to the participants after providing them with necessary explanations on the objective and methodology of the study. SPSS was utilized for data analysis using descriptive and inferential statistics and the Spearman's rho test.

RESULTS

The results of the study showed that most of the participants were female ($n=117$, 63.6%) and aged 30-39 years old (42.9). The majority of the study population were physicians ($n=99$, 53.8%). The highest frequency of work experience was for below 1 year. The highest frequency of computer skill level was related to medium computer skill ($n=73$, 39.7%) [Table 1].

Table 1: Respondent demographics

Characteristics	Number	Percent
Sex	Male	65 35.3
	Female	117 63.6
	No answer	2 1.1

Age	20–29	66	35.9
	30–39	79	42.9
	40–49	32	17.4
	50–59	4	2.2
	60 and above	1	0.5
No answer	2	1.1	
Job level	Physician	99	53.8
	Nursing	84	45.7
	No response	1	0.5
Work experience	Under 1 year	51	27.7
	1-10 year	37	20.1
	11-15	32	17.4
	15-20	19	10.3
	Over 20 years	13	7.1
No answer	32	17.4	
Computer skills	Poor	8	4.3
	Below average	9	4.9
	Average	73	39.7
	Above average	30	16.3
	Good	53	28.8
Excellent	11	6	

The correlations between the four demographic variables were calculated as follow:

Table 2: The correlations between the four demographic variables

	Age	Work experience	Computer skills
Age	-----	0.769*	0.132
Work experience	0.769*	-----	0.769*
Computer skills	0.132	0.769*	-----

A strong and significant correlation ($R^2=0.76$, $p<0.001$) was found between job level and age of respondents. Also a weak, but significant and positive correlation between age and computer skills of respondents ($R^2=13$, $p<0.01$) was determined.

Subjective norm and Voluntariness to use, which were 0.43 and 0.54 respectively, Cronbach's, for the remaining four dimensions were all above the 0.7 threshold and ranged from 0.71 to 0.84. Finding within table 3 indicated that the highest mean was dedicated to measurement items of Perceived usefulness; also the lowest mean was dedicated to measurement items of Voluntariness to use hospital information system. These findings suggest that "Perceived usefulness" and "voluntariness" respectively had the highest and lowest impact on the users of the hospital information system respectively.

Fig. 1 shows the findings of the evaluation of the relationship between the variables of the TAM indicating the positive effect of image in using IT applications (image) on PU and the positive effect of computer skill level on PEOU as well as its direct effect on BI. Social Norms only had a positive effect on PEOU. In fact, 67% of the PEOU changes were determined by computer skills and SN; as for the linear regression equation, the effect of computer skills and SN on PEOU was 0.40 and 0.65, respectively ($PEOU = 0.65*SN + 0.40*Computer$).

Moreover, 34% of the PU changes were determined by PEOU and image; the effect of image and PEOU on PU was 0.26 and 0.46, respectively. Its regression equation is as $PU = 0.46*PEOU + 0.26*Image$. In addition, PEOU only had a positive effect on PU with no effect on BI; the effect of PU and computer skills on BI was 0.61 and 0.30, respectively, and 52% of the BI changes were determined by computer skills and PU ($BI = 0.61*PU + 0.30*Computer$).

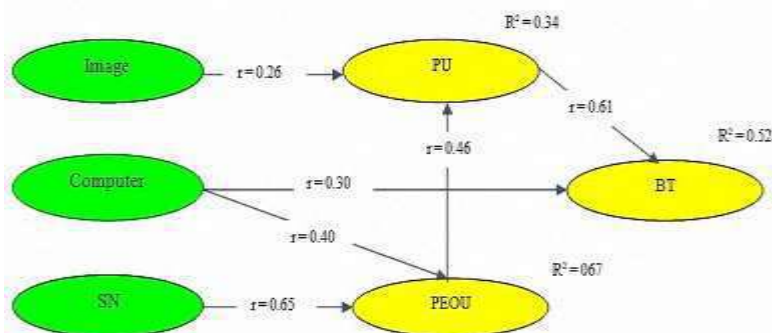


Fig. 1: The relationship between the variables of the TAM

Table 3: Summary of the measurement of hospital information system acceptance dimensions (based on five-point Likert scale ranging from 1: strongly disagree to 5: strongly agree)

Dimensions	Cronbach's α	Measurement items	Mean	S.D.	Min.	Max.
Behavioral intention (BI)	0.724	If no significant barriers exist, I	3.82	0.87	1	5

		would consider use IT.				
		Given the opportunity, I would like to use IT.	4.14	0.78	1	5
Perceived usefulness (PU)	0.842	Using IT could enhance my effectiveness.	4.21	0.79	1	5
		Using IT may improve my job performance.	4.22	0.77	1	5
		Using IT may enhance the quality of my work.	4.19	0.81	1	5
		Using IT is relevant to the delivery of residential care.	3.88	0.86	1	5
Perceived ease of use (PEOU)	0.798	Learning electronic documentation is easy for me.	3.59	0.94	1	5
		Using IT is compatible with all aspects of my work	3.56	0.96	1	5
Subjective norm (SN)	0.430	I find electronic documentation system easy to use.	3.32	0.93	1	5
		Electronic documentation system is not cumbersome to use.	3.03	1.02	1	5
		My manager influences my intention to use IT.	3.49	0.99	1	5
		My colleagues will encourage me to use IT.	3.30	1.02	1	5
Image	0.714	People at other aged-care facility who use IT have more prestige than those who do not use.	3.36	1.03	1	5
		People at other aged-care facility who use IT have a high profile.	3.14	1.03	1	5
		Using IT is a status symbol of my aged-care facility.	3.55	0.94	1	5
Voluntariness	0.541	Although it might be helpful, using IT is certainly not compulsory in my job.	3.03	1.11	1	5
		My supervisor does not require me to use IT.	2.82	1.09	1	5
		My use of IT is voluntary.	3.32	1.10	1	5
Work experience	----	How long have you worked in aged and community care sector?	9.93	7.18	1	36
Computer skill	----	How would you rate your current ability to use computers in general?	3.78	1.21	1	6

DISCUSSION

The results of the present study showed that the image of using the technology is an important determinant of PU, because there was a direct and positive relationship between the variables of image and PU ($R=0.26$, $P<0.01$). A study by Ping Yu et al. revealed that the image of IT use had no effect on PU [17]. Compare the results of present study with a similar study indicate lack of agreement of these studies. Moreover, the level of computer skills had a direct positive effect on PEOU ($R=0.40$, $P<0.01$) and BI ($R=0.30$, $P<0.01$).

The results of the study by Ping Yu et al. showed that the level of computer skills had a significant positive effect on BI ($R=0.41$, $P<0.001$) and PEOU ($R=0.18$, $P<0.05$) [17]. Wu et al. also reported that computer skills had a positive effect on PEOU of HIS applications [20]. The results of our study are in line with the findings of these studies.

Furthermore, SN only had a positive direct effect on PEOU ($R=0.65$, $P<0.001$). Langarizade et al. [24] and Ping Yu et al. [17] showed the significant positive effect of BI on SN ($R=0.38$, $P<0.01$ and $R=0.26$, $P<0.01$). It could be stated that SN is an important and influential factor on PEOU. PEOU had no effect on BI but had a direct positive effect on PU ($R=0.46$, $P<0.001$) although Langarizadeh et al. [24] reported that BI had a positive effect on PEOU ($R=0.46$, $P<0.01$). Furthermore, Ping Yu et al. [17] also reported a positive direct relationship on BI. The results of the present study are different from the findings of other studies in this regard; other studies showed the effect of PEOU on BI while we did not find such an effect.

Moreover, PU had a positive direct effect on BI ($R=0.61$, $P<0.001$). The study by Langarizadeh et al. [24] also revealed that BI had a significant positive effect on PEOU ($R=0.52$, $P<0.01$). In addition, Ping Yu et al. [17] confirmed a positive direct relationship between BI and PU ($R=0.30$, $P<0.01$). The findings of the present study are in line with these results. In our study, the voluntary use of IT had no significant relationship with other variables, which is similar to the results of the studies by Langarizadeh et al. [24] and Ping Yu et al. [17].

CONCLUSION

Finally, it could be concluded that most of the evaluated variables were more or less effective on the acceptance of information technology by users. In this study, image in using IT, the level of computer skills, SN, PEOU, and PU had a more prominent role in the acceptance of information technology by the users. Therefore, it is necessary to consider these variables while designing, purchasing, or revising hospital information systems. The voluntary use of information technology had no significant relationship with other evaluated variables.

CONFLICT OF INTEREST

The author declares no competing interest in relation to the work.

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FINANCIAL DISCLOSURE

None

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ARTICLE

EFFECT OF FOLIAR SPRAY OF CALCIUM AND ZINC ON YIELD, NUTRIENTS CONCENTRATION AND FRUIT QUALITY OF ORANGE

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ABSTRACT

Balanced nutrients application is essential to increase yield, improve quality and prevent contaminations. Orange orchards in the calcareous soil widely suffer from calcium (Ca) and zinc (Zn) deficiencies because they are immobile in plants while there are abundant of calcium carbonate in the soil. In order to supply Ca and Zn for orange fruits, a study was conducted in the south western of Iran. The foliar application of calcium and zinc on yield, calcium, zinc and boron concentration and some quality Characteristics of orange (cv.valencia) was conducted in two growing seasons as a factorial design based on completely randomized block with nine treatments and three replications. Treatments were spraying of calcium chloride at three levels (0, 0.25%, and 0.5%) and foliar of zinc spraying from zinc sulfate at three levels (0, 0.25%, and 0.5%) at fruitlet and repeated twice every 30 days. Results showed that interaction of calcium and zinc on yield has been significant ($p \leq 0.05$) and increased Ca and Zn levels in the foliage. Meanwhile, the boron (B) status decreased by Ca application in 0.25% concentration significantly. Ca and Zn foliar treatments both resulted on B reduction in the orange leaves. Since orange orchards in the region shows visual of high boron symptoms, combined Ca and Zn spraying reduced boron toxicity effect rather than solely application.

INTRODUCTION

Balanced nutrients application is essential to increase yield, improve quality and prevent contaminations. Calcium is vital nutrient that improves quality and yield [1]. Due to high calcium carbonate in the calcareous soils of the southwestern of Iran, it is expected to observe no calcium deficiency in the orchards [2]. Regarding the low soil moisture and light texture exchangeable calcium is not adequate in the soil solution and translocation of calcium in the developing fruits is not properly efficient due to poor evaporation. The concentration of calcium in the soil solution is not sufficient when the soil moisture is drastically low and consequently solubility of calcium decreases. Citrus orchards in the calcareous soil suffer from calcium (Ca) and zinc (Zn) deficiencies because they are immobile in plants while there are abundant of calcium carbonate in the soil [2]. The movement of calcium in the plant is very slow and it is mostly transmitted by xylem vessels [1]. Therefore, providing the necessary amount of calcium is not possible for fruits when they are developing fast. Fruits are faced with calcium deficiency which will rise physiological disorders due to calcium deficiency in the fruit during the growing season and cause low shelf life and decreases marketable yield [2]. Paul and Shaha [3] reported that fruits are not usually a good source of calcium. They also showed that only a few of citrus fruits significantly contain calcium out of which lemon has the most calcium. Sando et al. [4] observed that foliar application of calcium chloride did not change the fruit quality and only led to an increase in the middle skin of fruits. Dong and et al. [5] reported that the use of calcium and boron on the orange caused improvement of fruit tissues and cell membrane and influenced on the activity of enzymes such as polygalacturonase, pectinesterase and β -galactosidase. According to the report of FAO of 30 countries from all over the world, more than 30 percent of the agricultural soils are faced with available zinc deficiency problem for crop and horticultural plants [6]. The results of studies on agricultural soils of Iran and in the region where the study was conducted, confirm that zinc deficiency is common in these soils [7]. According to Malakouti et al. [7] Zinc deficiency is a widespread and common micronutrient deficiency in Iran due to lack of zinc in the soils, calcareous condition and high pH. After nitrogen, zinc deficiency is the most widespread nutritional disorder in citrus [8, 9].

Boron is one of the micronutrients whose sufficiency and toxicity range is very low, unlike other micronutrients [10]. Irrigation water has been a major proof of boron toxicity in the south of Iran [11]. Citrus is very sensitive to the boron deficiency and toxicity and their tolerance has reported as about 0.3 mg per kilogram of soil [12]. Zinc is used as an essential component for the activation of some enzymes in plant whose sufficient level is effective in the absorption and uptake of boron by plants [13]. Mahmoudi et al. [2] reported that foliar spray of calcium chloride increased calcium concentrations and decreased TSS in orange. Tariq et al. [14] reported that zinc sulfate treatment reduced the concentration of boron in the leaves of orange, but this reduction was not significant. In addition, the application of boron decreased the concentration of zinc in the plant, but it was also not significant. A similar result has been found in almonds by others [15, 16]. Contrasting results have been presented by researchers on the interaction of calcium and boron as Rostami et al. [17] reported that the application of boron significantly decreased calcium concentration in olive foliage. Some reports have indicated the positive interaction or lack of interaction of these two elements [18, 19]. In general, by attention to suffering citrus orchards in the calcareous soil from calcium (Ca) and zinc (Zn) deficiency. On the other hand, the concentration of boron is higher than sufficiency range (98 ± 29 mg per kg). Therefore, investigating the effect of calcium and zinc on boron concentration by aiming to the improvement of the toxicity of this nutrient is necessary.

KEY WORDS

Foliar application, Nutrients balance, Zinc, Calcium

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Accordingly, aim of this study is investigating the effect of foliar application of calcium and zinc on the yield and quality, control of boron concentration in the leaves.

MATERIALS AND METHODS

The experiment was conducted in an orchard located at gachsaran region with latitude 30°, 15' N and longitude 50°, 45' E in southwestern of Iran. This area is located in hot and semiarid climate, average rainfall of 425 mm, mean altitude 900 m and a loamy soil texture. At the first, among orange orchards one orchard has been selected in which boron concentration of leaf was higher than the desirable rang (98±29 mg kg⁻¹) that was determined by compositional nutrient diagnosis (CND) method [20]. Soil samples were collected from 0-30 and 31-60 cm depth and compose leaf sample was taken from non-bearing current shoots. The physical and chemical properties of the soil consisted soil texture was measured by the hydrometer method, electrical conductivity in saturated extract by conductimeter, pH with pH meter, calcium carbonate percent by titration, organic carbon by walkley-black [21], available K by ammonium acetate and flame photometer and available P using Olsen method [22]. Extractable Fe, Mn, Zn and Cu were extracted using DTPA solution and measured by atomic absorption spectrometry [23]. Boron was extracted with hot water and measured by Azomethien-H colorimetric method [23]. Total nitrogen content in leaf was measured by micro-kjeldahl, P by spectrophotometer and K by flame photometer. Concentration of Ca, Mg, Zn, Mn, Fe, and Cu in leaf was measured in dry ash extracted with Hcl by atomic absorption spectrophotometer. B concentration was determined by colorimetric Azomethin-H method with spectrophotometer [23]. The physical and chemical properties of soil and nutrient concentration of leaf are presented in Table 1 and 2, respectively.

The field experiment was laid out during 2013-2014 growing seasons as a factorial design on the base of completely randomized block with nine treatments, three replications and two trees per replication. Foliar treatments consisted of three levels of calcium chloride (0, 0.25 and 0.5 percent) and zinc sulfate (0, 0.25 and 0.5 percent), three times on the trees during fruitlet stage and repeated twice every 30 days. Other nutrients were applied according to soil and leaf analysis similarly in all treatments. Concentration of B, Ca and Zn in leaf was measured in the five-month leaves samples. At harvested time, yield and concentration of B, Ca and Zn in fruit were measured. Also, some quality characteristics of fruit juice such as TSS, TA were measured. Statically analysis variance on data was done with SAS and comparison of means was done by Duncan Multiple Rang Test.

Table 1: Physical and chemical properties of the orange orchard soil used in the study

Soil depth (cm)	Soil texture				Soil characteristics (mg kg ⁻¹)											
	Sand %	Silt %	Caly (%)	Texture	OC (%)	CCE (%)	pH	EC (dS m ⁻¹)	P	K	Fe	Zn	Mn	Cu	B	
30-0	48	52	20	L	0.86	49.75	7.3	0.55	4.9	224	4.68	0.42	13.4	0.46	0.682	
60-31	60	20	20	Sa-Cl-L	0.37	57.57	7.2	0.92	3.3	140	3.82	0.28	7.58	0.34	0.316	

Table 2: Concentration of nutrient elements in leaves orange orchard used in this study

N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
%					mg kg ⁻¹				
2.1	0.14	2.1	2.8	0.29	71	34	15	6	141

RESULTS AND DISCUSSION

Analysis of variance for yield, concentration of Calcium (Ca), Zinc (Zn) and Boron (B) in leaf and fruit of orange are shown in Table 3. The results indicated that interaction of foliar spraying of calcium and zinc on yield was significant (P≤0.01), [Table 3].

Table 3: Analysis of variance for yield, Calcium (Ca), Zinc (Zn) and Boron (B) concentration in leaf and fruit of orange

Source of variation	Df	MS							
		Yield	Ca		Zn		B		
			Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	
Replication	2	13.89 ^{n.s}	0.125 ^{n.s}	0.032 ^{n.s}	1549.65 ^{n.s}	0.015 ^{n.s}	346.36 ^{n.s}	0.085 ^{n.s}	
Calcium(Ca)	2	10.91 ^{n.s}	1.976 ^{*.*}	0.012 ^{n.s}	14761.27 ^{*.*}	0.003 ^{n.s}	2283.54 [*]	0.077 ^{n.s}	
Zinc(Zn)	2	37.24 ^{n.s}	0.353 ^{n.s}	0.009 ^{n.s}	395159.55 ^{*.*}	0.213 [*]	1634.08 ^{n.s}	0.660 ^{n.s}	
Ca×Zn	4	83.03 ^{*.*}	0.138 ^{n.s}	0.018 ^{n.s}	2644.729 [*]	0.006 ^{n.s}	1397.93 ^{n.s}	0.693 ^{n.s}	
Error	16	13.28	0.212	0.015 ^{n.s}	736.42	0.048 ^{n.s}	603.11	0.360 ^{n.s}	
C.V		12.99	11.57	23.45	10.25	22.07	18.62	22.6	

ns, * and **: respectively non-significant, significant at (P≤0.05) and (P≤0.01) probability levels

Comparison of mean yield using Duncan test from Table 4 shows that interaction of calcium and zinc has significantly increased yield (kg tree⁻¹) compared to control. The highest increasing of yield compared to control, has been with zero level of calcium and 0.25% of zinc sulfate with an average of 36.53 kg per tree

which has resulted in an increase of 14 kg in each tree compared to control. Obviously, in various levels of zinc, yield in second and third levels of calcium does not differ significantly.

Table 4: The interaction effect of calcium chloride and zinc sulfate foliar spray on mean yield (kg tree⁻¹) of orange

Treatment	Ca ₀	Ca ₁	Ca ₂	Mean
Zn ₀	22.63d [*]	30.43abc	31.43ab	28.16A
Zn ₁	36.52a	26.25bcd	27.37bcd	30.05A
Zn ₂	26.72bcd	23.71cd	27.52 bcd	25.98A
Mean	28.62A	26.80A	28.77A	

Means in each row and column with the same letters are not significantly different ($P \leq 0.05$) using DMRT
 Ca₀= control, ca₁ and Ca₂, foliar spray of calcium chloride with concentration of 0.25 % and 0.5 % respectively
 Zn₀= control, Zn₁ and Zn₂, foliar spray of zinc sulfate with concentration of 0.25 %, and 0.5 % respectively

Seyyed Kalayi et al. [9] reported that spraying zinc sulfate with a concentration of 0.3% has increased yield of orange significantly. In addition, they concluded that using zinc sulfate spraying possibly has increased auxin concentration. They mentioned increasing auxin concentration and decreasing fruit fall, is one of the reasons for yield increase. Tryptophan is the substrate of auxin synthesis and has been reported that zinc is important synthesis of this substrate [24, 9]. Asadi Kangar Shahi et al. [8] concluded that using zinc has increased orange yield significantly. Others have reported the positive effect of zinc, especially in the form of being sprayed, on yield of crops such as olive, almond, pomegranate, corn and wheat [9, 24, 25].

Concentration of Calcium, Zinc and Boron in leaf

Investigating the results from analysis variance of data related to calcium concentration from Table 3 shows that only the effect of foliar spraying of calcium on this trait has been significant ($P \leq 0.01$). The effect of using zinc and interaction of calcium and zinc on this trait was not significant [Table 3]. Comparison of mean calcium concentration by Duncan test at ($P \leq 0.05$) from Fig 1 shows that by increasing calcium, its concentration in leaf increased significantly in such a way that the highest concentration was related to calcium level of 0.05%. Obviously, no significant difference was observed between two levels of 0.25 and 0.05 % calcium chloride. Regarding that in foliar spraying this element is supplied for leaves directly, this concentration increase seems reasonable.

The results from analysis variance of zinc concentration in leaf from Table 3 show that the main effect and interaction of calcium chloride and zinc sulfate foliar spraying has been significant at ($P \leq 0.01$) and ($P \leq 0.05$) respectively.

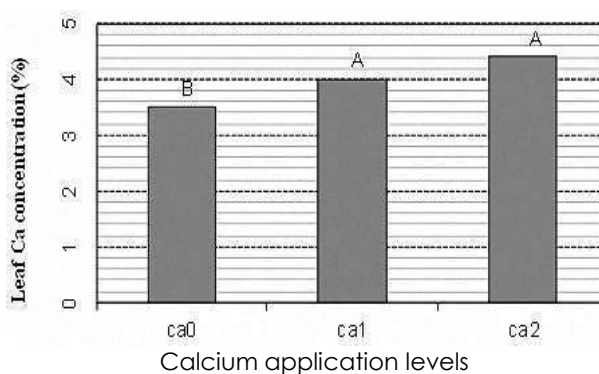


Fig. 1: The effect of different levels of foliar spray of calcium chloride on calcium concentration in orange leaf
 Ca0= control, ca1 and Ca2, foliar spray of calcium chloride with concentration of 0.25 % and 0.5 % respectively

Comparing mean concentration of zinc from Table 5 shows that combine application of calcium chloride and zinc sulfate has significantly increased zinc concentration in leaf compared to control. The highest mean of zinc concentration has been created by combine foliar spraying of calcium chloride and zinc sulfate with a concentration of 0.25 and 0.05 % respectively. Although there is no significant difference between second and third level of zinc sulfate treatment.

Table 5: The interaction effect of calcium chloride and zinc sulfate foliar spray on the mean zinc concentration (mg kg⁻¹) in leaf of orange

Treatment	Ca ₀	Ca ₁	Ca ₂	Mean
Zn ₀	16.27d [*]	36.45d	19.8d	24.17C
Zn ₁	293.37c	395.83a	399.8a	363.00B
Zn ₂	344.73b	440.23a	436.53a	407.17A
Mean	218.12B	290.84A	285.38A	

* Means in each row and column with the same letters are not significantly different ($P \leq 0.05$) using DMRT
 Ca₀= control, Ca₁ and Ca₂, foliar spray of calcium chloride with concentration of 0.25 % and 0.5 % respectively
 Zn₀= control, Zn₁ and Zn₂, foliar spray of zinc sulfate with concentration of 0.25 %, and 0.5 % respectively

Obviously, in various levels of zinc, there is no significant difference between second and third levels of calcium chloride. Considering the increasing of zinc concentration in leaf by spraying calcium chloride, it will be better to use calcium in low levels of zinc to increase zinc concentration in leaves [Table 5]. Mahmoudi et al. [2] reported that using calcium chloride in orange has increased zinc concentration; however, this increase has not been significant. One of the reasons of increasing yield is the positive effect of calcium and zinc on zinc concentration in orange. Considering the role and importance of zinc in physiologic activities of this plant, increasing yield is expected [2, 24, 8, 9].

Results from statistical analysis of leaf boron concentration from Table 3 show that only the main effect of calcium on this trait is significant ($P \leq 0.05$). Comparison of mean for boron concentration from Fig 2 indicates that calcium application levels have decreased leaf boron concentration. The highest decrease has been seen in 0.25 % concentration of calcium chloride.

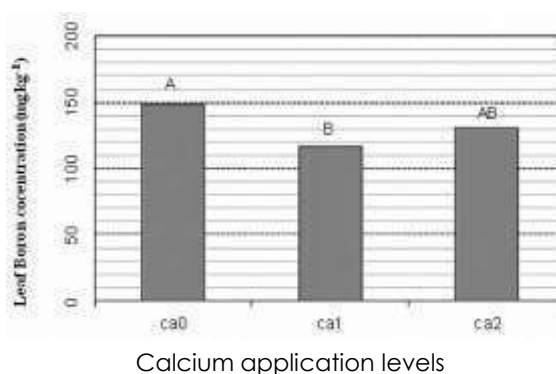


Fig. 2: The effect of different levels of foliar spray of calcium chloride on leaf boron concentration in orange
 Ca₀= control, Ca₁ and Ca₂, foliar spray of calcium chloride with concentration of 0.25 % and 0.5 % respectively

In addition, combined foliar of calcium and zinc in 0.25 % concentration has decreased boron levels in leaf samples to 100.3 mg kg⁻¹. The most decrease was belonging to this concentration. In this regard, Chakerolhosseini et al. (2016), desirable range of boron concentration in orange leaf using Compositional nutrient diagnosis (CND) was determined to be 98±29 mg kg⁻¹. Combined calcium and zinc application in 0.25 % doeses has been able to decrease the concentration of boron to sufficient range.

Rostami et al. [17] concluded that there is a significant negative interaction between boron and calcium in olive. Hosseini et al. [25] also reported this interaction between boron and zinc in corn. They recommended that in areas where boron concentration is higher than optimal level, especially when there is zinc deficiency, a sufficient amount of zinc should be administered for the plant. In addition, in studies conducted on wheat [18] and tomato [19], they were concluded that there is a positive interaction between these two nutrients.

Concentration of Calcium, Zinc and Boron in fruit

Results in Table 3 show that among these three concentrations, only the effect of zinc sulfate foliar spraying on concentration of zinc in fruit is significant ($P \leq 0.05$). In Figure 3, foliar application of zinc sulfate has significantly increased zinc concentration in fruit in two applicable levels compared to control. However, no significant difference was observed between two levels of 0.25 and 0.05 % zinc sulfate.

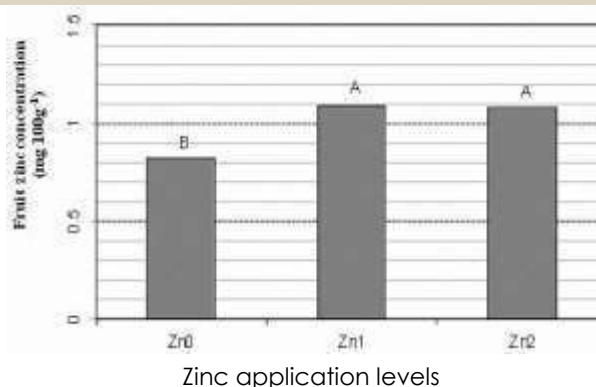


Fig. 3: The effect of different levels of foliar spray of zinc sulfate on zinc concentration in orange fruit Zn₀=control, Zn₁ and Zn₂, foliar spray of zinc sulfate with concentration of 0.25 %, and 0.5 % respectively

The reason for this increase is direct supply of zinc on fruit and indirect Zn uptake by leaf and transferring to fruits [16]. Comparison of mean for calcium and boron concentration in fruits analysis demonstrated that using calcium chloride has increased calcium concentration in orange fruit. This increase is not significant, but considerable. Using calcium and zinc has decreased mean boron concentration of fruit in all applicable levels compared to control; however, this is not significant. In this regard, Rostami et al. [17] reported that using boron has significantly decreased calcium concentration in leaf. However, some reports demonstrate positive interaction or no interaction between calcium and boron [18, 19].

Total Soluble Solids (TSS), pH and Total Acidity (TA) of orange Juice

The results from statistical analysis of these three properties (TSS, pH and TA) revealed that only the effect of calcium chloride spraying on TSS and pH of orange juice is significant ($P \leq 0.05$). Comparison mean of TSS and pH in Table 6 shows that calcium application levels decreased and increased these traits respectively.

Table 6: The effect of calcium chloride foliar application on the mean of TSS and pH in orange juice

Calcium application levels	TSS	pH
Ca ₀	11.69A	3.1B
Ca ₁	10.49B	3.3AB
Ca ₂	9.98B	3.4A

Means in each column with the same letters are not significantly different ($P \leq 0.05$) using DMRT
 Ca₀= control, ca₁ and Ca₂, foliar spray of calcium chloride with concentration of 0.25 % and 0.5 % respectively

Total soluble solids (TSS) indirectly includes the amount of sugars, organic acids, anthocyanin and other soluble materials and by creating alkaline conditions, increase pH [Table 6], calcium can decrease organic acids and consequently the amount of TSS decreases. In addition, by creating bonds with carboxyl groups, calcium stabilizes cell walls and reduces soluble compounds [26]. Another reason is, creating a relatively thick layer on fruit by calcium and postponing change processes including hydrolysis of polysaccharides and finally decreased concentration of soluble minerals [27].

CONCLUSION

The results of this study showed that the combined application of calcium and zinc has significantly increased yield compared to control. The effect of spraying calcium and zinc on calcium and zinc concentration in leaf and fruit was significant. By increasing calcium chloride, Ca concentration in leaf increased significantly, but no significant difference was observed between two levels of 0.25 and 0.05 % calcium chloride. Combined application of calcium chloride and zinc sulfate has significantly increased zinc concentration in leaf compared to control. The highest mean of zinc concentration has been created by combine foliar spraying of calcium chloride and zinc sulfate with a concentration of 0.25 and 0.05 % respectively. Although there is no significant difference between second and third level of zinc sulfate treatment. Considering the increasing of zinc concentration in leaf by spraying calcium chloride, it will be better to use calcium in low levels of zinc to increase zinc concentration in leaves. One of the reasons of increasing yield is the positive effect of calcium and zinc on zinc concentration in orange. The results also showed that calcium application levels have significantly decreased leaf boron concentration. Combined calcium and zinc application in 0.25 % doses has been able to decrease the concentration of boron to sufficient range. Foliar application of zinc sulfate has significantly increased zinc concentration in fruit. According to presented results, the 0.25% CaCl₂ + 0.25% ZnSO₄ as foliar spray was suitable combination for the most of measured characters in orange during the course of this experiment.

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ARTICLE

A COMPARATIVE STUDY OF THERAPEUTIC EFFECTS OF DOXEPIN AND CETIRIZINE IN PATIENTS WITH ALLERGIC RHINITIS: A RANDOMIZED DOUBLE-BLIND CLINICAL TRIAL

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ABSTRACT

Allergic rhinitis is a common disease presenting in 20% of the population. Major symptoms are including sneezing, rhinorrhea, nasal congestion, and nasal pruritus. It is seemed that tricyclic antidepressants blocking histamine receptors might be applied as an effective treatment in allergic rhinitis. In the current clinical trial, a total of 84 subjects with allergic rhinitis were enrolled and randomly assigned to 2 groups. Both groups were administered cetirizine and doxepin for 2 weeks. The subjects were evaluated in terms of sneezing, rhinorrhea, nasal congestion, and nasal pruritus after 2 weeks of taking the aforementioned medications. There was no difference in the clinical score of the patients after 2 weeks ($p = 0.261$). Sneezing was the only symptom that was affected by the type of remedy, it was significantly different between the groups ($p = 0.005$). The findings of the present study indicated that there is no substantial difference in taking cetirizine and doxepin in treating seasonal allergic rhinitis symptoms. Administering TCAs with more potency of blocking histamine receptors and larger population are necessary for future studies.

INTRODUCTION

Allergic rhinitis is a common disease presenting in 20% of the population [1]. Allergic rhinitis is a general term for seasonal allergic rhinitis, perennial allergic rhinitis, and perennial allergic rhinitis with seasonal severity. Seasonal allergic rhinitis and perennial allergic rhinitis are presenting in 20% and 40% of the cases, respectively, while 40% of the subjects have a combination of the aforementioned disorders. As for the high prevalence of allergic rhinitis, and co-morbidities such as atopy and asthma affect the society [2]. The seasonal allergic rhinitis is usually initiated with trees' and plants' allergens. The major symptoms are including sneezing, rhinorrhea, nasal congestion, nasal or pharyngeal pruritus [3]. The histamine is the most effective mediator in the preliminary phase of the disease, presenting of this factor was approved in the most symptoms [4]. The symptoms such as sneezing, pruritus, tearing and rhinorrhea are greatly adjusted by H₁ receptors [5]. There are different remedies for treating allergic rhinitis. The most common treatments for allergic rhinitis are including antihistamines, decongestions, and leukotriene regulators and inhaled corticosteroids [6]. In treating allergic rhinitis, topical corticosteroids including beclomethasone, fluticasone, and mometasone together with new generation of antihistamines including loratadine, cetirizine, fexofenadine, and ketotifen are administered as the first line of treatment [7]. Also, the histamine is considered as an important mediator in creating acute and chronic urticaria [8], the antihistamines are used as the selective treatment of urticaria [9]. The tricyclic antidepressants (TCAs) are effective medications in treating urticaria as well [10].

The TCAs are potent inhibitors of H₁ and H₂ receptors. The biochemical, pharmacological, and behavioral similarities were demonstrated in TCAs and some of the antihistamines. It should be kept in mind that TCAs are categorized in antihistamine medicines [11]. Doxepin hydrochloride is a TCA with the highest activity of antihistaminic feature that is stronger than diphenhydramine and hydroxyzine being 775 and 56 times than that, respectively [12]. Doxepin as an H₂ receptor inhibitor is 6 times stronger than cimetidine [13, 14]. In vitro and in vivo studies indicated that doxepin inhibits histaminic receptors in the wall of smooth muscles of the vessels; this feature can commonly be used to treat chronic pruritus and urticaria [7, 15]. Furthermore, anti-muscarinic, anti-serotonergic, and anti-adrenergic features were observed in doxepin [13, 16]. Literatures indicated that doxepin suppressed induced response by histamine [17].

Given consideration to the known pharmacological effect of doxepin as a tricyclic antagonist of H₁ and H₂ receptors, classic taking and its therapeutic indications in reducing allergic symptoms, also with individual experiences in treating allergic rhinitis, and similarity of the mechanism and interaction of the neurotransmitters in allergic rhinitis and classic therapeutic indications of doxepin (e.g., treating

KEY WORDS

Allergic rhinitis, Doxepin, Cetirizine, Treatment

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headaches relating to migraine, tension headaches, pains in face and head, sleep and behavioral disorders, and anxiety disorders) and high prevalence of the aforementioned diseases and their correlation with allergic rhinitis, also low rate of medication's side effects, low and daily dosage, possible effectiveness, and influence on chronic headache, we have decided to compare doxepin and cetirizine effects on patients with allergic rhinitis.

MATERIALS AND METHODS

This study is a clinical trial performed on the allergic rhinitis patients referred to the ENT clinic of Amir-Kabir hospital. At the first stage, the patients filled out the consent form and enrolled in the study. The subjects were randomly assigned to 2 groups based on block design. Both groups were administered 10 mg cetirizine and 10 mg doxepin daily for 2 weeks, respectively. The Total Nasal Symptom Score (TNSS) of the patients was recorded based on the severity and duration of the allergic rhinitis symptoms after diagnosing allergic rhinitis, the symptoms of allergic rhinitis are including rhinorrhea, nasal pruritus, sneezing, and nasal congestion. This score is in the range of zero to three (zero: no sign, one: The symptoms less than 30 minutes per day, two: The symptoms from 30 minutes to 2 hours per day, three: The symptoms more than 2 hours per day) [18-20]. After 2 weeks of treating the subjects, the symptoms were re-assessed based on TNSS in each group. To observe the blindness, the classifying of the groups and administrating medications were carried out by ENT specialist, documenting the clinical symptoms was undertaken by the author via calling to the subjects. Finally, the data obtained were analyzed by SPSS software version 19 via t-tests and compared by one-tailed variance.

The inclusion criteria were patients aged from 8 to 55 years old, history of allergic rhinitis for at least 2 years. The exclusion criteria were patients with the history of asthma, acute sinusitis, upper respiratory tracts infection, taking antihistamines in 2 weeks previously, patients with deformity of nose such as polyp, pregnant and feeder women, history of psychological disorders including schizophrenia, Post-Traumatic Stress Disorder (PTSD), and mania, also allergy to doxepin, mono-amino oxidase inhibitors and cimetidine.

RESULTS

A total of 84 patients with allergic rhinitis were enrolled in the current clinical trial, then, they were randomly assigned to 2 groups, and were administered doxepin and/or cetirizine. Forty five patients were female (53.6%) and the remainder were male. In the view of gender, there was no significant difference between two groups ($p=0.512$). The mean of age in doxepin and cetirizine groups was estimated as being 33.16 ± 11.06 , and 33.42 ± 13.88 , respectively. In terms of age, there was no significant difference between two groups ($p=0.54$).

The patients were assessed in terms of rhinorrhea, nasal pruritus, and sneezing, as well as nasal congestion, afterwards, the severity of the symptoms was recorded. The data obtained, indicated that there is no significant difference between two groups in terms of symptoms severity. The maximum score was 12 including all of the symptoms, the score 0 was considered for the patients with no symptom. There was no substantial difference between two groups in terms of score mean before receiving medication ($p=0.385$). Furthermore, the findings demonstrated that there is no significant difference between two groups in terms of score 2 weeks after receiving medication ($p=0.261$). The score mean in doxepin and cetirizine groups was 4.40 ± 3.43 , and 2.59 ± 3.19 , respectively. Moreover, the findings indicated that there is no significant difference between two groups in terms of gender segregation (males: $p=0.390$, and females: $p=0.488$).

The subjects were also evaluated in terms of symptoms. Firstly, rhinorrhea was investigated 2 weeks after receiving medication. In doxepin group, rhinorrhea was not observed in 18 subjects (42.9%), rhinorrhea was observed in 5 (11.9%), 8 (19%), and 11 (26.2%) patients less than 30 minutes, from 30 to 120 minutes, and more than 120 minutes, respectively. In cetirizine group, rhinorrhea was not observed in 18 subjects (42.9%) 2 weeks after treatment, whereas rhinorrhea was observed in 14 (33.3%), 3 (7.1%), and 7 (16.7%) patients less than 30 minutes, from 30 to 120 minutes, and more than 120 minutes, respectively. The data obtained showed that there is no significant difference between two groups in terms of rhinorrhea ($p=0.06$) [Table 1]. In doxepin group, nasal pruritus was not observed in 14 subjects (33.3%), while nasal pruritus was observed in 8 (19%), 10 (23.8%) and 10 (23.8%) patients less than 30 minutes, from 30 to 120 minutes, and more than 120 minutes, respectively. In cetirizine group, nasal pruritus was not observed in 24 subjects (57.1%), whereas nasal pruritus was observed in 8 (19%), 4 (9.5%), and 6 (14.3%) patients less than 30 minutes, from 30 to 120 minutes, and more than 120 minutes, respectively. There was no significant difference between two groups in terms of nasal pruritus ($p=0.102$).

Table 1: The severity of rhinorrhea in doxepin and cetirizine groups

Groups	Lack of rhinorrhea N (%)	Less than 30 min N (%)	Between 30 to 120 min N (%)	More than 120 min N (%)
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Doxepin	18 (42.9)	5 (11.9)	8 (19)	11 (26.2)
Cetirizine	18 (42.9)	14 (33.3)	3 (7.1)	7 (16.7)
Total	36 (42.9)	19 (22.6)	11 (13.1)	18 (21.4)

In doxepin group, sneezing was not observed in 13 subjects (31%), while sneezing was observed in 9 (21.4%), 13 (31%), and 7 (16.7%) patients less than 30 minutes, from 30 to 120 minutes, and more than 120 minutes, respectively. In cetirizine group, sneezing was not observed in 29 subjects (69%), whereas sneezing was observed in 6 (14.3%), 5 (11.9%), and 2 (4.8%) less than 30 minutes, from 30 to 120 minutes, and more than 120 minutes, respectively. There was no significant difference between two groups in terms of sneezing ($p=0.005$) [Table 2].

Table 2: The severity of sneezing in doxepin and cetirizine groups

Groups	Lack of rhinorrhea N (%)	Less than 30 min N (%)	Between 30 to 120 min N (%)	More than 120 min N (%)
Doxepin	13 (31)	9 (21.4)	13 (31)	7 (16.7)
Cetirizine	29 (69)	6 (14.3)	5 (11.9)	2 (4.8)
Total	42 (50)	15 (17.9)	18 (21.4)	9 (10.7)

Lastly, the nasal congestion was investigated in two groups. In doxepin group, nasal congestion was not reported in 31 subjects (73.8%), while nasal congestion was reported in 3 (7.1%), 5 (11.9%), and 3 (7.1%) patients less than 30 minutes, from 30 to 120 minutes, and more than 120 minutes, respectively. In cetirizine group, nasal congestion was not reported in 35 subjects (83.3%), whereas nasal congestion was reported in 3 (7.1%), 3 (7.1%), and 1 (2.4%) patients less than 30 minutes, from 30 to 120 minutes, and more than 120 minutes, respectively. There was no significant difference between two groups in terms of nasal congestion severity ($p=0.628$).

DISCUSSION

Comparison of doxepin and cetirizine effects was carried out on patients with allergic rhinitis. This is the first study, to our knowledge, investigated the effect of an antihistamine and a TCA on treating allergic rhinitis. The findings of the current study indicated that there is only significant difference between two groups in terms of sneezing. In this case, cetirizine had a more substantial effect than doxepin. Our findings demonstrated that cetirizine had a more remarkable impact than doxepin in reducing clinical symptoms score (cetirizine score as being 2.59 as compared to 4.40 for doxepin). Thus, there was insignificant difference between scores. Cetirizine as an antihistamine is the most common used medication for treating the symptoms resulted from over-release of histamine. In a study of comparing cetirizine and fexofenadine effects upon patients with seasonal allergic rhinitis, the same findings were reported [20]. A similar study was carried out by Charpinetal. on comparing azelastine nasal spray and cetirizine on reducing seasonal allergic rhinitis symptoms, the findings were reported the same [21]. In the study of Salmun et al. on comparing somnolence and motivation after taking loratadine and cetirizine, cetirizine led to more somnolence than loratadine as well as lower motivation [22]. Nevertheless, the effects of cetirizine and loratadine were evaluated in terms of reducing pruritus. More significant effects of hydroxyzine and doxepin versus cetirizine on reducing chronic pruritus were reported in the study of Shohratietal. [19].

Doxepin as a TCA has a potency in blocking H_1 and H_2 receptors. The studies reported more strength of doxepin than diphenhydramine and hydroxyzine in blocking H_1 receptors being 775 and 56 times than that, respectively [13]. In a study of comparing the effects of doxepin, hydroxyzine, and cyproheptadine as well as cinnarizine on patients with idiopathic cold urticaria by Neittaanmäki et al., more acceptable effect and lower side effect of doxepin than other medications were reported [23]. It should be noted that, low dosages of doxepin was administered in the most studies, although high dosages of this medication might lead to obscurity, dry mouth, constipation, and bladder outlet obstruction. In a study, the correlation of allergic rhinitis and migraine was surveyed, these disorders are the common factors of headache and facial pain involving inflammatory mediators with vasoactive feature, the prevalence of migraine without aura incidence in patients with allergic rhinitis was higher than subjects without allergic rhinitis [24].

As for diagnostic interference, the migraine was reported as the source of sinus pain as being 86% based on criteria of International Headache Society. Other studies reported migraine stimulators such as climate change (83%), seasonal change (75%), and allergens (62%), these factors are interfering with allergies and stimulating nasal lining. As for common mediators including histamine, IgE, alpha-peptide tumor necrosis factor depended on calcitonin gen, intestinal vasoactive peptide, D2 and F2 prostaglandins, interleukin and nitrous oxide between migraine and allergic rhinitis, doxepin can play an important role in preventing migraine incidence, taking of this medication is suggested in this study and other literatures [25, 26].

CONCLUSION

The same effects of doxepin and cetirizine were reported in the current study, approximately. It is seemed that administration of higher dosages of doxepin might lead to more prevention of allergic rhinitis symptoms. However, higher side effects of higher dosages should be considered. It is suggested to administer doxepin tablet for patients with the symptoms of the allergic rhinitis, tension headaches, migraine, depression, and anxiety disorders. It is also suggested to administer doxepin for patients with allergic rhinitis and various headaches.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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ARTICLE

CHALLENGES OF NURSING DOCUMENTATION IN CORONARY CARE UNIT: A QUALITATIVE STUDY OF NURSES' EXPERIENCES

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ABSTRACT

Evidence shows that there are challenges in nursing documentation in CCU. Studying nurses' experiences of documentation can help acquire a deep understanding about documentation-related challenges and develop strategies for promoting documentation. The purpose of the study was to explore nurses' experiences of documentation in CCU. This descriptive qualitative research was conducted in 2015 by using the conventional content analysis approach. A purposive sample of fifteen nurses was recruited. Unstructured interviews were held for data collection. Data analysis was done by using the Graneheim and Lundman's content analysis approach. Data trustworthiness was maintained through the criteria Lincoln and Guba. The study participants' experiences of documentation in CCU fell into three main themes including 'Documentation: an instrument for or a barrier to conscious and safe care', 'Reality-documentation-principles gap', and 'Dynamic management-person interaction and high-quality condition for documentation'. Nursing documentation has a contradictory nature for nurses and is perceived differently from 'an instrument for care provision' to 'a barrier to care provision'. However, the gap between realities and documentations diminishes the value of documentations. Managerial supervision and provision of necessary infrastructures for reality-based documentation can enhance the quality of nursing documentation in CCU.

INTRODUCTION

The profession of nursing has become more specialized and more complex compared with the past decades and hence, nurses' legal liability has also increased [1]. Nursing reports show the flow of medical treatments and patients' reactions to care services [2] and enable healthcare providers to communicate with each other and evaluate the quality, type, and continuity of services [3]. Besides, nursing reports have significant roles in nursing education and research as well as in care auditing and evaluation [4]. The quality of documentation in critical care units is of greater importance due to the hospitalization of critically-ill patients in these units, critical situations, administration of certain medications which may produce major side effects, and high mortality rate. However, studies showed that most nursing care services in critical care units are documented and reported by using non-standard narrative methods [1]. Besides, the quality of documenting the findings of nursing assessment and care evaluation is also poor. Different studies reported the inadequacy of documents on clinical care evaluation [5], mismatch between documentations and patients' actual conditions [6], the poor quality of documentations, and nurses' poor-to-moderate documentation-related knowledge, attitude, and practice [7]. Therefore, considerable emphasis is placed on improving the quality of nursing documentation. There are numerous studies on the methods for improving documentation quality, most of which have been done using quasi-experimental designs and in general hospital wards such as medical-surgical units. These studies dealt mainly with developing and implementing a directed program to improve documentation quality [8], launching a peer evaluation program [9], providing in-service educations to nurses [10,11] and assessing the effects of different methods for writing nursing reports [12]. Evidence shows that nurses have negative attitude toward documentation [13]. Moreover, there is a knowledge gap regarding nurses' experiences of documentation in the Iranian socio-cultural context. This study was made in Iran to explore nurses' experiences of documentation in coronary care unit (CCU).

METHODS

Study design

This was a descriptive qualitative study which was conducted in 2015 by using the conventional content analysis approach [14].

Setting and sample

This study was undertaken in the CCU of a teaching hospital located in X. A purposive sample of fifteen nurses was recruited. The selection criteria were having a minimum work experience of six months in

KEY WORDS

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critical care units, being interested in sharing experiences, and being psychologically stable for establishing communication.

Ethical consideration

The approval for conducting the study was obtained from the Ethics Committee of X University of Medical Sciences (LUMS.REC.1394047). The study aims and methods were explained to the participants and they were requested to provide written consent for participation. All participants retained perfect right to withdraw from the study. They were also ensured that their information would remain confidential and that they could have access to the study findings.

Data collection

In-depth unstructured interviews were held for data collection. All interviews were held personally in a private quiet room located in the study setting. The time of the interviews was determined according to the participating nurses' preferences. Initially, the interview questions were broad. For instance, 'What feelings do you have when documenting the care?' 'Would you please talk about your experiences of documentation?' 'In your opinion, what items should be documented in critical care units?' Interviews were recorded by a MP3 recorder. They ranged in length from 20 to 40 minutes. Data collection was performed in 2015 and ended once theoretical data saturation was achieved [15].

Data analysis

Data analysis was done concurrently with data collection by using the Graneheim and Lundman's five-step content analysis approach [14]. Immediately after holding each interview, it was transcribed, typed, and perused for several times. Then, primary codes were extracted, combined, and grouped based on differences and similarities among them. Finally, the latent content of the data was extracted. All codes were assessed, scrutinized, and grouped by authors in order to generate appropriate categories and themes.

Data trustworthiness was maintained through the criteria Lincoln and Guba. The credibility of the data was maintained through member checking, allocating adequate time to data collection, prolonged engagement with the data, and conducting the interviews according to the participants' preferences. On the other side, data conformability was ensured by sending the interview transcripts and the generated codes and categories to several reviewers and asking them to review the accuracy of data analysis. We also attempted to hold all interviews in a same place and to transcribe them immediately in order to establish the dependability of the data. Besides, the maximum variation sampling technique was deployed to enhance data transferability [16].

RESULTS

The study participants were twelve staff nurses, a matron, and two educational and clinical nursing supervisors. On average, the participating nurses' age and work experience were respectively 29 and six years and most of them were female (64%) and married (64%). The study participants had been employed permanently (14%), conditionally (57%), temporarily (7%), or based on the national post-education service law (21%). The study participants' experiences of documentation in CCU came into three main themes including 'Documentation: An instrument for or a barrier to conscious and safe care', 'Reality-documentation-principles gap', and 'Dynamic management-person interaction and high-quality condition for documentation' [Table 1].

Table 1: Critical care nurses' experiences of nursing documentation in CCU

Theme	Category
Documentation: An instrument for or a barrier to conscious and safe care	1. Documentation: An instrument for obtaining information and making decisions 2. Documentation: A factor behind effective care provision and rights protection 3. Documentation: A barrier to care and peace 4. Documentation: A marginal tool and task
Reality-documentation-principles gap	1. Censorship due to lack of professional autonomy 2. Documentation without implementation due to lack of resources and equipment 3. Inadequate documentation of essential nursing care services 4. Imprecise documentation 5. Traditionalism and non-adherence to principles
Dynamic management-person interaction and high-quality condition for documentation	1. Managers' support and supervision 2. Personal and situational characteristics

Documentation: An instrument for or a barrier to conscious and safe care

The participating nurses had different and even contradictory ideas about documentation. Some of them had a positive attitude towards it, emphasized its importance, and considered it as an instrument for exchanging care-related information. Moreover, they had experienced its positive role in protecting patients' health, particularly in critical care units, and also in protecting their own rights. The participants' positive experiences of documentation fell into two categories which are explained in what follows.

1. Documentation: An instrument for obtaining information and making decisions

Some participants noted that reading documents on patient assessment helps them obtain information about patients' conditions. Moreover, they referred to documentation as an instrument for exchanging information among healthcare professionals and making clinical decisions. On admission, I assessed the patient and documented his signs and symptoms such as ecchymosis. This helped my colleague in the next shifts consider the probability of bleeding. The attending physician also provided his orders based on our documentations (P. 3).

2. Documentation: A factor behind effective care provision and rights protection

As most patients who are hospitalized in CCUs have vital organ dysfunction, the participating nurses greatly valued the documentation of nursing assessments, hemodynamic monitoring, and advanced life support for vital organs. Besides, they highlighted the necessity to document data on patients' basic needs. Cardiac patients' level of consciousness is an important parameter and should be documented. Their breathing pattern and cardiac rate and rhythm are also among the priorities and need to be documented. These parameters show whether a patient is still suffering problems or is achieving recovery (P. 6). The study participants' experiences showed that accurate documentation of patients' clinical conditions and their responses to treatments can play a significant role in establishing right diagnoses, administering effective treatments, and saving patients' lives.

He had chest pain and was not responsive to oxygen therapy and we had documented this fact. The attending physician read it and prescribed more advanced diagnostic techniques for the patient. It was then revealed that the patient had severe coronary artery stenosis and hence, a stent was placed for him (P.11). During the process of documentation, our participants had clearly understood that documentation not only ensures patient safety, but also protects nurses' safety and rights and can strongly support them in lawsuits. At patient discharge, the patient's family members asserted that the staff nurse has not administered streptokinase to their patient. However, the nurse had documented the preparation and the administration of the medication and hence, family members' complaint was rejected (P. 1).

3. Documentation: A barrier to care and peace

Contrary to the above mentioned findings, some participants referred to documentation as a barrier to care provision which disturbs nurses' peace through creating a sense of insecurity and boredom. They found it as a fruitless repetitive and time-consuming task which hinders effective care provision. All of our reports are repetitive and alike and hence, I feel that writing reports is a time-consuming and boring task. It wastes our time and thus, we can't effectively care for patients. I become really tired of writing reports. I think that it isn't beneficial to patients (P. 2).

Besides considering documentation as fruitless and boring, some participants had a negative attitude towards it because of being scared of its potential legal consequences. When documenting, I always feel fear because some patients are critically-ill and hence, I may forget documenting one care measure and thereby, be punished or experience legal problems due to such failure to document (P. 5).

4. Documentation: A marginal tool and task

Some participants believed that documentation is important neither to nurses nor to physicians and has no pivotal role in clinical decision making. Nurses don't value their reports. Clinical care is more important [than documentation]. Nurses' decision making is even based on patients' clinical conditions. I haven't ever seen physicians to check vital signs charts and make their decisions based on them (P. 12).

Reality-documentation-principles gap

Two main findings of the study were the gap between the contents of documentations and what had been happened in real practice as well as violations against the principles of careful and accurate documentation. Such gaps have been perceived by the study participants in different ways which are explained below.

1. Censorship due to lack of professional autonomy

One of the study participants' experiences was screening and selective documentation which was related to their sense of insecurity and lack of professional autonomy. In other words, although the participating nurses implemented some measures for patients in critical situations where physicians were inaccessible, they avoided documenting such measures because of having fear over potential prosecution or punishment. For example, we had a patient who suffered from chest pain. There was no medical order for

administering opioid analgesics for him and I couldn't contact the attending physician at midnight. Therefore, I was compelled to administer an opioid analgesic without physician's order. However, I avoided documenting it due to the likelihood of physician's anger and subsequent punishment (P. 9). The solution is to delegate some sort of authority to nurses in the form of protocols. Then, nurses would not be compelled to avoid documenting their care services (P. 7).

2. Documentation without implementation due to lack of resources and equipment

The study participants' experiences showed that the availability of resources, workforce, and medical equipment also contributed to the quality of documentations. One of the participants referred to the impact of access to physiotherapy services on the accuracy of documentations by saying, Suppose that chest physiotherapy has been prescribed for a patient while there is no physiotherapist in the hospital. The prescribing physician also knows this fact; however, he prescribes physiotherapy due to the possibility of negative legal consequences for himself. Therefore, we will have no option but not to perform physiotherapy; however, we write in patient's records, 'Chest physiotherapy was provided' (P. 8).

3. Inadequate documentation of essential nursing care services

Most participants noted that essential care services such as therapeutic relationship, patient and family education, and patient support by nurses are not documented. Most of the times, patients are anxious and are scared of death. We, the nurses, attempt to support and calm these patients and their family members. However, we don't document such nursing care services at all (P. 6).

4. Imprecise documentation

One of the study participants' concerns and experiences was a lack of precision in documentation due to the shortage of resources and equipment. The monitor displays of the cardiac monitoring system do not work properly, have too much artifacts, and do not trace patients' cardiac rate and rhythm properly. Sometimes, sphygmomanometers also function improperly and hence, we're compelled to document blood pressure inaccurately (P. 15).

5. Traditionalism and non-adherence to principles

The study participants' experiences revealed that care services provided to patients are usually documented without performing careful patient assessment, critical thinking, and paying close attention to observational findings. Rather, documentation is performed traditionally and based on previous nursing reports and old routines. Nursing reports are written through imitating senior nurses' documentation styles. Freshman nurses may even never pay attention to patients' conditions and the data presented by monitoring systems and just copy other nurses' reports (P. 14). We document a series of repetitive and routine patient education materials about dietary regimen and physical activity without assessing patients' real educational needs. However, the sound practice is to document only the educations that are provided based on patients' unique educational needs, underlying conditions, and literacy levels (P.10).

Dynamic management-person interaction and high-quality condition for documentation

According to the study participants, nursing managers' attention to nurses' documentation practice, nurses and patients' personal characteristics, and the general atmosphere of CCU can considerably affect the quality of documentation. This main theme consisted of two categories which are explained below.

1. Managers' support and supervision

One of the main factors behind the quality of documentations was managers' support and supervision. Education, control, quality assessment, and feedback provision were among the most important experiences of the study participants regarding managers' role in documentation. Our manager used encouragement technique and introduced quality nursing reports to other nurses. Sometimes, hospital administrators held report writing workshops and reminded us of the importance and the legal consequences of documentation (P. 8).

2. Personal and situational characteristics

According to the study participants, nurses and patients' personal characteristics as well as the general atmosphere of CCU can also affect the quality of documentation. Participant 8 referred to nurses' professional characteristics as an important factor behind documentation quality by saying: Differences in nurses' personal characteristics such as knowledge, disciplined practice, conscience, interest, motivation, feeling of responsibility, and self-confidence can affect documentation quality. For instance, more knowledgeable or more accountable nurses usually write more comprehensive reports (P. 15). Other factors behind documentation quality were patients' health status and the severity of their problems. Once there is a critically-ill patient in the unit, all staffs attempt to write more detailed nursing reports to avoid being affected by legal consequences (P. 13).

Another patients' characteristic which can indirectly affect the quality of documentation is their cultural backgrounds which determine their behaviors. In our cultural context, some patients feel ashamed of expressing their chest pain. Therefore, a staff nurse may not notice patient's pain and does not document it (P. 9).

Finally, the general condition of CCU such as heavy workload and greater priority of care procedures may affect the possibility and the quality of documentations. When we have heavy workload due to great number of patients in the unit, we spend our time mainly on providing clinical care and hence, don't devote adequate time to reports writing (P. 4).

DISCUSSION

The study participants' experiences of documentation fell into three main themes of 'Documentation: an instrument for or a barrier to conscious and safe care', 'Reality-documentation-principles gap', and 'Dynamic management-person interaction and high-quality condition for documentation'. The theme of 'Documentation: an instrument for or a barrier to conscious and safe care' showed that the participants had perceived appropriate documentation as care and peace and found it beneficial. One of the benefits of the documentation of patient assessment data was to become aware of patients' conditions. Moreover, it is an instrument for exchanging information among healthcare professionals, making clinical decisions, and guaranteeing patients, physicians, and nurses' safety. Previous studies also reported the same findings [2, 3]. Irajpour et al. found that taking and documenting patients' past health history at the time of hospital admission help nurses understand patients' background and legally protect patients and nurses [17]. Besides, Kinnunen et al. reported that standardized wound assessment and documentation significantly affect the quality of wound care [18]. Ahmadi, Habibi Koolae also noted that real-time patient assessment and documentation in each working shift help nurses rapidly identify patients' problems, implement appropriate nursing interventions, and prevent cardiac emergencies [19]. Besides, Considine et al. reported nurses' abilities and documentations as significant factors in identifying, interpreting, and managing physiologic health problems and reducing mortality rate [20].

Previous studies showed that nurses' attitudes towards documentation affect its quality. For instance, Okaisu et al. reported that instead of considering it as a necessity for professionalization, nurses referred to documentation as a massive and second-hand task which distanced them from care delivery [13]. Another key finding of the present study was 'Reality-documentation-principles gap'. This finding is somewhat supported by the findings of previous studies. For instance, the results of a systematic review by Wang et al. indicated that despite the importance of documentation quality and attempts to its improvement, documentation is still performed inconsistently, inadequately, and dishonestly because of having limited information and understanding about health, quality of life, and psychosocial, cultural, and spiritual aspects of care. Besides, they found that despite using the nursing process in some studies, nursing diagnoses and interventions were documented inaccurately and items such as pain, cognitive problems, palliative interventions and evaluations, and heart failure assessment, treatment, and prevention were not documented et al. [21]. Gunningberg and Ehrenberg also reported that the documentation of pressure ulcers is not performed completely, adequately, precisely, and based on patients' real conditions [6]. Although nursing is a professional practice which needs precision, consciousness, and a sense of responsibility and it can significantly affect the quality of healthcare services [22].

The findings of the present study also indicated dishonest nursing documentation due to nurses' lack of functional autonomy. The participants highlighted that delegating the authority over managing some kinds of patients' problems to nurses would enhance nurses' honesty in documentation and the quality of their documentations. The prerequisites to this delegation are specialization of nursing, particularly in critical units, recruitment of competent and qualified staffs to nursing, developing more independent job descriptions for critical care nurses, and obtaining permissions from hospital administrators for more independent nursing practice. Toulabi et al. also noted that care quality in CCUs can be improved through providing patient-centered care, delegating some authorities to nurses, and developing protocols for giving more authorities to critical care nurses [23].

Another finding of the study was the documentation of patient education activities irrespective of patients' unique conditions or patient-centered care. In other words, patient education was documented routinely and monotonously without assessing patients' real educational needs. Sultani et al. also reported that the main barriers to patient education were poor planning for patient education, considering patient education as a routine nursing task, nurses' limited access to educational materials, poor managerial supervision and encouragement, low nurse-patient ratio, nurses' inability to provide patient education due to their heavy workload, their indifference toward their educational roles, their inability to communicate with patients, and their inability to develop educational programs. Therefore, patient education by nurses can be promoted through allocating one or two independent nurses to patient education, conducting educational programs and workshops for enhancing nurses' interest and competence in patient education, removing organizational barriers, assessing nurses' ability to perform their educational roles, and providing constructive feedbacks by administrators and managers to nurses to provide patient education [24]. Howse and Bailey also attributed nurses' disinterest in accurate documentation to psychological factors such as nurses' low self-confidence, poor writing skills, and negative attitudes toward documentation[25].

The third main theme of the study was 'Dynamic management-person interaction and high-quality condition for documentation'. This finding reflects the necessary conditions for accurate documentation and shows nursing managers' performance, access to resources, and an appropriate environment for direct care provision as factors affecting documentation. In line with our findings, the results of a study in Thailand on the complexities of nursing documentation also revealed that the main factors behind nurses' poor documentation practice were lack of managerial supervision, limited managerial and organizational support, and healthcare professionals', particularly physicians', indifference towards nursing reports [26]. Our findings also indicated nurses and patients' personal characteristics as well as nurses' lack of motivation for and indifference towards documentation as factors behind low-quality documentation.

One of the study limitations was that it was conducted in a local hospital and hence, the study findings may have limited transferability. It is recommended to explore nurses' experiences of documentation in other clinical settings such as general hospital wards, emergency departments, and intensive care units.

CONCLUSION

Three main themes were generated in this study, which were 'Nursing documentation: an instrument for or a barrier to conscious and safe care', 'Reality-documentation-principles gap', and 'Dynamic management-person interaction and high-quality condition for documentation'. These findings indicate that for nurses, documentation has a contradictory nature and is perceived differently from 'an instrument for care provision' to 'a barrier to care provision'. However, the gap between realities and documentations diminishes the value of documentations. Managerial supervision and provision of the necessary infrastructures for reality-based documentation can enhance the quality of nursing documentation in CCUs.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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ARTICLE

INVESTIGATING THE KNOWLEDGE AND ATTITUDE OF HIGH SCHOOL STUDENTS TO AIDS DISEASE IN ZABOL CITY IN 2014

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ABSTRACT

AIDS disease is the most serious health problem and deadliest infectious disease of the current century. The emerging of disease and insufficient knowledge of it easily expose the adolescents at risk. This study aimed to determine the knowledge and attitude of high school students to AIDS in Zabol city. In this cross-sectional study, 350 male and female high school students of Zabol city who were studying in the high school in 2014 were investigated. The questionnaire containing basic questions, knowledge and attitude about AIDS transmission ways to collect information. The majority of students (48.5%) had good knowledge and there is a significant relationship between their knowledge in terms of demographic variables and age, gender, field and grade of education and parental education. The rate of attitude towards the AIDS was assessed as follows: Weak in 114 people (32.6%), normal in 204 people (58.3%) and good in 32 people (3.1%). This study showed that despite the suitability of knowledge of students about AIDS and dominance of medium attitude in them, there is a weak attitude in 32.6% of students. Therefore, it is recommended to develop strategies in order to reduce the risk of AIDS among adolescents in secondary schools.

INTRODUCTION

AIDS is adventitious immune deficiency syndrome and it is a description of disorders caused by defects in the function of the human immune system that occurs by infection with human immune deficiency virus (HIV). This disease can range from mild viremia to severe safety impairment with life-threatening opportunistic infections and even malignancies associated with AIDS [1]. The transfer of HIV takes place from various ways, such as sexual contact, condensed coagulation factors, contaminated blood components and multiple non-sterile injections [2].

It is estimated that about 40 million people (36-44.5) infected with HIV are living in the world. About 5 million people (4-6) were infected with this disease every year and about 3 (2.8-3.5) million people lose their life. The statistics showed that more than 60% of people with AIDS are between the ages of 15-24 years [3]. The study of Center for Disease Control [CDC] on the risky behaviors of the youth showed that 50% of the American high school students have had sexual relations and on the other hand 50% of new cases of HIV infection occur in this age where every one minute 5 people and every 14 seconds a person between the age of 15-24 years is infected with the virus worldwide [4]. HIV prevalence in the general population is low and it is totally 1% in Iran [5]. According to the statistics collected by the University of Medical Sciences and Health Services as of March 2012, a total of 24 thousand and 290 people with HIV have been detected in the country of whom 90.8% are men and 9.2% are women [1]. The results of various studies showed that in the combat program with AIDS, training and subsequently correction of wrong attitudes in this field and promotion of correct hygienic behavior are the only ways to prevent the disease.

The first step of behavior change in line with a subject is having enough knowledge about that subject. Given that a large percentage of adolescents and young people attend in the schools, training of AIDS prevention in schools is of particular importance. So, the assessment of attitude and knowledge in the behavior of high-risks groups in all societies frequently becomes necessary.

The objective of this study was to evaluate the knowledge rate of Zabol students about AIDS. It assesses their beliefs and attitudes towards the subject and compares them with each other. In fact, this study tries to optimize the health of society.

MATERIALS AND METHODS

A questionnaire was used to collect data that is included demographic data, 25 questions with three-options of knowledge (Yes, No, I do not know) and also 11 questions with three-options of attitude (Agree, No idea, Disagree) about AIDS disease and its transmission ways. The validity of the questionnaire is determined based on the content validity method and reception of teachers' comments. Also, its reliability

KEY WORDS

Knowledge, Attitude, AIDS, Student

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was confirmed as 91% using Cronbach's alpha coefficient. In this study, the collected data are analyzed with SPSS 21 software and they are presented in the form of descriptive statistics (frequency, mean, standard deviation). The statistical tests such as t-test and ANOVA are used to analyze data and $p < 0.05$ is considered as significance level. To classify the level of knowledge-attitude, the total scores are divided into three parts. The scores are considered as follows: The score less than 33% is weak. The score between 33-66% is medium. The score higher than 66% is good. Finally, the results are shown in the table.

RESULTS

In total, 350 students are evaluated in this study of whom 50% were female and 50% were male and most of them (33.1%) were 19 years old. Many of these students (28.6%) were studied in the fourth grade of high school and 39.1% of them were enrolled in experimental fields and all of them were residents of Zabol city. Most of their parental education level were diploma and elementary school with 37.7% and 43.7%, respectively and most of their parental job were self-employed and homemaker with 38.6% and 90.6%, respectively.

In total, the knowledge of most of the students (48.5%) was good. The knowledge rate of (40.6%) students was moderate and knowledge rate (10.9%) of students was weak. The knowledge of the students in terms of demographic variables shows a significant difference between the average knowledge of the students in terms of age, gender, field and grade of education and parental education. So that 19 years old students show better knowledge than the other ages and the knowledge of girls was more than the boys. The students of third and fourth grade show a higher knowledge than the other grades and the students of experimental field had the higher average of knowledge. Also, the students with highly educated parents were obtained higher knowledge. The attitude rate of students towards AIDS was evaluated as follows:

- 114 (32.6%) people had weak attitude.
- 204 (58.3%) people had medium attitude.
- 32 (9.1%) people had good attitude.

The mean comparison test of attitude score in terms of demographic variables showed a significant difference between the attitudes of students in terms of age, gender, field and the grade of education. The mean of attitude rate in girls was higher than the boys. The 18 and 19 years old students showed the highest average of attitude. The average of attitude in the fourth grade was obtained more than the other grades. The students of experimental field had the highest rate of attitude.

Table 1: Comparing the mean scores of knowledge and attitude and demographic variables in students participating in study

The significance level	Average Rating attitude	The significance level	Average Rating attitude	Average Rating awareness	Demographic
p-value<0/05	3/91	p-value<0/05	11/67	14	Age (years)
	4/09		13/67	15	
	4/47		14/61	16	
	4/50		14/81	17	
	4/67		15/68	18	
	5/16		16/73	19	
p-value<0/05	4/20	p-value<0/05	14/29	Man	Gender
	5/00		16/27	Female	
p-value<0/05	4/10	p-value<0/05	14/73	First	Grade
	4/36		13/35	Second	
	4/70		16/17	Third	
	5/27		16/76	Fourth	
p-value<0/05	4/36	p-value<0/05	14/73	General	Field of study
	5/25		17/26	Experimental	
	4/06		13/47	human	

Investigating the sources of information about HIV/AIDS revealed that television- radio and teachers and relevant courses were the highest resources of information for the students with 48.5% and 16.4%, respectively. Internet, friends and relatives, newspapers and magazines were allocated 14.9%, 11.4% and 8.6% of the information resources, respectively.

Table 2: Absolute and relative frequency distribution sources all high school students in the city of Zabol

Intelligence sources	Number	Percent
Radio-TV	170	48/5
Gazette-Journal	30	8/6
Courses for teachers	58	16/6
Internet	52	14/9
Family and friends	40	11/4
Total	350	100

CONCLUSION

Among the students participating in this study, the rate of knowledge about the AIDS disease in 38 people (10.9%), 142 people (40.6%) and 170 people (48.5%) were evaluated as weak, medium and good, respectively. The rate of attitude towards the AIDS disease in 114 people (32.16%), 204 people (58.3%) and 32 people (9.1%) was weak, medium and good, respectively. In the Arabi. M and colleagues study on the female students in secondary schools in relation to AIDS, knowledge of transmission ways and overall knowledge of the patient were in the moderate level [5]. In this study, the findings show the good knowledge of students about AIDS disease. Also, In the Arabi. M and colleagues study, the mean scores of students' attitude towards AIDS was high and the majority of them had good attitude to the disease [5]. While in this study, the students' attitudes towards AIDS was reported as normal.

In Mazlumi and colleagues study on the high school students in Yazd province, the knowledge of the majority of the students (35.2%) was good [6]. Similar results were obtained in this study. In Mazlumi and colleagues study, Most students (36.3%) had positive attitude towards AIDS while according to the ratio of 32.4%, the negative attitude was also common among them [6]. However, in this study, the students' attitude towards the AIDS was normal. In this study, according to the ratio of 32.6%, negative attitude was also common among students. In Momeni and colleagues study on the pre-university students in Yasuj city regarding AIDS, the knowledge of 77.5%, 16.3% and 1.2% of people were good, excellent and weak, respectively [7]. The knowledge of students in Yasuj was in a favorable level compared to the current study. This could be due to the regional differences. In addition, the activities of health centers of education ministry in this region and the limitations created in the schools in Zabol due to the special faith and cultural conditions could be effective on the low level of the students' knowledge.

The knowledge and attitude of students in terms of demographic variables showed a significant difference between the average knowledge and attitudes of students in terms of age, educational grade and educational year. The mean scores of knowledge and attitude of students were higher with the increase of age and years of education. So that, there was a significant difference between the mean score of knowledge and attitude in terms of age and the year of education. This could indicate that students at higher levels of education and at older ages were more exposed to the information of different resources regarding AIDS disease or seek more information on this field and try more to self-learning and acquire positive attitude. This is in consistent with the study conducted by Dr. Arabi, Dr. Mazlumi and Dr. Imam Hadi [3,5,6]. Another finding of this study was that girl students had significantly higher mean score of knowledge and attitude compared to the boys. In other words, the knowledge and attitude of girls about AIDS and HIV patients were better than the boys. The study conducted by Ramazankhani, Rostami and Shokrollah confirmed this result [8].

According to the results of this study, there was a significant relationship between knowledge and attitude with experimental field that could arising from the different content of this field from the other fields of study and its close relationship with diseases. Also, the students with highly educated parents received higher knowledge. This may be due to the positive effect of scientific level of family on the knowledge of the children. Based on these findings, radio and television were the main source of information of students about AIDS disease. The study conducted by Tavusi et al [9] and Mazlumi et al [6] emphasized the role of social communication media especially radio and television to obtain information.

In the Zhao study [10], reception of information from school, family and peers about AIDS disease was less than social communication media. The results of several studies in Singapore and Yazd [11,12] are consistent with this finding. A significant proportion of students (14.9%) referred to the internet as one of the resources of information. This not only endorsed the role of indirect training and remote learning based on the computer, but also can be one of the significant methods of educational policymakers to promote the public knowledge level especially of young people in the schools. Since the social communication media especially television and radio are the most dominant source of information by students, they can be the best way to enhance students' knowledge. It should be said that battle mobilization against spread of AIDS is a basic necessity and schools must be one of the main centers in the reduction strategies of AIDS. Training of students regarding all aspects should attract the serious attention from the educational and governmental policymakers as an integral part of education in all schools of the country. Of course, the implementation of this requires the collective will of educational planners of parents and teachers community and (NGO) of country and voluntary and informed participation of parents.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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ARTICLE

INVESTIGATE AWARENESS, ATTITUDE AND PERFORMANCE TO GESTATIONAL DIABETES IN PREGNANT WOMEN REFERRED TO ZABOL'S HEALTH CENTERS IN 2014

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ABSTRACT

Gestational diabetes is disorder in carbohydrate tolerance that is diagnosed during pregnancy for the first time. The awareness and attitude of women about gestational diabetes will be effective in disease prevention and its complications and early detection. In this descriptive-sectional study, 300 pregnant women who have referred to Zabol's health centers during 2014 were studied. For data collection, a questionnaire was set containing demographic questions, knowledge (17 questions), attitude (9 questions), and performance (8 questions) that by pregnant women was answered. Of the mothers participating in the study, 175 patients (58.3%) had average awareness, 206 patients (68.7%) had good attitude and 168 patients (56%) had good performance. Factors such as education level, age of pregnancy, source of information in attitude of pregnant women and gestational age and job is influenced on performance of pregnant women than gestational diabetes and there was not observed a significant relationship between age, the number of pregnancy times and history of abortion in pregnant women with their attitude and performance to gestational diabetes. Mother's education level and source of information of mothers, age of pregnancy and job are the most important issues influencing their attitude and performance in relation to gestational diabetes.

INTRODUCTION

Gestational diabetes refers to any form of glucose intolerance that is diagnosed for the first time. Gestational diabetes usually starts in mid-pregnancy and continues until the end of pregnancy [1-3]. Its prevalence in different parts of the world is stated 1-14 percent that, on average happens in 2-5% of all pregnancies and is the most common metabolic disorder in pregnancy [4-6]. It almost affected 3.5% of pregnancies in England and Wales [7]. In a review article in Iran, the prevalence of gestational diabetes in eleven provinces was reported between 1.3 to 8.9 percent the lowest prevalence was related to Ardabil and the highest was related to Bandar Abbas [8]. Dramatic difference in statistics is used arising from the difference in the study population and diagnosis test. The prevalence of gestational diabetes steadily in developed countries including the United States of America, England, Australia and New Zealand is increasing [9]. Gestational diabetes as a silent disease affects the phenomenon of pregnancy, the mother and the fetus are adversely affected that is associated by severe complications such as preeclampsia, preterm delivery, caesarean section, Hydroamnios, fetal macrosomia and low birth weight and leads to adverse pregnancy outcomes and obstetric [10-15].

Among the risk factors for developing diabetes during pregnancy can be pointed by age, previous history of gestational diabetes, high body mass index, and history of hypertension in first degree relatives, smoking, belonging to particular breeds and short stature of mother [16]. Screening for gestational diabetes in pregnant women and identify risk factors can have an important role to minimize the risks and maternal and fetal complications [17]. Gestational diabetes can be divided into two groups: Asymptomatic and symptomatic and since most patients with gestational diabetes are in the group of asymptomatic and its diagnosis is difficult so that 70% of gestational diabetes are included, so in order to prevent and decrease implications in mother and fetus, early reviews of the disorder during screening is essential during pregnancy because uncontrolled diabetes to 90 percent in infants and 30 percent in mothers is associated with morbidity in addition to being at risk of developing diabetes after pregnancy as well. In gestational diabetes symptoms, such as visual disorders, bulimia, polyuria, weight loss and confusion is common and dangerous which should be controlled and in this field, collaborative role of pregnant woman to maintain her health and her fetus is very important [18].

Since pregnant women with diabetes in their care in the field of gestational diabetes due to high stress may mistake and therefore this subject disorders in the regulation of blood sugar; It must be provided necessary training to control the condition and ways to oppose it by health centers while adequate support of pregnant women in this context [19].

KEY WORDS

Awareness, Gestational diabetes, Performance, Attitude

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Also, given that the awareness, attitude and performance of women about gestational diabetes in prevention, early diagnosis and reduce its complications can be effective, therefore, awareness and attitude towards this issue and more informing to women is considered of effective strategies [20]. Including studies that are conducted on awareness, attitude and performance of pregnant women to gestational diabetes can be noted to Ghasemzadeh and colleagues in 2005-2006 on pregnant women referred to Military family hospital that its results show that 19% had awareness and 13% had appropriate performance and attitude 28.4% was assessed well [21] Also in the study of Balali Mobidi that was conducted in 2010 and on 1000 pregnant women referred to health centers in Medical Sciences University of Kerman that 14.2% had a good awareness, 15.6% positive attitude and 53.4% score of well performance and there was a significant relationship between awareness and performance with age, education and job and there was a significant relationship between attitude and education [20]. Considering the importance of the disease and its adverse consequences in babies and mothers with and also demographic differences and the importance of this issue in the next decisions of health system to improve health services and health promotion, the present study were conducted aimed to assess the knowledge, attitude and performance of pregnant women. In this study, it is tried to assess awareness, attitude and performance of pregnant women with gestational diabetes if needed, training programs to be developed based on the findings and by raising awareness and attitude of women towards gestational diabetes reduce the incidence of disease and its consequences.

MATERIALS AND METHODS

In this descriptive study, 300 pregnant women who were referred to health centers in 2014 using the formula of sample size ($n = Z_{1-\alpha/2}^2 P(1-P) / d^2$) and random sampling method were selected and studied. For data collection, a questionnaire including four parts was given to pregnant women. The first part included demographic questions such as age, education, job, gestational age, and the number of pregnancies, history of abortion and sources of information.

The second part consisted of 17 questions, two options with Yes and No options that based on true answer gets score one and false answer gets score zero that shows the awareness of pregnant women. Part III contains nine questions about the attitude of pregnant women which was 5 options that answer to each question is placed on 5-option Likert scale from strongly disagree to strongly agree and are awarded a score of 1 to 5, that higher score indicates a positive attitude to it and attitude of pregnant women assesses gestational diabetes and Part IV is performance questions which consists of 8 questions concerning the performance of pregnant women to gestational diabetes that was as third-choice with options Yes, sometimes and no that answer one gets score one and other responses get score zero.

To classify the awareness, attitude and performance, points were divided into three parts, points less than 33 weak, 33-66 average and higher than 66 was considered good. To the awareness, score less than 7 was considered weak, 7 to 12 considered average and higher than 12 considered good and for attitude, score less than 15 considered weak, 15 to 30 points considered average and higher than 30 considered good and for performance, score below 5 considered weak, 5 to 10 points considered average and higher than 10 considered good. The validity of questionnaire was approved by the view of epidemiology, internal medicine specialists and gynecologists and obstetricians and experts in social medicine. Reliability coefficient using Cronbach alpha was obtained 89% respectively. Data analysis was conducted in SPSS18 software and using Fisher's exact test and Chi-square. The significant level was considered $P < 0.05$.

RESULTS

In this study, investigating was conducted on 300 pregnant women that 64.7 percent of them were younger than 30 years and 35.3 percent of them had more than 30 years and the majority of the pregnant women were housewife with diploma education. The most important source of information was health personnel. Pregnant women demographic information in [Table 1] is presented in detail.

Table 1: Demographic information of pregnant women referred to Zabol's health centers in 2014

Percent	Number		Percent	Number		Percent	Number		
40/3	121	Radio and TV	6/7	20	Illiterate	75	225	Housewife	Job
10/7	32	Internet	34/7	104	Less than a diploma	25	75	Employee	
19/3	58	Friends	42/3	127	Diploma	64/7	194	Under 30 years	Age
4	12	Books and magazines	16/3	49	Higher Education	35/3	106	Over 30 years	
25/7	77	Health personnel	24	72	First three months	26	78	Once	Parity
76/7	230	No	36/7	110	Second three months	33	99	Twice	
23/3	70	Yes	39/3	118	Third three months	41	123	More than double	

Results of the survey conducted on pregnant women in terms of awareness, attitude, and their performance to gestational diabetes in the [Table 2] are provided.

Table 2: Awareness, attitude and performance of pregnant women to

gestational diabetes in referred to Zabol's health centers in 2014

Performance		Attitude		Awareness		
Number	Percent	Number	Percent	Number	Percent	
6	18	0	0	5	15	Weak
38	114	31/3	94	58/3	175	Average
56	168	68/7	206	36/7	110	Good
100	300	100	300	100	300	Total

People in terms of educational level are placed in four categories: no education, under diploma, diploma and higher education. Among them, 20 patients (6.7%) were illiterate that awareness in 4 patients (20%) was good, 14 patients (70%) were moderate and 2 patients (10%) weak, performance of 10 patients (50%) good, 8 (40%) average, 2 patients (10%) were weak, 5 patients (25%) had moderate attitude, and 15 patients (75%) had good attitude.

Education Level of 104 people (34.7%) was under diploma that attitude of 41 people (39.4%) was obtained good, 59 people (56.7%) average and 4 people (3.8%) weak and performance of 53 people (51%) good, 41 people (39.4%) average, 10 people (9.6%) were obtained weak and attitude of 47 people (45.2%) was reported average and 57 people (54.8%) were reported to be good. Education of 127 people (42.3%) was Diploma and Associate Degree that awareness of 44 people (6/34%) was obtained good, 78 people (61.6%) average and performance of 73 people (57.5%) good, 50 people (39.4%) average, 4 people (3.9%) weak and attitude of 33 patients (26%) average and 94 patients (74%) was obtained good. Education Level of 49 people (16.3%) was BA or higher that awareness of 21 patients (42%) was obtained good, 24 patients (49%) average and 4 patients (8.2%) weak, performance of 32 patients (65.3%) good, 15 people (30.6%) average, 2 people (4.1%) weak and attitude of 9 people (18.4%) average and 40 people (81.6%) was obtained good. The chi-square test results indicate that there is a significant relationship between the level of education of pregnant women and their attitudes toward gestational diabetes in the sense that with higher educational level, the attitude of pregnant women with gestational diabetes will improve (P-Value <0/05) but there wasn't observed any relationship with awareness and their performance (P-Value>0/05).

Occupational groups of pregnant women included housewife and employed. 225 people (75%) of them were housewives that 76% had good awareness, 60.9% average awareness, 12% poor awareness, 51.6% had good performance, 41.3% average performance, 7.1% had poor performance. Of 75 persons, 25% (45.3%) had good awareness, 50.7% had average awareness, 3% poor awareness, 28% had moderate attitude, 69.3 % good performance, 28% moderate performance, and 2.7% had poor performance. The chi-square test results indicate that there is a significant relationship between the job of pregnant women and their performance in gestational diabetes so that better performance was seen among employed women (P-Value <0/05). But with awareness and their attitude was not observed a significant association (P-Value > 0/05).

Of total 300 pregnant women, 70 women (23 percent) had abortion history. In mothers with a history of abortion: awareness of 30 women (42.9%) was obtained good, 35 women (50%) average, 5 women (7.1%) poor and performance of 36 women (51.4%) good, 31 women (44.3 percent) medium, 3 women (4.3%) poor and attitude of 26 women (37.1 percent) average and 44 women (62.9) was obtained good. In women with no history of abortion: Awareness of 80 women (34.8%) was reported good, 140 women (60.9%) average, 10 women (4.3%) poor and performance of 132 women (57.4%) good, 83 women (36.1%) average, 15 women (6.5%) poor and attitude of 68 women (29.6%) average and 162 women (70.4%) was reported to be good. The chi-square test results indicate that there is no significant relationship between the history of abortion in pregnant women, awareness, attitude and their performance to gestational diabetes (P-Value > 0/05).

In women who were pregnant in less than 2 times, awareness of 65 women (36.7%) was obtained good, 104 women (58.8%) average, 8 women (4.5%) poor and performance of the 101 women (57%) good, 35 women (36.7%) average, 11 women (6.3%) poor and attitude of 59 women (33.3%) average and 118 women (66.6%) was obtained good and in women who were pregnant more than 2 times, awareness of 45 women (36.6%) good, 71 women (57.7%) average, 7 women 5.7% poor and performance of 67 women (54.5%) good, 49 women (39.8%) average, 7 women (5.7%) poor and attitude of 35 women (28.5%) average and 88 women (71.5%) were reported to be good. The chi-square test results indicate that there is no significant relationship between the number of pregnancy times of pregnant women and awareness, attitude and their performance to gestational diabetes (P-Value>0/05).

Pregnant women who had gestational age less than 12 weeks, 31.9% has good awareness, 56.9% good performance and 70.8% had good attitude. The chi-square test results indicate that there is no significant relationship between pregnant women's gestational age and their awareness than gestational diabetes (P-Value>0/05), but there was not observed a significant relationship between attitude and their performance that whatever gestational age was more, better attitude and performance was seen (P-Value <0/05). Finally those who used resources, books, magazines and newspapers, 75% had average awareness and 91.7% good attitude and 83.3% had good performance which was significantly different than the others. The chi-square test results indicate that there is no significant relationship between the source of information in pregnant women and awareness and their performance than gestational diabetes

($P\text{-Value} > 0/05$), but according to Fisher's exact test with their attitude was observed a significant relationship ($P\text{-Value} < 0/05$).

DISCUSSION AND CONCLUSION

Of the 300 women participating in the study, 175 women (58.3%) had average awareness, 206 women (68.7%) had good attitude and 168 women (56%) had good performance as well as factors such as level of education, gestational age, source of information in attitude, gestational age and job are influenced on performance of pregnant women than gestational diabetes ($P\text{-Value} < 0/05$). In the study of Dr. Ghasemzadeh on 200 pregnant women, 62% had average awareness, 77% positive attitude and 69% had moderate performance, however, in this study, pregnant women older than 25 years and obesity is considered a risk factor for gestational diabetes and had a good attitude about the effect of maternal age in the incidence of gestational diabetes. In our study, pregnant women at every level of education had a good attitude toward gestational diabetes that this attitude in people with higher education was significantly higher than those under diploma that the results corresponded with the results of the study of Dr. Ghasemzadeh. It shows the impact of education on attitudes, it seems increasing the level of education of pregnant women at any stage caused to increase their awareness and thus create a positive effect on their attitude toward gestational diabetes actually it can be expected that having a regular training program is effective in increasing awareness and following changing attitudes of pregnant women [15].

Ahead study has shown that the majority of mothers in the use of any source of information had a good attitude and the attitude of people who have used books and magazines and newspapers than those who used other resources have been higher. In fact, it seems that the use of source of studies, books and magazines despite its low number among consumers, due to the reliability of the source of study and along with increasing the attitude with increasing education caused more impact of it than other sources of studies on the attitude of pregnant women than gestational diabetes. According to that 58.3 percent of pregnant women had moderate awareness, doing training programs, especially in terms of production, promotion of valid and understandable training books for the general public seems to be essential and given the delicate role of health personnel in raising awareness of women needs to be held such training programs. The subjects had good awareness about the definition and fasting blood glucose in gestational diabetes. But in the study of Dr. Ghasemzadeh [15] as well as most mothers did not have good awareness in this field, it can be said that changes in pregnancy blood sugar in textbooks in the field of obstetrics and Maternity in recent years and emphasis on the health system on correct definition of the problem [21] caused to raise awareness of pregnant women about this issue but still awareness about dimensions of problem and diabetes complications and strategies for dealing with it is remained in an unacceptable level that need to be investigated and further action. Most people considered urination as a sign of gestational diabetes and had a good awareness and attitude towards this issue, they also have a positive attitude about blood sugar control in the second trimester of pregnancy and showed a good performance in relation to this topic which represents the positive role of health system in following and suitable referring of pregnant women.

A high percentage of pregnant women reported that they act to their physician orders about health remedies that notes important role of doctors, especially family physicians. Most pregnant women not had positive attitude about the effect of increasing the number of pregnancy on gestational diabetes. It means that increasing pregnancies considered a factor to increase gestational diabetes while it has no scientific basis and it may be considered among the reasons for such thinking among pregnant women, high level of education and not wanting to be pregnant, it seems to be done in this field sufficient information. In the study of Ghasemzadeh [15], the majority of women in the second trimester of pregnancy had a good attitude to gestational diabetes, whereas in our study, most pregnant women in any age of pregnancy had a good attitude and good performance to gestational diabetes that the attitude and performance was significantly higher in the third quarter, perhaps the cause of increasing attitude of pregnant women due to pregnancy gestational diabetes by increasing age of pregnancy, especially in the third quarter can be stated as that by increasing gestational age, these women are covered by higher social protection from family and society in terms of their health status and their fetuses as well as increasing self-care behaviors, including blood sugar control, diet and exercise can also be a factor influencing this phenomenon.

In the study of Balali Mobidi that was conducted on 1000 pregnant women, 14.2% had good awareness, 15.6% positive attitude and 53.4% received a good performance score, which there was a significant relationship between awareness and performance with age, education and occupation and there was a significant relationship between attitude and education [20]. While awareness of pregnant women in our study had no relationship with any of the variables and just the job of pregnant women had relationship with performance than their gestational diabetes that a better performance status was observed in employed women that seems that given the level of higher education in both studies, employed women show better performance than diabetes, because these women with higher education that caused to increase awareness and their attitude and followed by it is an agent in order to improve their behavior (performance) in this field. In the study of Shrestha and colleagues that was conducted on 590 pregnant women in Nepal, 241 women (41%) of the participants had heard gestational diabetes about diabetes and in aged over 30 years old, 75% of them had no knowledge of gestational diabetes. Also education had a positive impact on awareness and their attitude ie people with higher education had a higher knowledge

level. While in the present study, age and education had no significant effect on awareness of participants on gestational diabetes, but the level of education had a positive effect on their attitudes and this indicates that the more the education of pregnant women is higher, it will have a positive effect on attitude and their performance in the field of gestational diabetes [22].

Finally, given the positive correlation between education level and attitude of pregnant women with gestational diabetes, planning is considered essential to increase the overall level of education and education of mothers. It also appears to be due to the recent policy of the Ministry of Health to increase the quality of services to pregnant women, health care system of area in identifying and the timely referral of pregnant women has acted as desirable as the performance of pregnant women has improved, but in the field of increasing awareness of mothers, measure or long-term planning is necessary. It also seems family physicians in level one and specialists in level two should consider more time for training of pregnant women.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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ARTICLE

A COMPARATIVE STUDY ABOUT THE IMPACT OF SENSORY STIMULATION PERFORMED BY FAMILY MEMBERS AND NURSES ON VITAL SIGNS OF PATIENTS AT ICU: A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Some studies have shown the effects of sensory stimulation on vital signs of patients at intensive care unit (ICU). However, little knowledge is available about family role compared to the role of nursing staff in this issue and current results are controversial. The aim of this study is to compare the impact of sensory stimulation performed by family members and nurses on vital signs of patients at ICU. In this study, 6969 patients were categorized into two intervention groups and one control group by stratified block randomization method. Dyang sensory stimulation was provided by family members and nursing staff. No intervention was performed for control group. Sensory stimulation was performed 2 hours a day for 6 consecutive days. The vital signs were assessed 5 minutes before and 30 minutes after intervention. Data analysis was performed by ANCOVA, ANOVA and repeated measures. The results showed that there was significant difference between the experimental groups in terms of vital signs before and after the intervention ($p < 0.001$). Of this aspect, family group was the best, nursing group was the second and control group was the last group in classification. The effect of sensory stimulation on vital signs of comatose patients was greater when provided by family members.

INTRODUCTION

Admission in the intensive care unit (ICU) provides great physical and psychological tension to the patient [1, 2]. Being away from family members during admission period at ICU and also short duration of visiting time are considered as risk factors of psychological tension for patients [3, 4]. In most hospitals around the Europe and also Iran, some limitations have been defined for visiting the patients in the ICU [5]. Despite scientific advancements and progresses in the medicine and nursing profession, visiting the patient by family members is one of the most important issues in the hospitals that inappropriately have been neglected. Approximately, it is near to 40 years that visiting regulations have not been revised in Iran [6]. The results of studies since 1970 to 1980 showed that visitors may cause the blood pressure and heart rate of the patients to be increased; but recent studies indicate that no significant changes occur in cardiovascular state of patients during visiting by family members [7]. Besides, the effects of visiting by family members on heart rate, blood pressure or other ventricular events have not yet established well [8]. Mitchell et al showed that hemodynamic indices of patients with cerebrovascular accident who were admitted in ICU had not significantly changed before, during and after visiting time [9]. Loyalty et al reported a statistically significant difference in systolic blood pressure and heart rate of patients admitted in ICU before, during and after visiting time [10]. In addition, Fumagali et al in a 2-year follow-up study on 226 patients concluded that visiting not only does not impair the cardiovascular status, but also the elongation of visiting time will reduce cardiovascular events and alleviate patients' anxiety [11]. Currently, little papers are available focusing on the impact of presence of visitors at ICU on patients' medical condition [4]. Moreover, researchers believe that depriving the human from receiving stimulus and also over stimulation as well, may impair the physical and emotional balance [12, 13]. In the United States, 66% of patients who had been admitted in ICU for at least 10 days have experienced the consequences of stimulus deprivation during hospitalization and also after discharge. Stimulus deprivation can impact vital signs throughout changing physiological rhythms of the body [14].

The majority of the patients in the ICU in Teaching hospital affiliated with the Lorestan University of Medical Sciences come from the surrounding villages and tribes, and there are deeper emotional communications between these patients and their families. From religious and humanistic perspectives, visiting a patient is considered as a humanistic duty with spiritual rewards. The results of different studies are in favor of the effects of sensory stimulations on vital signs of patients at ICU, but the role of family members versus nursing staff and also stimulating more than one day have been less evaluated.

The present study was conducted to compare the effect of sensory stimulation by family members compared to nursing staff on vital signs of patients at ICU

KEY WORDS

Family member, Nurse, Intensive care unit, Sensory stimulation, Vital signs

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MATERIALS AND METHODS

This clinical trial study was conducted on 69 patients hospitalized in the ICU in Teaching hospital affiliated with the Lorestan University of Medical Sciences in April to November 2014. A total number of 69 patients were recruited and 23 patients were allocated to each group. After evaluation of patient's records and calculation of Glasgow Coma Scale (6-12), patient information sheet was filled and family members were interviewed. After receiving written informed consent and assuring them about safety of sensory stimulations, the eligible patients were selected and were categorized into two test groups (nurse group & family group) and one control group by stratified block randomization method based on their age. The inclusion criteria were hospital admission for less than 3 days; providing informed consent by family members for participation in the study; the visitor to be first degree relative (father, mother, brother, sister, spouse or child) and > 18 years old; head trauma patients (all types of cerebral hematomas and other cerebral traumas except for diffuse axonal injury) who have pupil reflex at the time of entrance into the ICU; GCS between 6-12; age between 16-65 years and no history of delirium, dementia and hospitalization at psychiatry hospital. The exclusion criteria were withdrawal from the study for any reason, death, being transported to other medical centres and getting psychosis during hospitalization.

Dyang sensory stimulation program for test groups was performed 2 hours a day with 3 hours interval (started at 4:00 to 5:00 pm and repeated 8:00 to 9:00 pm) for 6 consecutive days. The family member who was selected for doing the intervention was the same one along with the 6 days. He/she was told to perform just sensory stimulations for 1 hour as educated by the researcher; and was being supervised from this aspect. It should be noted that the intervention was done while the half-life of analgesic medications had been passed. Dyang sensory stimulation program (1987) was educated to family members by researcher (nurse). For family group, the sensory stimulation was performed by a family member and for nurse group, it was done by the researcher, No intervention was performed for control group.

Dyang sensory stimulation program includes olfactory, hearing, visual, motor and tactile stimulations. Olfactory stimulation is implemented by holding an alcohol-soaked cotton in front of patient's nostrils for 5 seconds; visual stimulation by turning on and off a flashlight in front of patient's eyes for 2 seconds, hearing stimulation by telling patient's name, time, location and date near to patients ears for 3 times, tactile stimulation by hand pressure, massage and rubbing cotton and gauze against skin (one side of the body and then another side) and motor stimulation by moving the joints in the hand, foot, wrist, hip, and shoulder by flexion and extension and moving them upward and downward alternatively for 15 times. Each of the stimulations was done one time during one hour [13, 14].

The vital signs (pulse rate, respiratory rate, systolic and diastolic blood pressure and body temperature) were measured by research assistant (someone other than the stimulator), using cardiopulmonary monitoring device and auxiliary thermometer. The measurements were done for all subjects in three groups in 2 stages; 5 minutes before intervention and 30 minutes after the end of intervention (total of 12 interventions for each patient). Calibration of applied devices was being checked by medical device engineer of hospital on weekly basis.

Data were analysed using SPSS software (SPSS Inc, Chicago, Illinois, USA). Descriptive and analytical statistics were used to analyse the data. Mean and standard deviation were calculated for quantitative variables. Repeated measurement test was used to compare mean vital signs of the patients in each group before and after intervention, and one-way ANOVA was used to compare mean vital signs in 3 groups of patients. The Chi-squared test was used for some variables such as sex, level of education and diagnosis. This study was approved by the Ethics Committee of Lorestan University of Medical Sciences (N.20066375), and registered in the Iranian Clinical Trial Website with the IRCT201204149469N1 code. The objectives of the study were explained to all participants and all of them signed a written informed consent and were assured of the confidentiality of their individual information as well as the voluntary nature of participating in the study. In all stages the researchers were committed to observe the ethical issues in accordance to the Helsinki ethical declaration.

RESULTS

The results of this study showed that the subjects in all groups had no significant difference in terms of age and basic vital signs. In addition, there was no significant difference in terms of sex, level of education, occupation, cause of coma, diagnosis at time of admission and location. Most of the studied subjects (89.9%) in 3 groups were male. There were statistically significant difference between mean systolic blood pressure in three groups before 10th and 11th intervention; body temperature before and after 9th intervention; mean respiratory rate of patients after 9th intervention and mean pulse rate of the patients before 6th and after 12th intervention. But, regarding the diastolic blood pressure and other interventions related to other variables, no significant difference was noted between variables before or after intervention in 3 groups [Table 1].

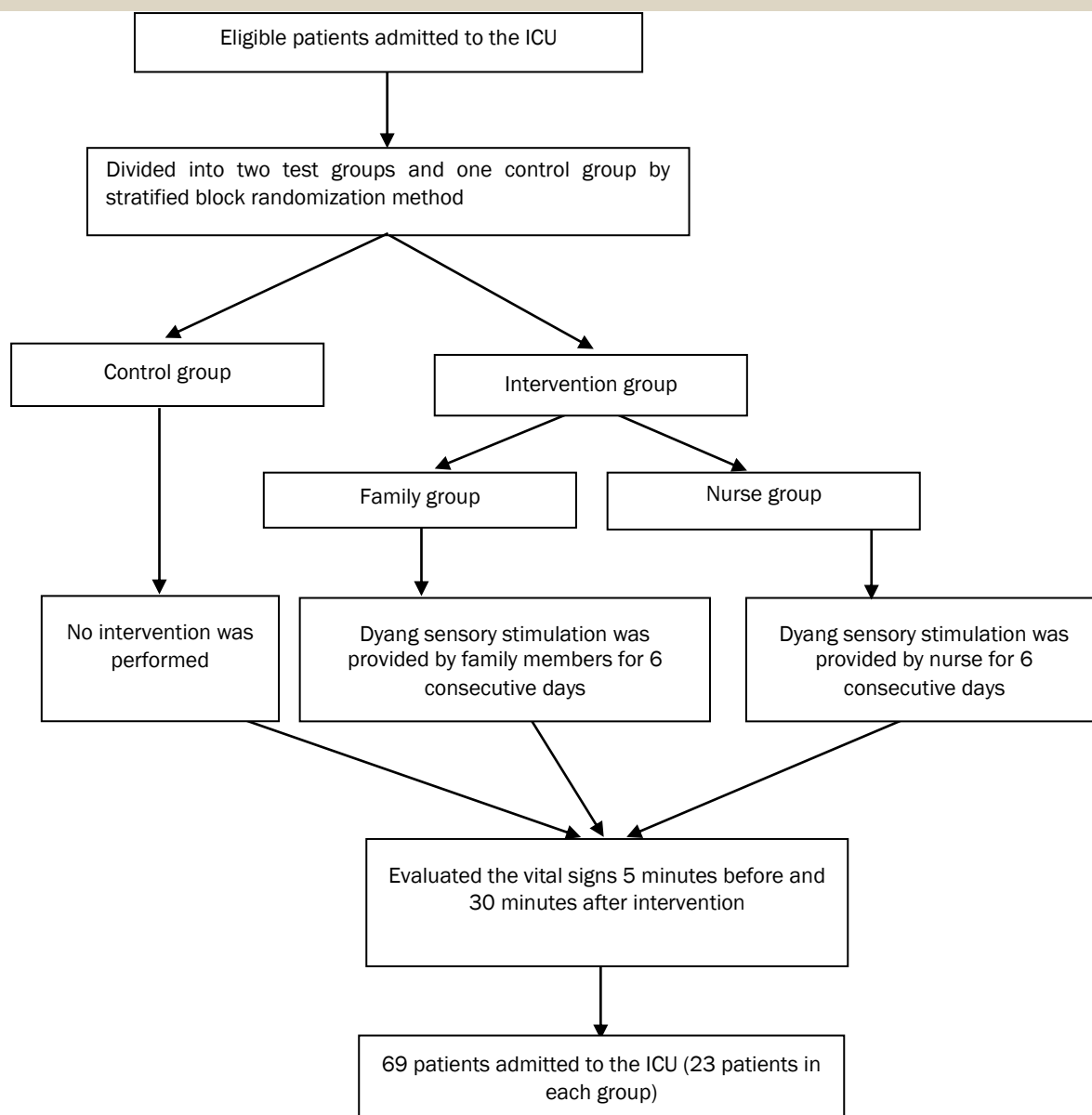


Diagram 1: The flowchart of study groups

Table 1: Comparison of the three groups in terms of variable means before and after each intervention

Type of variable	Number of intervention	Before or after the intervention	Family group N (Mean ± SD)	Nursing group N (Mean ± SD)	Control group N (Mean ± SD)	Number N (Mean ± SD)	P-Value
Systolic blood pressure	Tenth	Before	23 (120.47 ± 10.89)	23 (125.78 ± 7.71)	23 (129.91 ± 17.53)	69 (125.39 ± 13.12)	0.048
	Eleventh	Before	23 (116.48 ± 10.31)	23 (122.95 ± 12.00)	23 (127.43 ± 14.35)	69 (122.27 ± 12.97)	0.013
Temperature	Ninth	Before	23 (37.14 ± 0.44)	23 (37.47 ± 0.38)	23 (37.13 ± 0.57)	69 (37.22 ± 0.49)	0.027
	Ninth	After	23 (37.15 ± 0.41)	23 (37.49 ± 0.40)	23 (37.19 ± 0.50)	69 (37.27 ± 0.46)	0.024
Respiratory Rate	Ninth	After	23 (21.21 ± 4.33)	23 (21.30 ± 3.16)	23 (18.52 ± 2.50)	69 (20.34 ± 3.34)	0.010
Pulse Rate	Sixth	Before	23 (79.60 ± 14.75)	23 (92.04 ± 20.61)	23 (89.73 ± 13.76)	69 (87.131 ± 7.28)	0.032
	Twelfth	After	23 (89.82 ± 19.53)	23 (90.47 ± 16.24)	23 (78.60 ± 17.27)	69 (86.30 ± 18.31)	0.045

In addition, the ANCOVA results showed that there were statistically significant difference between mean systolic blood pressure of patients before and after 3rd, 7th to 12th interventions; mean diastolic blood pressure before and after 9th and 11th interventions; body temperature before and after first intervention; respiratory rate before and after 2nd, 5th, 8th and 12th interventions and pulse rate before and after first, 5th to 7th, 9th and 12th interventions; but the difference was not significant in other items [Table 2].

Based on the results of repeated measurement test, there was no significant difference between mean vital signs except for respiratory rate of patients before and after intervention in different days. In other words, in general, the impact of intervention in different days was the same on vital signs, except for respiratory rate. Besides, considering the results of this test, there was no interactive effects between mean vital signs before and after intervention and between the test groups. In other words, the impact of intervention was the same on different days in 3 groups [Table 3].

But according to ANCOVA results, there was statistically significant difference between test groups in terms of mean vital signs of patients before and after intervention except for body temperature ($p < 0.001$). The Tukey paired test showed that there is significant difference between test groups in terms of mean vital signs before and after intervention except for body temperature. In other words, in all subjects, family group was the best test group and nursing group was the second and control group was the last group in classification [Table 4].

Table 2: Comparison of three groups in terms of mean vital signs before and after intervention

Type of variable	Number of intervention	Family group N (Mean \pm SD)	Nursing group N (Mean \pm SD)	Control group N (Mean \pm SD)	Total N (Mean \pm SD)	P-Value
Systolic blood pressure	Third	23 (3.56 \pm 6.08)	23 (2.27 \pm 5.97)	23 (2.04 \pm 7.91)	69 (1.24 \pm 7.04)	0.017
	Seventh	23 (7.43 \pm 6.94)	23 (0.13 \pm 9.85)	23 (0.13 \pm 5.97)	69 (2.39 \pm 8.45)	0.001
	Eight	23 (4.39 \pm 4.87)	23 (0.82 \pm 6.25)	23 (1.04 \pm 6.29)	69 (1.53 \pm 6.15)	0.012
	Ninth	23 (5.95 \pm 5.76)	23 (1.13 \pm 6.48)	23 (0.82 \pm 6.69)	69 (1.33 \pm 7.04)	0.000
	Tenth	23 (4.47 \pm 5.46)	23 (0.00 \pm 5.72)	23 (1.26 \pm 4.96)	69 (1.07 \pm 5.86)	0.002
	Eleventh	23 (5.69 \pm 7.44)	23 (2.04 \pm 9.09)	23 (2.39 \pm 4.27)	69 (1.11 \pm 7.88)	0.001
Diastolic blood pressure	Twelfth	23 (5.30 \pm 6.75)	23 (3.39 \pm 7.79)	23 (0.65 \pm 5.82)	69 (2.42 \pm 7.66)	0.000
	Ninth	23 (4.82 \pm 8.47)	23 (1.65 \pm 5.74)	23 (1.39 \pm 7.56)	69 (1.69 \pm 7.68)	0.021
Temperature	Eleventh	23 (2.47 \pm 6.02)	23 (1.60 \pm 5.33)	23 (0.60 \pm 5.50)	69 (0.08 \pm 5.81)	0.043
	First	23 (0.07 \pm 0.10)	23 (0.03 \pm 0.11)	23 (0.02 \pm 0.17)	69 (0.02 \pm 0.14)	0.022
Respiratory rate	Second	23 (2.65 \pm 2.40)	23 (0.47 \pm 2.76)	23 (0.60 \pm 2.82)	69 (0.84 \pm 2.96)	0.000
	Fifth	23 (1.26 \pm 2.71)	23 (0.65 \pm 2.70)	23 (0.65 \pm 2.46)	69 (0.42 \pm 2.71)	0.048
	Eight	23 (2.00 \pm 2.79)	23 (0.00 \pm 2.95)	23 (1.52 \pm 2.52)	69 (1.17 \pm 2.85)	0.044
	Twelfth	23 (1.86 \pm 2.11)	23 (0.56 \pm 3.02)	23 (0.39 \pm 2.88)	69 (0.68 \pm 2.82)	0.022
Pulse rate	First	23 (4.60 \pm 4.47)	23 (1.08 \pm 11.26)	23 (3.56 \pm 7.45)	69 (0.71 \pm 8.76)	0.05
	Fifth	23 (5.39 \pm 5.77)	23 (2.26 \pm 11.06)	23 (0.52 \pm 4.75)	69 (2.37 \pm 7.97)	0.040
	Sixth	23 (5.43 \pm 11.43)	23 (2.30 \pm 14.71)	23 (2.21 \pm 5.76)	69 (0.30 \pm 11.68)	0.033
	Seventh	23 (5.60 \pm 5.49)	23 (4.39 \pm 21.02)	23 (3.52 \pm 5.13)	69 (1.57 \pm 13.42)	0.026
	Ninth	23 (5.47 \pm 7.91)	23 (0.47 \pm 9.07)	23 (1.65 \pm 8.46)	69 (1.11 \pm 8.94)	0.013
	Twelfth	23 (8.95 \pm 23.99)	23 (0.82 \pm 5.53)	23 (4.34 \pm 16.12)	69 (1.81 \pm 17.62)	0.033

Table 3: Analysis of variance for mean vital signs before and after after intervention in different days

Vital signs	The variable name	Degrees of freedom	F	P-Value
Systolic blood pressure	Overall effect of the intervention	11	1.095	0.362
	Interaction between the intervention and control group	22	0.847	0.667
	Interaction between the intervention and control group	11	0.951	0.490
Diastolic blood pressure	Overall effect of the intervention	11	0.257	0.993
	Interaction between the intervention and control group	22	0.540	0.958
	Interaction between the intervention and control group	11	0.321	0.981
Temperature	Overall effect of the intervention	11	0.189	0.998
	Interaction between the intervention and control group	22	0.852	0.660
	Interaction between the intervention and control group	11	0.619	0.813
Respiratory rate	Overall effect of the intervention	11	1.289	0.226
	Interaction between the intervention and control group	22	1.099	0.342
	Interaction between the intervention and control group	11	1.511	0.122
Pulse rate	Overall effect of the intervention	11	0.272	0.991
	Interaction between the intervention and control group	22	1.176	0.262
	Interaction between the intervention and control group	11	0.317	0.982

Table 4: Analysis of variance between the mean vital signs before and after Intervention

Type of variable	The variable name	Degrees of freedom	F	P-Value
Systolic blood pressure	The overall effect of the test group	2	38.677	<0.001
	The overall effect of age	1	10.282	0.673
Diastolic blood pressure	The overall effect of the test group	2	14.424	<0.001
	The overall effect of age	1	1.069	0.305
Temperature	The overall effect of the test group	2	0.930	0.404
	The overall effect of age	1	0.243	0.624
Respiratory rate	The overall effect of the test group	2	6.691	0.02
	The overall effect of age	1	0.118	0.732
Pulse rate	The overall effect of the test group	2	2.656	<0.001
	The overall effect of age	1	4.937	0.030

DISCUSSION

Despite the homogeneity of the patients' vital signs in three groups on the first day before study initiation, the results showed the effectiveness of Dyang sensory stimulation on mean vital signs of comatose patients except for body temperature. However, the long-term impact of sensory stimulations on reducing or increasing mean vital signs was not obvious. Along with the current research, results of the study done by Rahmani et al who evaluated the effect of planned meeting on the physiologic indicators of the patients who suffer from Acute Coronary Syndrome, showed that heart rate and systolic/diastolic blood pressure of study subjects were increased after the start of visiting compared to pre-visiting time and this increase was

continued throughout the visiting time. However, they found that these values were decreased at the end of visiting; as during half an hour after visiting, the heart rate, systolic/diastolic pressure are decreased to the level that is lower than the pre-visiting stage [15]. This indicates that overall effects of time on the impact of intervention on mean vital sign is not significant. Kamranifar et al also in a separate study found the same results [16]. Although higher difference of mean vital signs after intervention in this study compared with the studies of Rahmani and Kamranifar may be caused by scheduled Dyang sensory stimulations, presence of a constant and the closest family member on the bedside and continued intervention during different days. However, unlike the results of the current study, Mitchell et al found that there is no significant difference between mean arterial blood pressure before, during and after visiting time [9]. This fact may be caused by shorter duration of visiting time and shorter duration of intervention in their study. As well as, this study was conducted in NICU.

In this study, no significant difference was noted between body temperature of patients before, during and after intervention. So, it can be concluded that the intervention did not provide any impact on body temperature of subjects. This fact may indicate that the intervention has not increased the metabolic rate of the patients and so is beneficial for the patients because increased metabolic rate can increase the body temperature [17]. But, in the study of Kamranifar et al, there was significant difference in mean body temperature before, during and after visiting [16]. This difference may be caused by using Dyang sensory stimulation program; because no specific program had been utilized in Kamranifar study and family members had performed the sensory stimulations in any desired format and might over-stimulate or under-stimulate the patient. The results of this study showed that there is significant correlation between mean respiratory rates before, during and after intervention and respiratory rate has been increased after visiting and after sensory stimulation but has been decreased 30 minutes after visiting and intervention. The recently mentioned findings are in accordance with Rahmani and Kamranifar studies, but in this case, Hart et al showed that there is no significant difference between mean respiratory rate before, during and after visiting [18]. This fact may be caused by shorter duration of visiting time in this study and the above mentioned researches. In addition, our findings showed that regarding the impact of sensory stimulation on vital signs, the family group was the best, nursing group was the second and control group was the last group in classification. This result can be utilized on the best way for patients who are admitted in ICU. Tavangar et al showed that performing sensory stimulation by family members for 10 consecutive days could increase patients' GCS at the end of 10th day compared with the first day ($p=0.0001$), but, no significant changing in GCS was noted in the control group (without receiving sensory stimulation) [19]. Besides, the presence of family members at the bedside can improve general health of the patients; because the family and family life is an essential part of every person's health. So, considering the importance of family and its impressive role for patients, it should be considered as a very important issue in nursing plans. Today, caring environment includes both patient and family and general care includes patient and family care together [20, 21]. Leon et al and Alvarez et al showed that the presence of family members at ICU and engaging them in the treatment process can reduce anxiety and help families cope with the current crisis [22, 23].

So it can be concluded that the vital signs are influenced by visiting and sensory stimulations; but these effects are transient and usually reset after the visiting time and are not clinically significant. If family members perform sensory stimulations, a greater impact on the vital signs will be observed at short time. Moreover, formal sensory stimulations by Dyang will provide less negative and more positive impact on physiological parameters of the patients.

CONCLUSION

The effect of sensory stimulation on vital signs of comatose patients was greater when provided by family members. It is suggested that in future studies, a closest member of patient's family to be trained to perform sensory stimulation on appropriate time for the patient at ICU.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ARTICLE

THE PREVALENCE OF GINGIVAL ENLARGEMENT IN CHILDREN AND SOCIO-ECONOMIC AND DEMOGRAPHIC FACTORS, IRAN, 2016

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ABSTRACT

There are different types of periodontal diseases with the common aspect of destructing periodontium. Most of patients are children and one of these diseases is gingival enlargement. Demographic factors like gender and age, socio-economic conditions, inflammatory factors, systemic conditions, medications and many other factors are the etiology of gingival enlargement. Due to physical and emotional complications of this problem, it is felt necessary to evaluate the prevalence of gingival enlargement and etiologic factors affecting it. 1113 girls and boys [7 to 13-year-olds] were selected from 10 primary schools of Gorgan-Iran. After filling the consent form by parents, a questionnaire was filled. Children were examined by McGraw index to assess the presence of gingival enlargement, location and severity. Achieved data were analyzed through SPSS16 statistical software and chi-square test and T-test. 716 children were normal. 397 children had gingival enlargement: 143 cases with minor gingival enlargement, 251 cases with moderate gingival enlargement, and 3 cases with severe gingival enlargement. Mother's education in 264 cases were academic and in 132 cases were non-academic and father's education in 250 patients were academic and in 141 patients were non-academic. Incidence of gingival enlargement was more in boys, But other factors were not significantly associated with its incidence.

INTRODUCTION

Several thousands of people in the world are suffering from periodontal diseases. The prevalence of these diseases is high and even exceeded the prevalence of dental caries [1,2]. There are different types of periodontal diseases, the common aspect of them is changing the ideal condition and destructing the periodontium [3]. This is despite the fact that a lot of people with these diseases are children [2-5]. Periodontal diseases are the main cause of permanent tooth loss. Inflammation, bleeding, gingival recession, bone loss, tooth mobility and displacement, pain and many other complications are symptoms of periodontal diseases [6]. It is believed that periodontal disease in adults is partly accelerated due to inflammation of the gingiva formed in childhood and early adolescence. In this case with no treatment, non-interference non-destructive gingival inflammation in children can progress to be more serious in adulthood [7]. One of the commonest periodontal diseases in children is gingival enlargement which starts from the cervical part of crown like a collar and will continue to mucogingival Junction [8]. Healthy gingival is coral pink with a view of orange peel [stippling] and in some breeds with dark skin, pigmentation may be seen [9,10]. If the gingival has increased in volume, the view will be red and inflamed with a smooth shiny surface or suspended light pink, firm and non-edematous [7,9].

Conditions such as gingival enlargement can lead to bad breath and difficulty in speaking, chewing, tooth eruption and pain [11]. On the other hand, health of gingival has effective role on the physical and mental health and psychic diseases such as anxiety and depression in children by interfering with the beauty [12]. In most patients gingival enlargement are ignored and the appropriate treatment are not presented; Even if gingival enlargement is mild and is not easily recognizable, it can act as a site for the accumulation of pathogens leading to gingivitis or periodontitis and damage surrounding tissue [13,14]. In severe cases it may require that the additional volume of gingiva being removed through surgery. This method has limitations and complications and its use in children is not easy [14]. The best approach for these problems is prevention and early diagnosis along with appropriate treatment [15,16]. Studies show that factors such as demographic factors like gender and age, socio-economic conditions, inflammatory factors, systemic conditions, medications and many other can cause gingival enlargement in children [17-27]. The most common cause of gingival enlargement is inflammatory changes in the gingival tissue due to poor oral hygiene. Local factors such as unfavorable anatomy of teeth and dental crowding affect the quality of oral health [18,19]. This increase in volume can be exacerbated by hormonal changes at puberty or mouth breathing, especially in the anterior part of maxilla [17,20-24].

The prevalence of gingival enlargement in children has been reported with different values [2,25]. According to a study it conducted in children and students below high school age, the prevalence of periodontal disease was three times more than adults [17]. However, other studies have shown that the prevalence of periodontal disease increases according to age and is not noticeable in children [22,28]. The role of gender in the prevalence of gingival enlargement is contradictory [3] [30,32]. In other studies, the effect of factors such as living conditions, education and socio-economic position on development of

KEY WORDS

Gingival enlargement,
McGraw index,
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gingival enlargement in patients have been reported [32,33]. However, in some studies, the relationship between individual and social characteristics associated with gingival enlargement is not clear [34]. Numerous studies showed conflicts and various results about gingival enlargement and its associated risk factors among children and In Iran also the exact number is not known. Due to physical and psychiatric complications of this problem, it is felt necessary to evaluate the prevalence of gingival enlargement and etiologic factors affecting it, while recognizing the positive samples, prevent the occurrence of more cases and try to improve the conditions of patients.

MATERIALS AND METHODS

In this cross-sectional study, 1113 girls and boys [7 to 13-year-olds] were selected from 10 primary schools of Gorgan city- Golestan state-Iran. The samples were selected randomly by use of questionnaires and based on inclusion and exclusion criteria. The inclusion criteria include: not suffering from any kind of diseases affecting gingival, not taking drugs affecting gingival, not having orthodontic treatment during examination or last 3 months, not having periodontal treatment during examination or last 6 months, no antibiotic use for 7 days in last 3 months, not having mouth breathing, not using partial dentures as a space maintainer, not having extensive tooth decay, and not having open bite. According to the similar study, in this study with primary estimation of %15 prevalence, %3 accuracy, %95 assurance, and $n=z^2pq/d^2$ relation, at least 545 samples were needed, but according to sampling structure and considering correction factor, 1113 samples were selected to be analyzed. The case selection method was multi-step random sampling, in each region of the city a male and a female school were selected as a cluster, and in each school from each grade, the appropriate number of students were selected by random sampling.

For this purpose, after coordinating with Golestan Office of Education and obtaining permits from the committee on ethics in academic research, the list of male and female elementary school children accompanied by the information related to the geographical position and the number of students per school was taken. Then by cluster random sampling, 10 elementary schools [5 male schools and 5 female schools] were determined in 5 parts of the city [North, South, West, East and central based on the administrative division of the municipality of Gorgan]. The demanded number of students were selected randomly proportional to the population per school based on number of students in the class. So that, from each school an average of 110 forms of personal information were taken and in total 1113 children came into this study.

A number was assigned to each elected child, and with the cooperation of selected schools' agents who were informed before during the explanatory meetings, a numbered envelope containing a form to obtain parental consent and a questionnaire was given. The form includes information such as age, gender, living location, used drugs, systemic diseases, history of dental treatment, history of antibiotic use, parents' education and job. Phone number of parents were also taken to contact if needed. After collecting the forms, the cases lacked the inclusion criteria were discarded, and in order to replace the cases, random sampling was repeated. After completing the required number of samples, clinical examination was performed with a disposable mirror and probe and explorer to investigate the presence or absence of gingival enlargement, location and severity by using McGraw index.

Table 1: McGraw gingival enlargement index

Grade	Explanation
0	Absence of gingival enlargement
1	Gingival enlargement is only in dental papilla
2	Gingival enlargement covers less than 1/3 of dental crowns
3	Gingival enlargement covers more than 1/3 of dental crowns

In the case of gingival enlargement, its location [localized/ generalized, anterior/ posterior, left/ right, mandible/ maxilla] and the severity [1-2-3] was determined. The total prevalence of gingival enlargement and its prevalence associated with age, gender, living location, parents job and education were analyzed through SPSS16 statistical software and chi-square test and T-test. Pvalue was 0.05.

RESULTS

According to children examination based on McGraw index, 716 children [64.3%] were normal and without gingival enlargement and 397 children [35.7%] were identified with gingival enlargement [Fig 1].

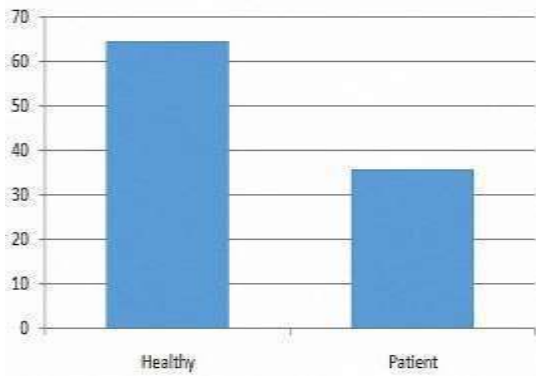


Fig. 1: Gingival enlargement prevalence

Among the 716 children without gingival enlargement, 357 were girls [49.9%] and 359 [50.1%] were boys and also among the 397 children who were diagnosed with gingival enlargement, about 185 of them were girls [46.6%] and 212 were boys [53.4%] [fig 2].

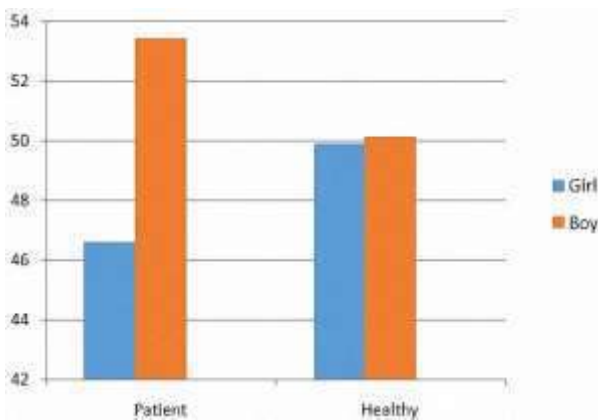


Fig. 2: The percentage of patient and healthy children according to gender

Of 397 known cases, 143 cases [36%] had minor gingival enlargement, 251 cases [63.2%] had moderate gingival enlargement, and 3 [0.75%] had severe gingival enlargement. Among patients with gingival enlargement, 60 cases were girls [42%] and 83 cases were boys [58%], of patients with moderate gingival enlargement, 122 cases were girl [48.6%] and 129 cases were boys [51.4%], and the patients with severe gingival enlargement, all were female [100%]. Mother's education of patients in 264 cases [66.7%] was non-academic and in 132 cases [33.3%] was academic, while mother's educational of healthy students in 478 cases [67.1%] was non-academic, and in 234 cases [32.9%] was academic.

Father's education of patients in 250 cases [63.9%] was non-academic and in 141 [36.1%] was academic, while father's education of children without the disease in 468 cases [66.3%] was non-academic and in 238 cases [33.7%] was academic. Mothers of patients in 295 cases [74.3%] were housewives and in 102 cases [25.7%] were employed, while mothers of healthy children, in 511 cases [71.5%] were housewives and 204 [28.5%] had a job. Fathers of patients in 238 cases [61%] had non-government job and in 152 cases [39%] had government job, whereas the fathers of healthy children, in 443 cases [63.3%] had non-government job and in 257 cases [36.7%] had government job. The mother's and father's jobs of both patient and healthy children are shown in Fig 3 and 4.

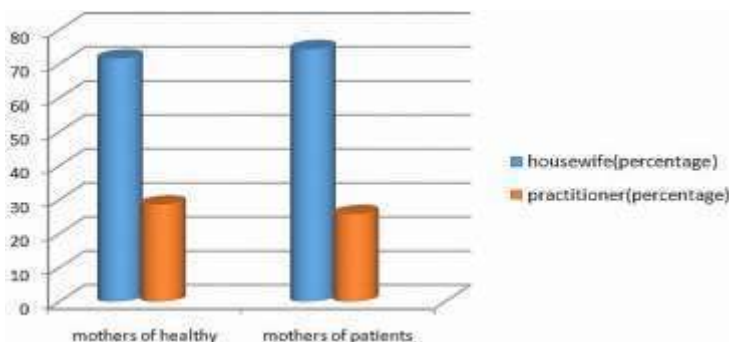


Fig. 3: Parents' education in patient children category

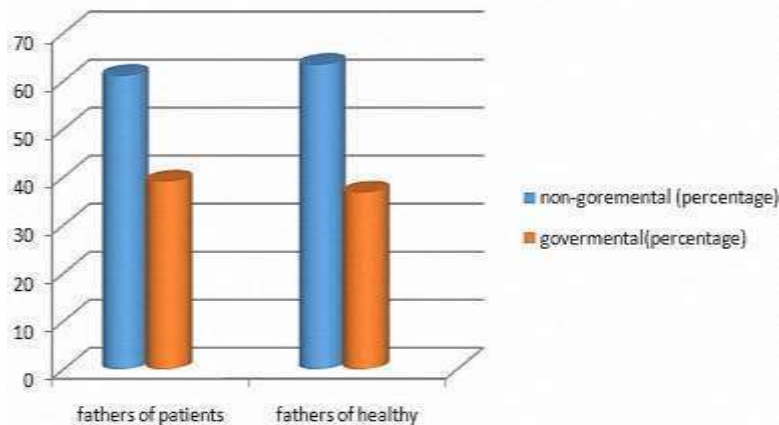


Fig. 4: Parents' job in patient children category

The habitat of patients, in 89 cases [22.4%] were in the north of city, in 83 cases [20.9%] were in downtown, in 78 cases [19.6%] were in west, in 64 cases [16.1%] were in Eastern section and in 24 cases [6%] were in the center of city.

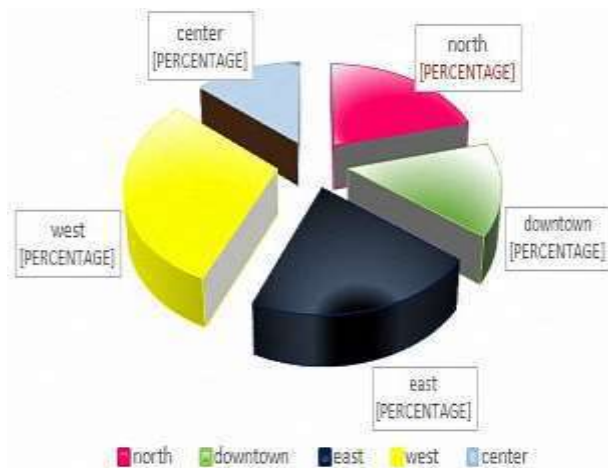


Fig. 5: Geographic distribution of patients

DISCUSSION

The results of this study show that the incidence of gingival enlargement was 35.7% and it was happening more in boys [p.value =0.014]. More boys had moderate and severe gingival enlargement. But factors such as living location, occupation and parental education were not significantly associated with the incidence of gingival enlargement in children. One can only conclude that boys are more likely to develop the condition than girls. The studies showed that factors such as demographic factors like age and gender, socio-economic conditions, inflammatory factors, systemic conditions, medications and many other factors cause gingival enlargement in children [17-27,30,32]. Monique and colleagues stated that age and sex are closely related to gingival inflammatory disease and gingival enlargement. They showed that gingival inflammatory disease had more incidence in men than in women and increased with increasing age [12].

Louisa and colleagues conducted a study in Colombia examined the association between social factors and gingival enlargement, they concluded that those with better social status suffer less [17]. While this study did not show statistically significant associations between these factors with gingivitis. In some studies it has been shown that the prevalence of periodontal disease increases with age and is not noticeable in children [22,28]. While this study did not show any significant association between age and gingival enlargement in children. Numerous studies showed conflicts and various results about gingival enlargement and associated risk factors among children and In Iran also the exact number is not known. Since gingival enlargement in children can be accelerated and modified by hormonal changes at puberty or mouth breathing and can be detected as gingivitis and gingival enlargement, especially in the anterior part of maxilla [7, 20-24], So it is necessary to instruct oral hygiene and oral health promotion in schools more centralized in boy schools]to avoid this problem in children and long-term effects in the future which is more cost effective for families. Because the exact number of this disease is not known in Iran, so wider

research is necessary to achieve more accurate results for authorities in order to plan with more certainty about improving oral health hygiene in schools.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FINANCIAL DISCLOSURE

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ARTICLE

FACTORS LIMITING CANOLA YIELD AND DETERMINING THEIR OPTIMUM RANGE BY BOUNDARY LINE ANALYSIS

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ABSTRACT

Golestan Province, especially its eastern parts, is a main canola (*Brassica napus*) growing region in Iran. Inappropriate yield is the main factor limiting its planting area. So, factors affecting canola yield were studied in a survey in 2013-2014 and 2014-2015 growing seasons in eastern part of Golestan Province, including Qonbad, Kalaleh and Galikash, in which all managerial data of 332 canola farms were collected and then, were analyzed by boundary line approach. It was found that there was 59% gap between farmers' mean yield (1417 kg ha⁻¹) and potential yield (3407 kg ha⁻¹) which is equal to a yield gap was 1987 kg ha⁻¹. Examining fertilization rates by boundary line analysis indicated that most farms did not fertilize their canola crops adequately. In fact, 80, 93, 95 and 93% of farmers applied less-than-optimum rates of nitrogen, phosphorus, potash, and sulfur fertilizers, respectively. The minimum optimal rates found by boundary line analysis were estimated to be 122 kg ha⁻¹ N, 49 kg ha⁻¹ P₂O₅, 34 kg ha⁻¹ K₂O, and 40 kg ha⁻¹ S. Also, it was revealed that it would be better to use 42 kg ha⁻¹ of 122 kg ha⁻¹ N as basal fertilizer. Plant density and sowing dates were two other factors limiting yield. The best plant density was estimated to be 83-90 plants ha⁻¹ whilst only 23% of farms had densities in this range. The best sowing date range was estimated to be October 27 to November 8. In order to obtain potential yield, the weed population should be less than three plants m⁻² and the plants showing advanced symptoms of disease in their stem should be less than 4% of the plants. Using findings of the study it is possible to narrow yield gap. The approach can be applied in other regions and crops.

INTRODUCTION

Golestan Province, especially its eastern parts, is one of the most important canola (*Brassica napus*) production regions in Iran, where canola is often produced in rotation with wheat. Canola is considered as a good rotation crop for wheat yield stability. The advantages of canola rotation include its deep root system and the increased porosity of subsoil on the one hand [1] and its capability in breaking the cycle of wheat diseases and weeds on the other hand [2]. Despite the fact that wheat planting area is annually about 400,000 ha in Golestan Province, canola planting area annually averages only about 30,000 ha and so, it has not gained its real niche in local agricultural pattern. After 2004-2005 growing season when planting area was culminated to 70,000 ha, it was started to decline due to declining yield. Yield and production limiting factors need to be well understood if canola yield is intended to be increased so much that its economical advantages encourage farmers to grow it. Then, the optimum range can be determined for each factor to pave the way for yield improvement.

A lot of factors affect canola production and yield in all regions, including high temperatures [3] diseases especially blackleg (*Leptosphaeria maculans*) and white mold (*Sclerotinia sclerotiorum*) [4] and soil, climatic and managerial factors [2] that are the main natural limiting factors of canola growth and yield.

The present survey was aimed at examining some canola yield limiting managerial factors, including nitrogen, phosphorus, potash, and sulfur fertilization, plant density, and sowing date, investigating the impact of weeds and diseases on yield, and determining their optimum range by boundary line analysis. Boundary line analysis is a technique by which the yield response to an environmental or managerial factor can be quantified under the conditions that all other factors are variable. In fact, this technique specifies yield response to a specific factor under the conditions that all other factors are suitable [5]. Since natural conditions are constantly changing and natural factors are uncontrollable and unpredictable, the results might be affected and the precise evaluation of the relationship between two variables might be impossible [6]. So, an approach that could cope with these problems would be invaluable. Boundary line approach, first introduced by [7] possessed this feature. The technique was successfully used to describe the relationship between soil nutrient concentrations and soybean yield [8] and the leaf yield of are canut [9] to determine the relationship between yield and plant density of corn as well as some soil characteristics [10] to estimate the range of N₂O emission from soil [11] to specify the relationship between pea yield components [12] and in studies on wheat yield gap [13] and soybean yield gap [14].

Since yield is affected by various factors, the use of boundary line technique will allow recognizing the yield response to just one variable out of the various collected data. Rather than fitting regression lines from the middle of data dispersions, the technique studies the upper edge of the data dispersion. This boundary shows the highest obtained yields (yield potential) and/or the best yield as affected by different levels of a certain factor or input. The technique assumes that these yields are the highest yields possible in the absence of other limiting factors and that all points located at lower spots are limited by other factors.

KEY WORDS

Yield gap, Boundary line model, Potential yield

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MATERIALS AND METHODS

Surveyed region

The surveyed region was located to the east of Golestan Province around Qonbad, Kalaleh and Galikash cities covering the area between the latitudes of 37°02'50" and 38°05'29" N. and the longitudes of 54°33'01" and 56°02'01" E. The highest altitude of the surveyed region was 500 m. Qonbad has 159,000 ha arable land, out of which over 52% is rain-fed. Kalaleh is to the east of Golestan Province bordered Qonbad. It has 68,000 ha arable land, 80% under rain-fed. Galikash is bordered to the north of Kalaleh and Qonbad. Its area is 1460 km² with 29,000 ha arable land, out of which about 56% can be irrigated.

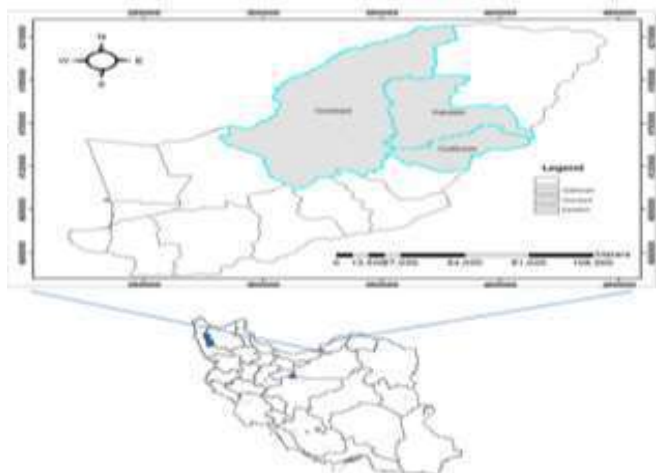


Fig. 1: The location of survey region in eastern Golestan Province, Iran

The surveyed region has Mediterranean climate with dry summers. Table 1 shows local climatic data.

Table 1a: Growing season climate data of Kalaleh

Date	Mean T _{min}		Mean T _{max}		Rainfall (mm)		Evaporation (mm)	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
October	12.9	12.5	26.3	24.9	45.1	26	120.9	130.7
November	10.6	7.3	21.4	17.1	34.2	63.9	69.7	58.7
December	3	4.6	12.9	16.8	49.9	29.5	40.6	50.5
January	2.9	3.2	13.3	14	27.6	43.1	51.1	51.4
February	-0.4	5.6	12.9	14.8	33.7	90.9	37.3	39
March	5.2	5.7	17.4	15.6	84.3	72.8	78	62.6
April	10.1	9.4	24.1	23.1	20.1	22.4	96.6	96.1
May	16.3	15.3	32.5	30.5	19.4	13.4	24.3	194.5

Source: Kalaleh synoptic station

Table 1b: Growing season climate data of Gonbad

Date	Mean T _{min}		Mean T _{max}		Rainfall (mm)		Evaporation (mm)	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
October	13.6	12.7	26.5	25.6	43.4	31.8	95.3	88.2
November	10.8	7.6	21.4	17.7	43	54.3	47.3	36
December	2.7	4.4	13	16.4	34.9	28.4	21.7	29
January	2.7	2.8	13.9	14.3	12.9	39.7	28.8	30.7
February	-0.5	6.4	13.1	15	28	50.3	34.9	29.7
March	5.7	5.9	17.1	16.5	92.6	56	52.7	47.4
April	10.6	9.3	23.9	24.2	14.9	15.5	79	90.6
May	17.2	15.9	33.1	32	15	3.3	177.3	183.2

Source: Gonbad synoptic station

Data collection

The survey was carried out in the eastern part of Golestan Province in Qonbad, Kalaleh and Galikash in 2013-2014 growing seasons. In each growing season, more than 60 canola farms were surveyed in each region. The survey just considered the impact of the managerial factors, ignoring soil and plant-related

data. The collected data were related to the managerial practices including field preparation, agronomical pattern like rotation, planting data, cultivar, and pests, diseases and weeds.

Boundary line analysis

The scatter diagram of the yield data of 332 farms as dependent variable was drawn against managerial variables as independent variables. Boundary line analysis (BLA) is based on the premise that the line fitted on the outer edge of data body (boundary line) is indeed the variable function of the independent variables [15]. It is assumed that such a line is an independent function and is only limited by a single dependent variable or factor. Thus, keeping these facts in mind, all other points under this line (lower yields) have been influenced by other limiting factors [15].

The scatter diagram of data was drawn between dependent variable of seed yield in 332 canola farms and N, P, K and S fertilizer application. First, outlier data were removed and then, points appropriate for boundary line fitting on upper edge of data were selected that had more or less same distance to x-axis. The same procedure was repeated for independent variables of plant density and planting date. Among other factors affecting canola yield, two variables of weeds density and diseased plants percentage were also studied and the boundary line was fitted on distribution of yield data as influenced by these factors. In the present study, after drawing the scatter diagram of the yield in each farm as dependent variable against independent variables (agronomical managements), a function was fitted on upper edge of data distribution by SAS Software Package and nlin procedure. If the functions resulted from the fitting of the boundary line against these points were polynomial, they would be fitted as two-piece functions or, if required, as three-piece functions to better describe the ascending or descending relationship of dependent and independent variables and to determine the optimum range. Then, the optimum range was determined for each factor and the yield gap was calculated by boundary lines equations.

RESULTS

Nutrients and fertilizers

Among 332 surveyed farms in two years of the study, the lowest yield was 200 kg ha⁻¹ and the highest yield was 3450 kg ha⁻¹. The distribution of yield data against managerial variables of fertilization showed that the response of yield as dependent variable to the application of main nutrients as independent variables exhibited similar function so that the application rate of N, base N, P₂O₅, K₂O and S followed a two-piece function [Fig. 2].

The resulted two-piece function [Fig. 2a] shows that if N fertilizer is not applied, seed yield will not reach even 1 t ha⁻¹ (928 kg ha⁻¹ was the maximum yield under no N fertilizer). Also, fitted boundary line reveals that the highest possible yield is obtained by the application of at least 122 kg pure nitrogen. In this respect, the maximum limited N yield was estimated to be 3425 kg ha⁻¹ [Table 2]. Whilst the minimum optimum rate for pure nitrogen fertilizer was 122 kg ha⁻¹, 80% of farmers applied lower N rates to their canola farms so that mean N rate was 92 kg ha⁻¹ in surveyed farms [Table 3]. Also, in spite of the fact that the application of 48 kg ha⁻¹ pure N as basal resulted in the yield of 3407 kg ha⁻¹ [Fig. 2b], 93% of the surveyed farmers used lower rates of basal N fertilizer [Table 2]. In addition, although N rate varied in the range of 0-250 kg ha⁻¹ pure N, its mean application rate was only 10.5 kg ha⁻¹ [Table 3].

Nitrogen is an important nutrient for canola playing a crucial role in boosting its yield. The number of branch per plant, the number of pods per plant, plant height, and biological yield are increased by N application [16]. Bahmanyar and Poshtmasari (2010) reported that the highest yield was obtained from the treatment of 150 and 225 kg N ha⁻¹ and that canola's response to the increase in N rate varied with environmental factors, climate, and soil type. The application of N fertilizer influenced seed yield in all surveyed regions significantly. Probability levels were $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$ and the root of mean squares of error (RMSE) were 487, 504, and 535 kg ha⁻¹ for Qonbad, Kalaleh and Galikash, respectively. The slope of boundary line [Fig. 1a] shows that seed yield could be increased by 20.4 kg ha⁻¹ per 1 kg ha⁻¹ increase in N rate up to N level of 122 kg ha⁻¹. Since 80% of farmers applied less-than-optimum rates of nitrogen (122 kg ha⁻¹) and 93% of them no use basal N fertilizer or applied less-than-optimum rates (48 kg ha⁻¹), it can be concluded that lower dose of N application is one of the most important limiting factors of growth and production in the studied region.

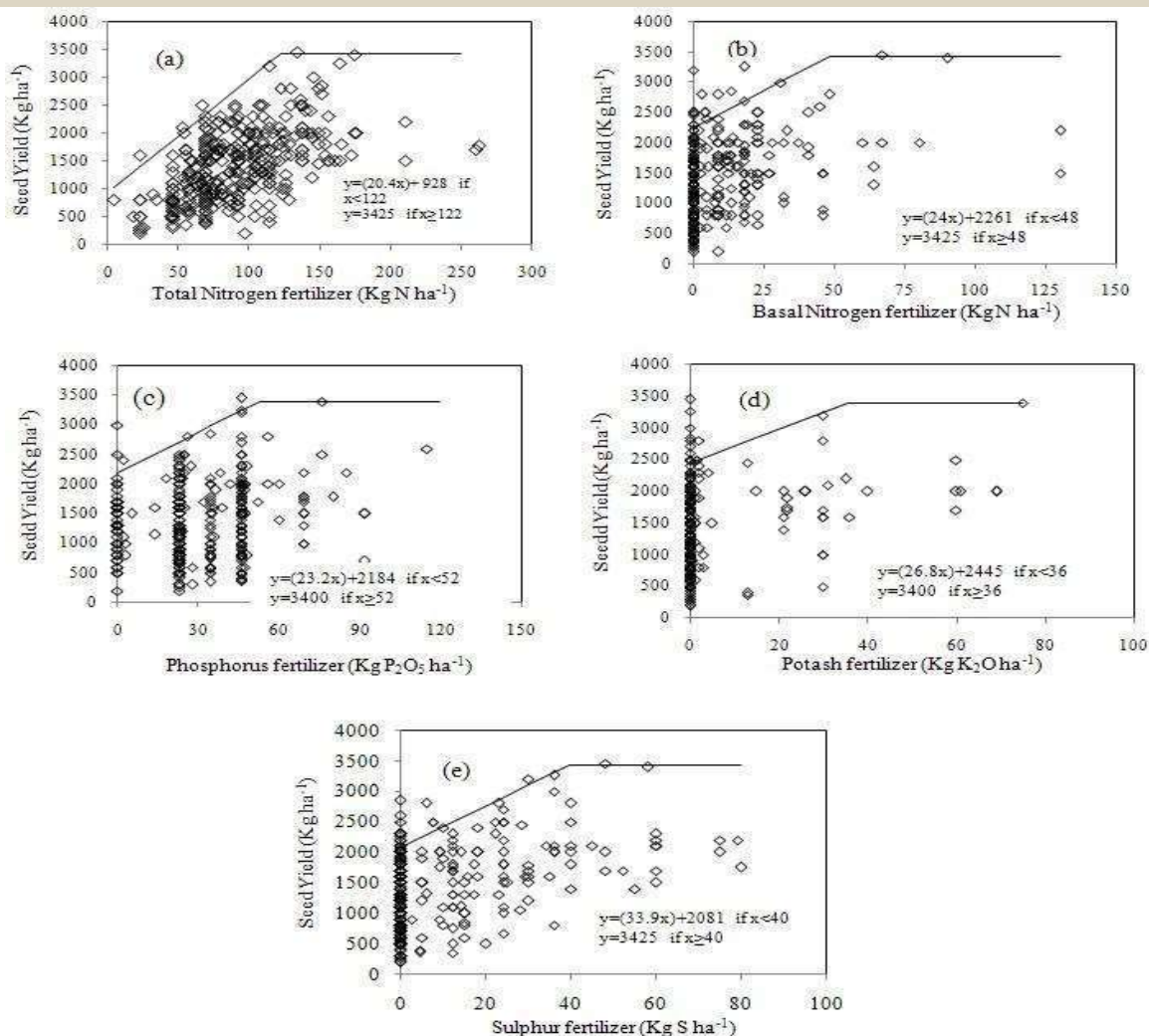


Fig. 2: Scatter graph of the yield data against the rate of nitrogen (N) (a), basal N (b), phosphorus (c), potash (d), and Sulfur (e) fertilizers as well as the fitted boundary line

Table 2: The results of boundary line analysis as well as estimated potential yield and yield gap of canola in eastern parts of Golestan Province

	BasalN (kg ha ⁻¹)	N (kg ha ⁻¹)	P ₂ O ₅ (kg ha ⁻¹)	K ₂ O (kg ha ⁻¹)	Sulfur (kg ha ⁻¹)	Density (plants m ⁻²)	Sowing date	Disease (%)	Weeds (plants m ⁻²)	Average
Minimum optimal level	48	122	52	36	40	83-90	36-48	<4	<3	-
Farmers out of optimum (%)	93	80	93	96	92	77	56	90	66	-
Yield at optima (kg ha ⁻¹)	3425	3425	3400	3400	3425	3279	3425	3450	3450	3407
Average yield (kg ha ⁻¹)	1417	1417	1417	1417	1417	1417	1417	1417	1417	1417
Yield gap (kg ha ⁻¹)	2008	2008	1983	1983	2008	1862	2008	2033	2033	1990
Yield gap (%)	59	59	58	58	59	57	59	59	59	58

Table 3: Management range of yield limiting factors in the surveyed farms

	BasalN (kg ha ⁻¹)	N (kg ha ⁻¹)	P ₂ O ₅ (kg ha ⁻¹)	K ₂ O (kg ha ⁻¹)	Sulfur (kg ha ⁻¹)	Density (plants m ⁻²)	Sowing date	Disease (%)	Weeds (plants m ⁻²)
Minimum	0	4.5	0	0	0	33	282	0	0
Average	10.5	92	32.2	5.4	9.7	78	310	6.2	4
Maximum	250	365	190	170	200	113	355	16	26

The minimum optimal rates were lower for P and K (52 and 36 kg ha⁻¹ absorbable P and K, respectively). The application of these two nutrients had positive impact on yield. Higher P and N application rates improved canola yield significantly through enhancing the number of pods per plant and the number of seeds per podas reported by [17] who stated that 60 kg P₂O₅ ha⁻¹ gave rise to the best result. Whilst most farms (85%) were fertilized with P, only 8.5% of them were treated with K fertilizers. Nonetheless, potassium is an important nutrient that plays a vital role in assimilates mobilization and the tolerance of stresses. Figure 2c depicts that, at most, a yield of 2 t ha⁻¹ can be obtained without phosphorus application. However, a yield of 3400 kg ha⁻¹ can be produced by the application of 49 kg ha⁻¹ phosphorus. In the surveyed regions, 93% of farmers used unfavorable rates of phosphorus [Table 2]. Potash fertilizer was, also, applied in only 26 farms, i.e. 8.5% of all surveyed farms. Figure 2d shows that the application of 36 kg k₂O ha⁻¹ resulted in the yield of 3400 kg ha⁻¹. Although some farms had been applied with as high as 170 kg k₂O ha⁻¹, the mean application rate was very slight in the surveyed region [Table 3]. Table 2 reveals that 95% of the farms received unfavorable potash fertilizer.

Sulfur is another important nutrient for the growth and yield of canola so that its application improves plant height, branch number, pod number per plant, seed number per pod, and biological and seed yield [18]. It influenced yield in the present study, too. In eastern parts of the province, mean yield in farms treated with sulfur was 1763 kg ha⁻¹, whilst it was only 1239 kg ha⁻¹ in farms that were not. According to Figure 2e, the application of at least 40 kg S ha⁻¹ made it possible to obtain a yield of 3425 kg ha⁻¹. At the same time, Table 2 indicates that the rate of S application was lower in 92% of the farms. In total, about half of the farms were not treated with sulfur fertilizer. About one-third (34%) of farmers used S fertilizer with average application rate of 10 kg ha⁻¹ in eastern parts of the studied province [Table 3].

Sowing date

Delayed planting reduced both seed yield and oil yield so that two weeks delay in planting resulted in 309 kg ha⁻¹ loss of yield (i.e. almost 22 kg ha⁻¹ d⁻¹) [19]. They related the yield loss to the coincidence of flowering with higher temperatures. They found that the yield loss was 289 kg ha⁻¹ for each °C higher temperature. Canola farms were planted in early-October to mid-December in the surveyed region. In fact, half of the farms were not planted in the sowing interval (October 6 to November 10) recommended by research centers. Boundary line analysis showed that the optimum range for sowing date was October 27 to November 8 in the surveyed region. It implies that canola should be sown in this interval in order to produce the maximum yield. Figure 3a shows that the boundary line fitted on the upper edge of data is a three-piece function. The yield will be 3425 kg ha⁻¹ if the sowing is carried out in the optimum interval. Table 2 indicates that 56% of the farms were not sown in this sowing interval. The sowing dates varied in the range of October 9 to December 21 in the surveyed region. However, a lot of farmers planted their farms in the optimum range, so that the mean sowing date was early-November [Table 3]. The yields in farms sown before or after optimum sowing range were lower than those in farms sown within this range, ignoring all other limitations. In early-sown farms, pests and birds reduce plant density. Also, weeds spread faster creating more intense competition. Flowering and seed filling period coincides with higher temperatures in late-sown farms affecting yield. Also, precipitation survey shows that reproductive phase coincides with drier conditions in late-sown farms [Table 1].

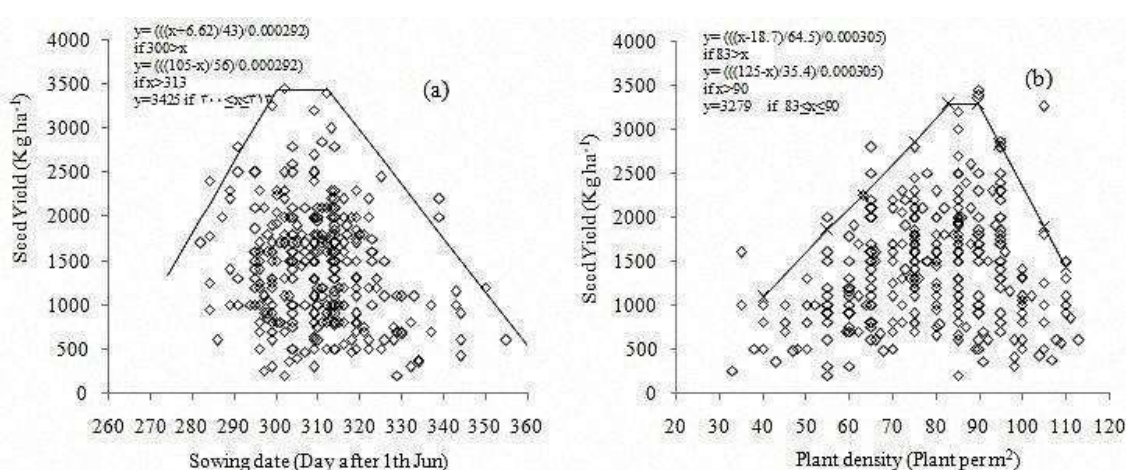


Fig. 3: Scatter graph of yield against sowing date (a) and plant density (b) as well as fitted boundary line

Plant density

Canola yield is widely affected by agronomical pattern, sowing method and plant density [20]. The increase in density only up to optimum level improves yield. Boundary line analysis determined optimum plant density as 83-90 plants m⁻². Due to the fact that no herbicides were optimally applied and since the planted cultivars were not of those resistant to general herbicides and no pre-planting herbicides were

applied, weeds used space fast in lower plant densities and reduced yield by creating competition. Under similar conditions, optimum plant density is higher for maximum yield than that in other parts of the world. At densities higher than 90 plants m^{-2} in addition to the increased elongation of the stems, the stems become weaker increasing the possibility of the lodging of the plants and the outbreak of diseases. Also, higher inter-species competition reduced the availability of the resources affecting yield. Plant density varied in the range of 33-113 plants m^{-2} in the surveyed farms [Table 3]. As can be seen in Figure 3b, boundary line had three-piece trend. Optimum density was found to be 83-90 plants m^{-2} for maximum yield of 3279 $kg\ ha^{-1}$ [Fig. 3b]. Table 2 indicates that 77% of farms had out-of-range those plant densities.

Diseases and weeds

The main diseases in the surveyed regions were blackleg (*Leptosphaerium maculans*) and white mold (*Sclerotinia sclerotiorum*), and mean infection in surveyed canola farms was a little higher than 6% [Table 3]. These two diseases are the main diseases of canola in other parts of the world [4 and 21]. However, the yield loss in infected regions can be reduced to as low as 10-15% by using genetic resistance, keeping distance from the residue of previous crop, and applying fungicides [21]. [2] observed infection in as high as 40% of plants in wetter regions with lower fungicide application. The fitting of a boundary line on upper edge of yield data revealed that yield response to the diseases incidence percentage and weeds population followed a two-piece function. Less than 4% infection of plants to the disease was the optimum level for maximum yield of 3450 $kg\ ha^{-1}$ [Fig 4a]. Since the planted cultivars were not genetically resistant to the diseases, the incidence rate was higher and 90% of farms showed symptoms of over 4% infection rate [Table 2].

Regression relationship between seed yield and infection percentage was significant for Kalaleh farms at the probability level of $Pr < 0.0005$ and for Galikash farms at the probability level of $Pr < 0.0001$, whilst only 11 and 18% of yield variations was accounted for by disease infection in Kalaleh and Galikash, respectively. According to the slope of regression equation inside yield data against plants infection percentage in Kalaleh and Galikash, where there was a high disease incidence rate, the yield loss was, on average, 67 $kg\ ha^{-1}$ for each percent increase in disease infection. When boundary line analysis was used for whole region, the regression equation showed 184 kg loss of potential yield for each percent increase in disease infection.

It implies that ignoring all other limitations, the yield loss caused by the outbreak of diseases plays an important role in the reduction of potential yield. The rate of blackleg incidences was increased with delayed sowing [4]. They used regression analysis to find the possible relationship between yield and the damages of *Sclerotinia* and found that blackleg and white mold, individually or together, reduced yield by 0.39-1.54 $t\ ha^{-1}$ and that the lower the infection was, the higher the yield was significantly. Also, they reported that for each percent decrease in blackleg infection, the yield was increased by 5% while it was 1.3% for *Sclerotinia sclerotiorum*.

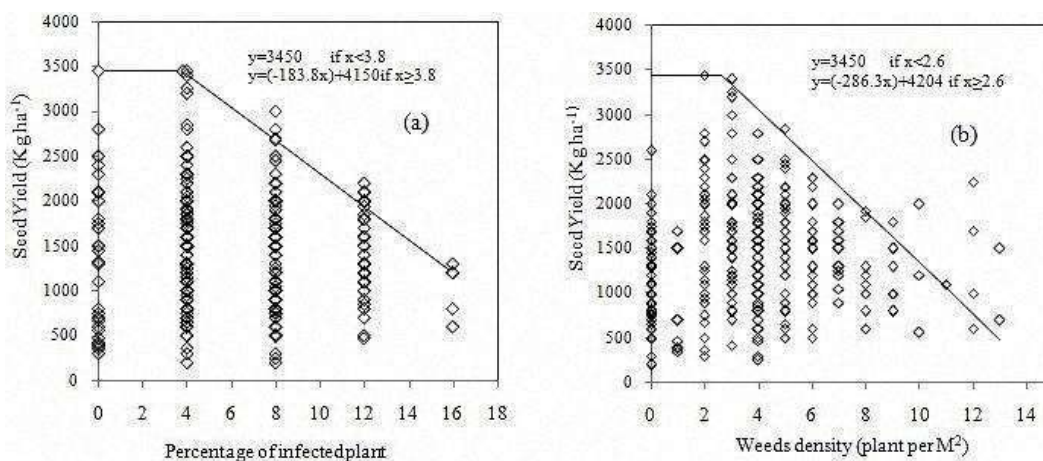


Fig. 4: Scatter graph of yield against infected plants percentage (a) and weeds density (b) as well as fitted boundary line

The function of yield response to weeds population indicated that the maximum yield of 3450 $kg\ ha^{-1}$ can be obtained from weeds population of less than three plants m^{-2} [Fig. 4b]. Since the cultivars were not resistant to general herbicides and no pre-planting herbicides were applied, 66% of the surveyed farms were attacked by a high population of weeds [Table 2]. Weeds reduce crop yield by competing with the crop. The damage of weeds from the family of canola to this crop yield is reported to be 11-16% under weed population of 4-7 plants m^{-2} [4]. Seven wild mustards, nine wild spear thistles, twelve wild oats, and four wheat and barleys per unit area can result in 16, 14, 10, and 11% loss of canola yield [23]. According to boundary line analysis, canola can tolerate at most three weed plants m^{-2} to produce the maximum yield

under the conditions of the present survey and higher weed populations can reduce potential yield with a relatively high slope (-286 kg ha⁻¹ per weed plant) [Fig. 3b].

CONCLUSION

Some factors limiting canola yield were surveyed in eastern part of Golestan Province by boundary line approach and the yield gap caused by each factor was estimated. Whilst mean yield produced by farmers was 1417 kg ha⁻¹ seed, the obtainable yield was estimated to be 3407 kg ha⁻¹, implying a yield gap of 1987 kg ha⁻¹. This is, in fact, the gap between local farmers' mean yield and the optimum yield estimated by boundary line analysis. It came to be known that most farms were not adequately fertilized. In the surveyed region, 80, 93, 95, and 93% of farmers were using non-optimum rates of nitrogen, phosphorus, potash, and sulfur fertilizers, respectively. The optimum rates of fertilizers that can be recommended to reduce or eliminate yield gap caused by inadequate fertilization were estimated to be 122 kg ha⁻¹ N, 49 kg ha⁻¹ P₂O₅, 34 kg ha⁻¹ K₂O, and 40 kg ha⁻¹ S. Furthermore, it was found that it would be better to use 42 kg ha⁻¹ of 122 kg ha⁻¹ N as base fertilizer. The best plant density was estimated to be 83-90 plants ha⁻¹, and only 23% of the surveyed farms were in this plant density range. The best sowing date range was October 27 to November 8. Also, the population of weeds should be less than three plants m⁻² and the density of diseased plants should be less than 4% in order to obtain maximum yield.

CONFLICT OF INTEREST

There is no conflict of interest

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ARTICLE

THE COMMITMENT IN DIAGNOSTIC REPORTS DOCUMENTATION IN CASE OF PRIVATIZATION THE HEALTH INFORMATION TECHNOLOGY

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ABSTRACT

By taking into account that the employees are always involved in the privatization process, it seems that the approach and subsequent to it the behavior of the employees are key factors in this process' being a success or a failure. Therefore, this study aims to investigate employees' commitment in diagnostic reports documentation in case privatization the health information technology. This study was performed in affiliated hospitals of Semnan University of medical sciences in Semnan, Iran. In this study, a census method was used. The data collection tool was a researcher made questionnaire. 45.4% reported the privatization increases physicians' commitment to complete the patient's diagnostic reports. 39.4% believed the privatization process equips the health information technology department. 42.7% reported the privatization increases nursing staffs' commitment to complete the patient's reports. The results showed that employees had various approaches to the privatization of the health information technology. Some of them embrace this process and some believe that it cannot make the clients satisfied or improve the commitment in diagnostic reports.

INTRODUCTION

Nowadays, privatization is a quite significant approach. It has been used by some organizations with the purpose of improving the quality of their services, reducing the costs and to increase the effectiveness of their services in general [1]. When privatization was first introduced, it meant when the employees choose outside sources for doing some of their activities in the organization. In fact, they are not involved with the process any longer. This privatization leads to the fulfillment of the activities. This is actually traditional outsourcing. On the contrary, in modern privatization the employees remain in their position and the organization gets use of the new service in a set of processes of that organization [2, 3]. There are numerous causes for privatization and downsizing the governmental sector. Low-quality services, necessity of improving the satisfaction of the clients to whom the services are offered, motivating employees and contractors work wise for the organization to have access to desirable and suitable sources, escalating desirable services and a higher level of satisfaction are some of the most important causes [4]. Studies have shown that management consideration, solving the problems associated with the employees, motivation for improving the performance of employees, strategic considerations, technological considerations, getting access to new technologies, learning the new technologies and achieving a better performance are factors which affect the privatization decisions of an organization [1, 2]. It is interesting to note that in the framework of the reformations of the health system, privatization is considered as a strategy which can be of help for promoting the efficiency of the health system. Privatization can be used in units where services are provided and where the payment method is reformed in a way that it would create competition in providing services, increasing efficiency and also making the customers satisfied [5, 6]. There are some units in any organization which have a special place when it comes to informational value. In the hospitals and health centers, the health information technology (HIT) unit has this special place [7]. The HIT unit is one of the most significant reference as for gaining information about the diseases of the patients, how to treat them and about the method of management as well as the performance of employees of the health care centers. This unit is effectively efficient when all of the professional standards are observed including structure, policies and instructions, equipment, human force, performance and services [8, 9]. The medial file is of great value when it comes to taking good care of the patient, preventing diseases and improving their health. A medical file dramatically depends on the quality of what the file contains recorded by the employees of this unit in terms information, researches, scientific information and statistical information [10, 11]. Mahjoob et al. also investigated the extent to which the selected forms in the medical files are filled out in the hospitals of the city Jahrom. They have concluded that the reviewed documents do not contain a sufficient amount of information especially when it comes to clinical information which plays a major role in the rest of the treatment of patients. It is necessary to note that the investigated documents were some of the basic and important documents in the medical files [12]. On the other hand, numerous studies have shown that there are some factors that can affect the escalation of the quality of effective documentation. Some of these factors are: Awareness, approach, training, instructions, standards and regulatory levers [13, 14]. Ferdosi et al. reviewed the privatization of the HIT in Isfahan's Ayatollah Hospital and deduced that quality has been improved in various aspects. 76.2% of this improvement has been seen in medical processes. The level of satisfaction of internal customers has rose for 59% and a reduction of 37.4% is seen in the elimination of the defects of medical files [15]. Tabibi et al. conducted a research called evaluation of the strategy for privatization support services in hospitals affiliated to the Ministry of Health Care and Medical Education. In this study,

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they concluded that privatization services triggers a rise in the efficiency of the hospital and in the level of satisfaction of the employees [16]. Only a handful of studies have been done on the privatization of the HIT unit and none of them have focused on investigating clients' satisfaction and commitment in documenting medical files in case the services of the HIT are outsourced [15, 17]. By taking into account that the employees are always involved in the privatization process, it seems that the approach and subsequently to it the behavior of the employees are key factors in this process' being a success or a failure especially when it comes to the information systems [18]. Therefore, this study aims to investigate employees' approach to clients' satisfaction and commitment in documenting medical files in case privatization the services of the HIT.

METHODS

Participants in this study were employees working in affiliated hospitals of Semnan University of Medical Sciences in Iran. Census sampling was used in this study. A total of 840 questionnaires were distributed; 604 were returned, which represented a response rate of 71.9%. Ethics approval was obtained from the Medical Ethics Committee of Semnan University of Medical Sciences. A covering letter was prepared for distribution with the survey document, which described the purposes of the study and explained that a response to the survey would indicate the consent of the participant to take part in the research. It also assured participants of the confidentiality of their responses. The data collection tool was a researcher made questionnaire. The questionnaire consisted of 15 questions in 2 sections. The first part contained 5 questions related to demographic information including Job, gender, age, work experience and education; the second part included 10 questions related to impact of privatization on customers. The participants' attitudes on each item were measured on a 3-point Likert type scale, where, disagree=1, neither disagree nor agree=2 and agree=3, respectively. The questionnaires have been distributed and returned to the researcher within 72 hours. Means and standard deviations, in dependent- samples T, and one way ANOVA were used to analyze the data.

RESULTS

The results indicated that 47.2% of the study subjects' job was allied health. 69.1% was female. 40.8% of the participants aged 20-30 years. 26.6% had less than 5 years work experiences, and 60.7% had bachelor degree. The results showed 41.8% of the employees believed that the privatization increases the consent of the patients or their relatives from medical record department. 33.4% believed that the privatization increases physicians' satisfaction from medical record department. 46.3% reported that the privatization increases managers' sensitivity towards medical record department. 45.4% reported that the privatization increases physicians' commitment to complete the patient's diagnostic reports. 39.4% believed that the privatization process equips the medical record department. 42.7% reported that the privatization increases nursing staffs' commitment to complete the patient's diagnostic reports [Table 1].

Table 1: The study subjects' attitudes towards the impact of the outsourcing

Attitudes	Agree N (%)	Neither disagree nor agree N (%)	Disagree N (%)	Mean ± SD
The increases of nursing staffs' commitment to complete the patient's diagnostic reports	253(42.7)	181(30.5)	159(26.8)	2.84±0.81
The increase of the consent of the patients or their relatives from HIT department	249(41.8)	204(34.2)	143(24)	2.82±0.79
The increase of the equipment of HIT department	233(39.4)	206(34.9)	152(25.7)	2.86±0.79
The increase of managers' satisfaction from medical record department	225(37.8)	207(34.8)	163(27.4)	2.90±0.80
The increases of physicians' commitment to complete the patient's diagnostic reports	269(45.5)	179(30.2)	145(24.5)	2.79±0.81
The increase of allied health staffs' satisfaction from HIT department	218(36.8)	204(34.5)	170(28.7)	2.92±0.80
The increase of managers' sensitivity towards HIT department	273(46.3)	199(33.7)	118(20)	2.74±0.77
The increase of medical students' cooperation with HIT department	219(37.6)	202(34.7)	161(27.7)	2.90±0.80
The increases of medical students' commitment to complete the patient's diagnostic reports	260(43.8)	197(33.2)	136(22.9)	2.79±0.79
The increase of physicians' satisfaction from HIT department	199(33.4)	237(39.8)	160(26.8)	2.93±0.77

There was not significant relationship between the study subjects' demographics characteristics and their attitudes [Table 2].

Table 2: Relationship between the participants' characteristics and their attitudes

Characteristics	Groups	Mean ± SD	P-value
Sex	Male	2.82±0.64	0.260

	Female	2.85±0.59	
Age (Year)	<20	3.43±0.23	0.335
	20-30	2.83±0.57	
	30-40	2.84±0.59	
	40-50	2.84±0.57	
	50<	2.94±0.56	
Job	Physician	2.87±0.52	0.791
	Nurse	2.83±0.57	
	Worker of HIT	2.94±0.55	
	Allied Health	2.86±0.61	
Work experiences (Year)	>5	2.82±0.56	0.575
	5-10	2.88±0.61	
	10-15	2.97±0.61	
	15-20	2.79±0.60	
	20<	2.82±0.61	
Education level	PhD	2.87±0.52	0.684
	Master degree	2.70±0.60	
	Bachelor degree	2.84±0.57	
	Diploma	2.83±0.54	

DISCUSSION

The purpose of this study has been to investigate the employees' approach to clients' satisfaction and commitment in documenting medical files in case privatization the services of the HIT. The obtained results indicated that less than half of employees have stated that by privatization the HIT, managers become more sensitive to the performance of this unit. On the contrary, in the health centers it might be believed that privatization is the reason why managers can focus on more important issues in an organization and try to meet the goals of the organizations without any additional concerns. If this is the case, rise of efficacy is directly affected. This idea is basically seconded by Mohammad Karimi et al. in the research conducted by them. In this research, they have cited that the time of executive managers is freed of daily responsibilities if the services were to be outsourced. In their opinion, 80% of the time of executive managers is spent on managing affairs which are not that important in normal cases and only 20% of their time is spent on developing strategies. However, when the services are successfully outsourced, managers will have enough time for searching new areas for earning income, accelerate other projects and focus on the clients. All of these will ultimately improve efficacy [19]. In 2010, Kavosi et al. conducted a research which aimed to investigate the level of employees' and managers' tendency based on features of different units. The results obtained by them was an indication of the strong tendency managers had to outsource radiology and laboratory units [20]. The findings of this research showed that the majority of their statistical population did not come to terms with the idea that by privatization the HIT, patients or those accompanying them become more satisfied with the services of the HIT. On the contrary, Tabibi et al. have done a study called "evaluation of the strategy for privatization support services in hospitals affiliated to the Ministry of Health Care and Medical Education. In this study, they concluded that privatization services triggers a rise in the efficiency of the hospital and in the level of satisfaction of employees and patients [16]. In addition, studies have shown that privatization affects the performance in terms of presenting health care services and improving the quality of treatments and making patients more satisfied [21, 22]. In 103 health care centers of a city in Pakistan, a rise in the level of satisfaction of the patients was observed when the services were outsourced [23]. In most statistical populations, it is believed that privatization is neither considered as a factor playing an important role in providing services with higher qualities nor as a factor which escalates how satisfied patients or those who accompany them are with the services of this unit. Perhaps, the majority of the statistical populations believe that patients are dissatisfied because the privatizing firms do not keep patients' information confidential. Confidentiality of the information is a priority no matter how big or small the center providing health care services is [24, 25]. Generally speaking, the first thing that immediately comes to mind when most people hear about privatization is that private companies do not provide clients with suitable services. These concerns become more significant when the focus is on the sensitive and confidential information of the patients such as their medical file [26]. In fact, it is these concerns about the violation of the confidentiality and development of new standards that has been identified as the factor preventing the members of the population from accepting privatization [27, 28]. Anciaux et al. it was not guaranteed that the electronic health files are not secure enough outside the range of health care services [29]. It was by reviewing the results of this study that it was concluded that the studied health centers need to ensure that privatizing authorities can both provide high-quality services to patients or their escorts and protect patients' information and keeping them confidential. In doing so, the latest improvements and executable principles must be considered.

Studies shown that reducing the costs as much as possible and making the customer satisfied is the most important factor affecting the decision to do privatization [30, 31]. However, the findings of this study showed that a few of the employees believed that privatization the services of the HIT makes internal customers more satisfied including doctors, nurses and other personnel. In 2012, Ferdosi et al. conducted a research called "effectiveness of giving the services of the imaging center of Isfahan's Ayatollah Kashani Hospital to the private sector". In this study, they came to the conclusion that privatization has had positive

influences. It has caused a 78% improvement in the performance of the unit and a 95% promotion in the satisfaction of internal customers [15]. It is believed that in the opinion of the majority of the statistical population, privatization the services of the medial records department is factor causing a fall in the regulatory levers. In their view point, this fact negatively affects the performance of the employees of this department and as a result, the employees become more dissatisfied. It might also be the case that the statistical population is concerned with the hunch that privatizing companies might not be able to integrate medical data in health centers. This is a problem leading to shortcomings and human errors in the clinical environment [32, 33]. It is necessary for the members of this population to be reassured that it is in fact by privatization the HIT that accurate information can be provided for those who provide health care services. And in addition, in this case, if the services are outsourced, the patients would be excellently cared for and clinical specialists and staff would become more efficient. By reviewing the findings, it becomes apparent that there is this thought that privatization is considered to be an investment in education and it is what makes individuals more skilled and reduces problems. All in all, more than one third of the statistical population have cited that privatization the HIT makes the students more satisfied since they are more involved.

Studies show that managers are not able to overcome this problem by only focusing on training and educating human resources in the organization. Therefore, the best way to reduce the problems and to obtain desirable results is to give this responsibility to an outside source [34-36]. If outsourcing, as a part of the economic development project of the society, was to create a substrate for individuals' creativity and innovations to blossom and to provide equal and proper facilities for the education process in the organization, the work-related needs of the employees would be met [37, 38]. The results showed that most employees were uncertain when it came to the impact the privatization of the HIT has on equipping this department and consequently making the employees satisfied. Conversely, Susomrith and Brown conducted a research and concluded that it is for using more specialized and qualitative services that privatization is quite significant [39]. In addition, Jacobson et al. studied the privatization of information technology in the hospitals of USA in 2004. In this study, they showed how privatization makes technologies more accessible [40]. The study Salmani et al. did aimed to evaluate the experience of privatization health services in the city. In this study, they concluded that since supervisors have put greater importance on the physical structure and equipment standards, it has also been more observed by the private sector [5]. In contrast, nowadays health centers insist on investing in making the specialized force associated with the health-related information improve and on using new technologies. Their goal is to present the employees with secure and accessible information when they are needed for them to take care of the patients and to improve the results [41]. However, by reviewing the results of this study, it can also be concluded that the members of the statistical population of this study probably think that the employees cannot gain access to new technologies, skills and specialties only through outsourcing. While the members of this population must be confident that privatizing companies can reduce the costs of the HIT, increase the security of the patient and significantly affect the quality of health care services by introducing new technologies. The results of this study indicated that some employees probably believe that privatization improves documentations by making the activities identical. Less than half of the employees have cited that by privatization the staff becomes more responsible in the documentation of medical files. Karimi conducted a review study and concluded that by standardizing the jobs, accurately defining the job and its result, privatization becomes successful [10]. Numerous studies confirm the results of the present study. For instance, Omrani concluded that privatization improves the behavior of employees and makes them more responsible [42]. In addition, Salmani also came to the result that documenting and recording information in outsourced databases has been more desirable than in governmental databases [5].

The results showed that approximately one third of the respondents were not informed of the advantages of privatization the HIT or they did not look at them optimistically. If these approaches were to develop, those health centers that have limited sources will face problems. On the other hand, if the ambiguities are not eliminated, the confidence and trust in the organizational decisions will be lost [43]. It is essential to note that if there are complexities and ambiguities, the costs of the services won't be evident and the health centers will lose their organizational competence. This will also escalate financial, political and cultural risks that the organization might face in case of privatization the HIT. Thus, it is quite important to analyze the risks of privatization when you want to select and use tools to reduce the risks. This analysis is a key factor that plays an important role in the success of outsourcing. If we seek to find out about the risks, we will also be confronted with additional advantages of privatization [44].

It seems that informing the staff about the opportunities brought by privatization the HIT might be beneficial for those of health centers that have a limited number of sources [45]. On the other hand, the position of the employee working in the HIT in a hospital is important. When the employee directly reports to the head of the hospital, that employee will be a manager in the hospital who is in charge of making decisions. In this case, they might be given a better position to have a positive impact on the strategy of the organization and then on the decision to outsource the services of the HIT [46].

It must also be noted that information technology is strategically important in a hospital; since it might be reason why the hospital outsources its information system [47]. The geographical location of the hospital, whether it is located in the city or in the capital of the province, is an effective factor on outsourcing. The size of the hospital and the intervention of the doctors in the activities of the information might also affect privatization [48].

CONCLUSION

The results showed that employees had various approaches to the privatization of the HIT. Some of them embrace this process and some believe that it cannot make the clients satisfied or improve the commitment in medical documentation. The results showed that the majority of the statistical population were concerned with the probability of unpredicted risks happening as a result of outsourcing. If the employees believe that privatization is in compliance with their own cultural – value systems and don't see it as a threat, they won't resist it and they will cooperate with the organization in this regard. Therefore, it can be concluded that for any evaluation program to be successful, the employees of any organization need to be aware of them and have a positive approach to it. In fact, employees who embrace the changes that guarantee their welfare and happiness. Otherwise, they will resist it. Thus, the organization have to inform the employees about the privatization programs and purposes, and its advantages and benefits. They must also create an intellectual correlation about privatization between various levels of the organization and therefore, create a cultural and intellectual system which is in proportion with privatization in the organization. The results obtained from this study is crucial for managers because it will inform them about the probable risks of privatization and prepare them for facing and then overcoming these risks and concerns. In the end, it must be noted that when it comes to outsourcing-related decision makings, managers must ask the employees for their opinion and try to find out their tendency towards this before executing the project for them to be accepted and embraced more and also to prevent failure.

CONFLICT OF INTEREST

None declared.

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ARTICLE

THE APPLICATION OF PATIENTS' MEDICAL RECORDS IN TEACHING HOSPITALS

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ABSTRACT

The aim of maintaining the medical records is to meet the legal, training, research, and treatment requirements. Accordingly, a question arises that to what extent the medical records at teaching hospitals can meet the multiple needs of clients. In other words, what kinds of objectives are mostly intended with the medical records? In order to answer the question, a study was conducted to evaluate the different applications of medical records of hospitalized patients. 400 medical records of patients who were admitted in hospitals affiliated to Semnan University of medical sciences have been evaluated in 2016. A checklist was developed by the authors, after reviewing related literatures. The results showed that 55% of medical records of patients who were admitted in internal medicine ward have used for legal purposes and 20% of medical records of patients who were admitted in psychiatrist ward have used for therapeutic purposes. The finding showed that the medical records were more used for legal purposes. The results showed that the medical records of hospitalized patients are used for various purposes. The nature of hospitalization wards, duration of patient stay, and type of services offered to patients affect the amount and usage type of the records.

INTRODUCTION

Medical records are valuable documents that are arranged and maintained for continued patient care, training, research, legal issues, hospital statistics, and evaluation of care and services provided to patients by physicians and other health care providers [1, 2]. The accumulation of information in the medical records will increase the quality of care [3]. Medical records department having clinical records can be a major source of information for patient care, qualitative research, training, research, and management planning. The accurate and appropriate use of medical records reduces reworking, the time, and the cost consumed [4]. Convenient collection, accurate record, correct application of capabilities at medical records department, and rapid recovery provide an efficient approach to be applied by managers, researchers, physicians, and other providers of health services [5]. The basic features to be considered when using medical records are completeness, accuracy, and quality of data listed in the medical record [6]. Different tools are employed in hospital promotion plans in order to evaluate the method of encoding diagnosis and the quality of patient care. One of these tools is the use of medical records of patients [7]. Nowadays, the medical record is not only a means of communication for all people involved in the treatment, but also reflects the quality of care and treatment of patients [8].

Access to accurate and up-to-date medical information is a determining factor in implementation and development of research as well as proper use of environmental technology products for the treatment of patients. Limited access to important data of patients may retard and/or even hinder the research process [9]. In a study, Safdari et al. found that a considerable amount of nursing sheet information was used to accelerate the cure in 52.5 percent of cases. In addition, the use of medical records was 54 percent in response to legal issues [10]. Rangraz Jeddi et al. investigated the use of medical records in Iran and showed that more than 50 percent of medical records information was used in legal issues, most which in were associated with surgery, specialized wards, and emergency room [11].

The aim of maintaining the records is to meet the legal, training, research, and treatment requirements. Accordingly, a question arises that to what extent the medical records at the affiliated hospitals of Semnan University of Medical Sciences can meet the multiple needs of clients. In other words, what kinds of objectives are mostly intended with the medical records? In order to answer the question, a study was conducted to evaluate the different applications of medical records of hospitalized patients.

METHODS

400 medical records of patients who were admitted in hospitals affiliated to Semnan University of medical sciences in Iran have been evaluated. The research was conducted from July 2016 to November 2016. A checklist was developed by the authors, after reviewing related literatures [10-12]. The checklist consisted of 50 questions in four sections. The first section included 14 items and focused on kinds of hospital wards. The second section comprised 19 items and contained types of patient's reports. The third section consisted 7 items and measured the reasons of the use of patient's reports. The fourth part included 10 items and was related to kinds of the units requesting patient's reports. The primary check list was reviewed for content validity and evaluated by experts in relation to the simplicity and clarity of questions,

KEY WORDS

Application, Patient medical record, Teaching hospital

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and the relationship between questions. Next, further revisions were made and some statements were rephrased. Lastly, the final version of the checklist was used. Frequency and percentage was calculated by descriptive statistics. Ethics approval was obtained from the Semnan University of Medical Ethics Committee (IR.SEMUMS.REC. 1394.229).

RESULTS

The results showed that 55% of medical records of patients who were admitted in internal medicine ward have used for legal purposes, 20% of medical records of patients who were admitted in Psychiatrist ward have used for therapeutic purposes, 5% of medical records of patients who were admitted in psychiatry and post catheterization wards have used for research purposes and 60% of medical records of patients who were admitted in eye ward have used for administrative aims [Table 1].

Table 1: The use of patients' medical records for different purposes among clinical departments

Wards		Reason to use				Total
		Administrative	Research	Therapeutic	Legal	
Internal medicine	N	19	1	7	33	60
	%	32	2	11	55	15
Emergency	N	22	0	3	20	45
	%	49	0	7	44	11.25
CCU	N	12	0	6	13	31
	%	38	0	19	41	7.75
ICU	N	29	1	8	24	62
	%	47	2	13	38	15.5
Chemotherapy	N	11	1	3	7	22
	%	50	4	13	31	5.5
Surgery	N	29	2	13	34	78
	%	37	3	17	43	19.5
POST CCU	N	9	0	0	12	21
	%	42	0	0	54	5.25
Post catheterization	N	7	1	2	10	20
	%	35	5	10	50	5
Ophthalmology	N	25	0	7	9	41
	%	60	0	17	21	10.25
Psychiatrist	N	7	1	4	8	20
	%	35	5	20	40	5

The results showed that the medical records were more used for legal purposes [Fig. 1].

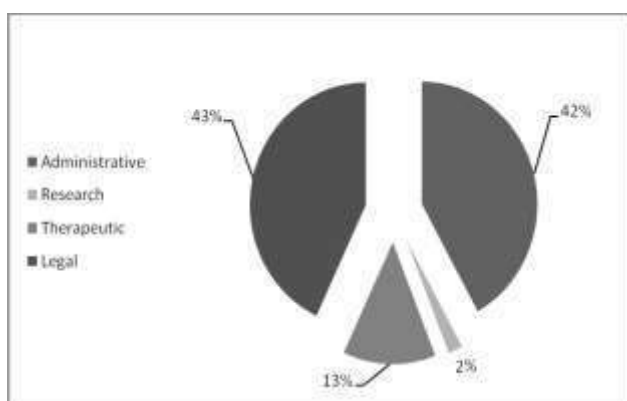


Fig. 1: The percentage of using of patients' medical records for different purposes.

The findings indicated that admission and summary sheet, record summary sheet (100%) and emergency sheet (86.8%) have more used than other records. Patient education sheet (1%) had the least use among patient's medical records [Fig. 2].

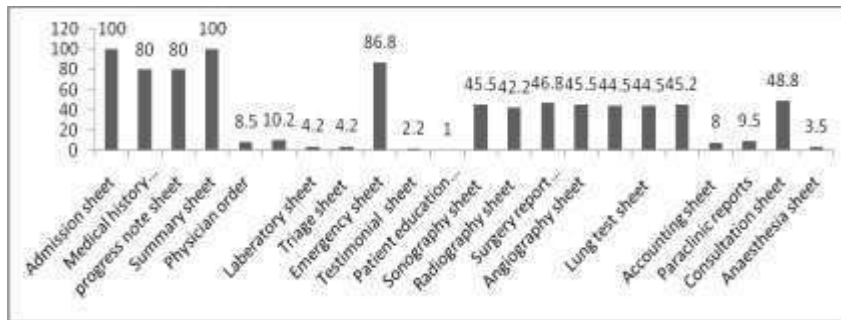


Fig. 2: The percentage of using of patients' medical records' papers.

DISCUSSION

This study was performed to evaluate the use of medical records of hospitalized patients. The findings suggest that most of the medical records studied were used in legal and administrative cases each with 170 cases and 42.5 percent with the least amount of 7 (1.75%) usage in the training-research area. This findings are inconsistent with other studies [13, 14]; however, the differences are justifiable given the differences in the research populations studied. Regarding the high research uses of records, it can be stated that research information is often extracted through archival records; moreover, any survey needs a dozen of cases that raises the frequency of medical records usage.

Furthermore, concerning the frequency of medical records usage depending on the application and the specialized sector in the research population [15], the results of this study showed the highest usage at the male surgical ward with 78 cases (19.5%) and also at the Internal medicine ward with 60 cases (15%), but the lowest use was observed at the post catheterization and psychiatrist wards with 20 cases (5%). The high levels of records available at the specialized surgical ward, including orthopedic, urology, and neurosurgery are mainly due to the fact that orthopedic, urology, and neurosurgery wards need longer treatment periods leading to increased use of the records. Therefore, it is recommended that necessary measures be taken to familiarize students with the importance and roles of the records in promotion of their scientific levels. In addition, the use of medical records will not be finished after 10-15 years but legal and administrative cases will make most use of them [16].

Considering the fact that correct documentation makes medical records a primary tool for evaluating the performances and health-treatment cares with an important role in legal and medical studies, hospitals should seek to identify factors influencing the improvement of documentation quality in order to enhance the strength of clinical records' accountability in legal medical cares. The study by Jeddi also showed that more than 50 percent of medical records information was used in legal cases [11]. The finding shows that the admission and summary sheets, among all medical records of hospitalized patients, are used more than the other ones for different purposes. The greater use of these sheets seems to be because of the fact that they are among the main sheets in patients' records, which must be attached for any use of the records.

The results showed that the records of hospitalized patients are used for various purposes. The nature of hospitalization wards, duration of patient stay, and type of services offered to patients affect the amount and usage type of the records. Our findings demonstrated that the patients' medical records are mostly used in the surgical ward compared to the other clinical wards. The results also denote that the sheets of medical records are not equally applied with some sheets used far more than others. The results of this study should be interpreted with caution because the study was conducted using a researcher made check list method and potential problems, such as, poor understanding of questions and probably bias answer threaten the results of the study, that is resolved with regard to questionnaire validity and reliability. Also, non-generalizable results of the study because of being done in a city, is of the other limitations of this study. But, however, the study results were in line with other studies in this field.

CONFLICT OF INTEREST

There is no conflict of interest.

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FINANCIAL DISCLOSURE

None

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ARTICLE

PREVALENCE AND CHARACTERISTICS OF DIETARY SUPPLEMENT USERS IN KHORRAMABAD CITY, SOUTHWEST OF IRAN: A DESCRIPTIVE CROSS-SECTIONAL STUDY

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ABSTRACT

In recent years, using a variety of dietary supplements in the daily diet has increased rapidly around the world. The aim of this study was to determine the frequency of dietary supplements consumption and tendency causes and related factors with its usage in adults referred to health care centers of Khorramabad city in 2015. In this cross-sectional study, the study population included all adults (over 18 years) referring to health service centers in Khorramabad in a 6 month period. The method of sampling was multistage sampling and the data collection instrument was a multi-sectional questionnaire. In this study, 171 people (48.9 percent) of subjects had a history of regular usage at least one of dietary supplements that this usage was once a day in 138 people (80.7 percent) and two or three times a day in 28 people (16.4 percent). In examining distribution frequency of different supplements usage in consumers of dietary supplements, it is also observed that the most frequency of dietary supplements usage is related to iron (70.5%) and Folic Acid (70.5%), then, calcium (30.1%), multivitamin (25.4%), calcium- vitamin D (17.3 %), vitamin B complex (16.8%), vitamin D (15%), vitamin B6 (15%), vitamin C (9.5 %) and vitamin A (9.2 %), respectively. On the other hand, the least frequency of dietary supplements usage was related to Chrome, Magnesium, and Potassium (each 2.3%). Differences in taking dietary supplements based on sex, educational level, marital status, occupation, place of residence, body mass index, smoking history, current physiological conditions, and chronic diseases were statistically significant ($P < 0.05$). According to the fact that tendency to take dietary supplements was more in lower ages, therefore it is necessary to provide training and nutritional strategies appropriate for different age groups about the proper use of nutritional supplements.

INTRODUCTION

Today, tendency to use supplements such as multi-vitamins and minerals is increasing in different communities [1-5]; so that in 2001, the prevalence of dietary supplements usage is reported 5.56% in general population of America [6], in 2007, it was reported 26% in Sweden population, and in 2008, it was reported 49% in Italy general population [7, 8]. Since enactment of Dietary Supplement Health and Education Act of 1994 (DSHEA) in 1994, it was reported that there are about six hundred plants of dietary complement manufacturers that they nearly produce four thousands types of this product. Annual sales of these products is estimated 4 billion USD [9]; in 1998, this amounts to 13.9 billion USD and, in 2006 it was 21.3 billion USD [10].

In fact, using dietary supplements have been always demanded as correct or incorrect beliefs for strengthening body security system, relieving fatigue and reducing stress or boredom as arbitrarily and/or to describe or prescribe by feeding counselors and doctors. Results of a study about causes of using dietary supplements have shown that 48% of consumers believed that using these products is an easy way to stay healthy and preventing disease [11]. On the other hand, medical and feeding knowledge determines using dietary supplements for people in special age and in terms of need, on the same basis, using iron supplements is recommended for women of fertile age and pregnant women and nursing mothers in our care country program, they are freely available for these vulnerable groups. Also, using calcium and vitamin D is recommended for preventing and reducing the risk of brittle bone in adults, middle-aged, and in some other cases in terms of the conditions. Results of previous studies have shown that taking supplements is effective for preventing some diseases, for example, prescribing folic acid during pregnancy is effective for reducing the risk of creating neural tube deficiency in embryo [12].

The results of other studies have shown that in healthy individuals, using small amounts of some minerals, including Selenium may reduce the incidence of cardiovascular and some cancers [13, 14]. Dietary complements can be used based on need as an aid in meeting food needs along with unbalanced diet. In fact, access to a daily balanced diet may not be available all days of the week or months and in the long-term effects of some nutrition shortage will be obvious, but what is important is arbitrary usage of these supplements that sometimes they are along with special effects and drug interactions [15]. Certain groups of society, such as athletes or people who have weight loss diet, may use arbitrarily dietary complements. In a study in Southern African, the cause of arbitrary use of dietary supplements is mentioned to maintain physical health and preventing the occurrence of diseases [16].

As it is clear, there are some motives for explosive growth of dietary supplements; therefore, many studies are conducted across the world about dietary supplements and causes of tendency and related factors of

KEY WORDS

Dietary supplements,
Health care centers,
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its usage; among studies in these areas, Najmabadi et al., study can be referred. These researchers in their study entitled nutritional supplement use among adults in different areas of west Tehran showed that 42% of population used one of dietary supplements, which this amount was 11.3% in men and 88.7% in women. Also, taking different types of multi-vitamins, multi-vitamin combined with minerals reported 38% cases, taking calcium supplements and vitamin D in 30.1%, iron supplements in 27.1% and omega supplements in 4.9% cases. In this study, age group under 30 and age group 31-41 were the highest users of dietary supplements and recommendation to take dietary supplements in 75.5% cases were done by doctor or dietary consultants [17]. Radimer et al., study in America is among other studies that are done in this context, it was done on national health and feeding data in America during 1999 through 2000, its results was that 52% of participants had a record of receiving one type of supplement during the past month, 35% of them had a record of taking multi-vitamin. In this study, female gender, older age, education higher than diploma, non-Hispanic white race, average physical activity, non-smoking and well-being feeling by taking supplements were the most important prediction factors of taking dietary supplements, and most people used supplements daily at least for 2 years [18]. Another study that is referable in this context is the study of Hui-Jing Shi et al., in 2005 that showed residence in the city, female sex and high education level has significant relevance with more taking dietary supplements in Japan. Also, reducing stress and improving fatigue and maintaining body health are among reasons to take dietary supplements. In this study, multi-vitamins (49.5%), vitamin Bcomplex (27.1%), vitamin C (26.7%) and minerals (29%) were the highest amount for consumption, low percentage of people mentioned about taking Magnesium, Zinc and Selenium. Also, in this study, results showed that people who faced 2 mental stressors or more during the day and faced lack of social protection mentioned taking dietary supplements 2 times more than other people [12].

According to what has been referred so far, in the present study, it was tried to determine the frequency of dietary supplements consumption and tendency causes and related factors with its usage in adults referred to health care centers of Khorramabad city in 2015.

MATERIALS AND METHODS

In this cross-sectional, descriptive study, under study population included all adults (up to 18 years) referred to health centers in Khorramabad city from middle of June through the middle of December in 2015, they referred for receiving service and health care in this period of time. Criteria for entry into the study were an age older than 18 years and giving consent to participate in the study.

The sample size was determined 348 people based on previous studies [6] with approximate estimate of 41% taking dietary supplements in patients referred to health centers and with 5% type I error and the accuracy of 0.04 and considering Design Effect about 1.2.

$$n = \frac{z \left(1 - \frac{\alpha}{2}\right)^2 p(1-p)}{d^2} \approx 350$$

$$z \left(1 - \frac{\alpha}{2}\right) = 1.96p = 14\% \quad 1 - p = 86\%$$

The sampling method was multi stage sampling. In such a way that at first, Khorramabad city was divided into 5 categories of North, South, East, West and Central; then each point of every health care center was considered as a cluster and one cluster was randomly selected among clusters and then in next stage based on times of referrals of each center, samples were determined proportional to referred population. Then under study samples were determined based on available referrals to different units of health care centers and each day one of units including vaccination unit, family health unit, environment and occupational health unit, laboratory, consulting, etc. were investigated.

In this study, a multi-part questionnaire was a tool for collecting information; first part includes participants' demographic data such as age, sex, marital status, education, occupation, place of residence, etc. Also, in this part, there are questions regarding history of smoking, aerobic/physical activity during last seven days, history of using food diets for reducing and increasing the weight and history of being affected (such as hypertension, blood fat, the diabetes, brittle bone, joint disease, heart disease, depression, etc.), as well as the current physiological conditions of an individual (including pregnancy, nursing mother, professional sports). In the second part of the questionnaire, some questions about taking dietary supplements are raised that at first three general questions about taking any of dietary supplements during life, during last year and during last month were asked and then consuming any of dietary supplements were asked in separated times.

In third part of the questionnaire, willingness causes to take dietary supplements are listed along with reviewing articles and scientific resources and counseling with feeding experts and participants were asked to determine importance degree of any willingness reason to use dietary supplements. Importance degree of each cause is considered as Likert scale of 5 items from very much to not at all. Score 1 is assigned to at all option and score 5 assigned to very much option.

Before completing questionnaire, questioner gave total descriptions to participants about different types of supplements and their trade and medicine names. To collect the information, questioners were trained during several meetings in the field of established questionnaire properties, details of dietary supplements

classification, method of collecting dedicated information, and how to do the job. It should be noted that feeding specialists and a pharmacist doctor were counseled for information about different types of supplements in the market and a list of dietary supplements with their trade names were prepared. At the end, after collecting data and entering them into SPSS, ratios, central indices and data dispersion were calculated. Also chi square, Man-Whitney U test and independent t tests were used to analyze data, results were reported in a 0.05 significance level.

RESULTS

In this study, 350 people were examined, 258 people (73.7%) were women and 92 people (26.3%) were men. The mean age of these participants in the study was 33.5 ± 10.5 , the youngest participant was 18 years old and the oldest one was 73 years old. In this study, 69 people (19.7%) of patients were between 15 through 24 years, 154 people (44%) between 25 through 34 years, 86 people (24.6 percent) between 35 to 44 years, 16 people (4.6%) between 45 through 54 years and 25 people (7.1%) were more than 55 years. Most of subjects (74%) were married and most participants' level of education in the study (41.7 percent) was a degree of high school and diploma. In this study, 99 people (28.3%) had a university degree. Also, most of the people in the study were housekeeper (53.7 percent) and citizen (78 percent). In this study, 171 people (48.9 percent) of subjects had a history of regular usage at least one of dietary supplements that this usage was once a day in 138 people (80.7 percent) and two or three times a day in 28 people (16.4 percent). Table 1 presents frequency distribution of taking dietary supplements in terms of demographics of subjects.

In this study, the history of taking dietary supplements in smoking people was 22.8 percent, and it was 53.9 % in non-smoking people that this difference was statistically significant based on chi square test ($p < 0.01$). Also, history of taking dietary supplements in people with chronic diseases (hypertension, diabetes, osteoporosis, rheumatologic diseases, heart disease, depression and etc.) was 69.9% and in healthy individuals was 42.8% that this difference was also statistically significant ($P < 0.01$). Table 2 shows frequency distribution of taking dietary supplements in subjects by history of smoking, history of affected by chronic diseases, drug history and the history of regular physical activity (three times a week, at least 30 minutes). In addition, in this research, the most usage of dietary supplements was seen in nursing mothers and the least was related to pregnant women that these findings are statistically significant ($P = 0.029$). In men, the most usages was reported in sportsmen, especially professional ones that this difference was also significant ($P < 0.01$).

Table 1: Frequency distribution of taking dietary supplements in terms of demographics of subjects

Type of variable		History of taking dietary supplements				P-Value
		N (%)	Yes N (%)	No N (%)	Total N (%)	
Age	15-24	69 (19.7)	41 (59.4)	28 (40.6)	69 (100)	0.093
	25-34	154 (44)	78 (50.6)	76 (49.4)	154 (100)	
	35-44	86 (24.6)	32 (37.2)	54 (62.8)	86 (100)	
	45-54	16 (4.6)	8 (50)	8 (50)	16 (100)	
	≥55	25 (7.1)	12 (48)	13 (52)	25 (100)	
Sex	Male	92 (26.3)	8 (8.7)	84 (91.3)	92 (100)	<0.001
	Female	258 (73.7)	163 (63.2)	95 (36.8)	258 (100)	
Marital status	Married	259 (74)	139 (53.7)	120 (46.3)	259 (100)	0.006
	Unmarried	76 (21.7)	26 (34.2)	50 (65.8)	76 (100)	
	Other	15 (4.3)	6 (40)	9 (60)	15 (100)	
Education	Junior high school or less	105 (30)	44 (41.9)	61 (51.8)	105 (100)	0.006
	High school or high school diploma	146 (41.7)	71 (48.6)	75 (51.4)	146 (100)	
	University	99 (28.3)	56 (56.6)	43 (43.4)	99 (100)	
Occupation	Unemployed	42 (12)	9 (21.4)	33 (78.6)	42 (100)	0.001
	Housewife	188 (53.7)	122 (64.9)	66 (35.1)	188 (100)	
	Self-employed	32 (9.1)	13 (40.6)	19 (59.4)	32 (100)	
	Office employee	29 (8.3)	13 (44.8)	16 (55.2)	29 (100)	
	Laborer	8 (2.3)	3 (37.5)	5 (62.5)	8 (100)	
	Farmer or Stock breeder	11 (3.1)	1 (8.7)	10 (90.9)	11 (100)	
	Student	32 (9.1)	9 (28.1)	23 (71.9)	32 (100)	
	Other	8 (2.3)	1 (12.5)	7 (87.5)	8 (100)	
Place of residence	Urban areas	273 (78)	140 (51.3)	133 (48.7)	273 (100)	0.015
	Rural areas	69 (19.7)	28 (40.6)	41 (59.4)	69 (100)	
	Other	8 (2.3)	3 (37.5)	5 (62.5)	8 (100)	

Table 2: Frequency distribution of taking dietary supplements in subjects by history of smoking, history of chronic diseases, drug history and the history of regular physical activity

Type of variable		History of taking dietary supplements				P-Value
		N (%)	Yes N (%)	No N (%)	Total N (%)	
History of smoking	Yes	57 (16.3)	13 (22.8)	44 (77.2)	57 (100)	<0.001
	No	293 (83.7)	158 (53.9)	135 (46.1)	293 (100)	

History of chronic disease	Yes	79 (22.6)	55 (69.6)	24 (30.4)	79 (100)	<0.001
	No	271 (77.4)	116 (42.8)	155 (57.2)	271 (100)	
History of regular physical activity	Yes	141 (40.3)	60 (42.6)	81 (57.4)	141 (100)	0.053
	No	209 (59.7)	111 (53.1)	98 (46.9)	209 (100)	
History of drug therapy for chronic diseases	Yes	70 (20)	50 (71.4)	20 (28.6)	70 (100)	<0.001
	No	280 (80)	121 (43.2)	159 (56.8)	280 (100)	

Reviewing distribution frequency of taking supplements in terms of body mass also indicates that the most usage of dietary supplements was regularly in people with a body mass index under 18 and the least usage was in people with a body mass index of 25 to 29.9; again this difference was statistically significant ($P < 0.05$).

In examining distribution frequency of different supplements usage in consumers of dietary supplements, it is also observed that the most frequency of dietary supplements usage is related to iron (70.5%) and Folic Acid (70.5%), then, calcium (30.1%), multivitamin (25.4%), calcium- vitamin D (17.3%), vitamin B complex (16.8%), vitamin D (15%), vitamin B6 (15%), vitamin C (9.5%) and vitamin A (9.2%), respectively. On the other hand, the least frequency of dietary supplements usage was related to Chrome, Magnesium, and Potassium (each 2.3%).

In evaluating causes of tendency to dietary supplements usage in subjects (based on score), it is also observed that prevention diseases and health enhancement (1.77 ± 3.13), feeling of lack of salts used in daily food (1.47 ± 2.26), using in order to strengthen body security system (1.37 ± 1.97) and using in order to add weight (1.24 ± 1.71) were considered as the most important factors of tendency to use them, on the other hand, taking supplements with aim of weight loss (0.47 ± 1.19) and using in order to increase sexual powers (0.53 ± 1.35) were considered as the most insignificant factors of tendency to use dietary supplements.

In examining the references frequency for recommending the usage of dietary supplements in under study people, it was also observed that specialists (38.7%), general practitioners (23.7%) and feeding experts (16.8%) were considered as the main recommending reference and the insignificant recommending reference was also recommendation by friends (2.3%), media (2.3%) and the Internet (2.9%).

DISCUSSION

This study was conducted in adult population referred to health care centers of different areas in Khorramabad city and the findings of the study showed that 48.9 percent of under study population use one of synthetic dietary supplements in a regular basis and especially during last year; this finding is consistent with a little difference with study conducted by Najmabadi et al., in western region of Tehran city on adult population referred to health care centers; so that Najmabadi et al., found that 42% of population used regularly at least one dietary supplement during last year [17]. While, in a study of Babanejad et al., in south of Tehran city, 26.3 percent of participants used dietary supplements that this difference is likely related to economic social situation of under study units in south part of Tehran city. In addition, consistency of this study findings with study conducted in west of Tehran can be attributed to similarity purchasing power of the populations examined, this confirms role of economic social factors [19] in dietary supplements usage.

In this study, the most frequency of different supplements consumption was related to iron and Folic Acid, calcium, multi vitamin and vitamin D. In study of Najmabadi, 38 percent of dietary supplements consumers used multi vitamins or multi vitamin along with salts, 30.1% calcium supplement with vitamin D and 27.1% used iron supplement or different kinds of hematinic, the reason of difference of this study results with our study can be resulted from gender composition of two study population, because in this study more than 70% of participants were women that naturally use iron and hematinic supplements more than men, this issue is a reason for more frequency of using iron supplements (ferrous sulfate) in the present study. In fact, taking dietary supplements is under influence of various factors such as age, sex, marital status and similar factors. Results of our study showed that there is a significant difference between taking supplements in women participants than men participants in the study. In Najmabadi study, also, difference in taking dietary supplements between men and women was statistically significant [17]. This is also seen in studies in other areas of the world such as consumer population of dietary supplements in America and studies in the United Kingdom and Poland [20, 21]. Given that iron deficiency and bone fractures in higher ages is among problems that in many cases are seen in women more than men, tendency to take calcium supplements and vitamin D in this group of society is approximately predictable. Read et al., study in seven states of America by assessing usage frequency of dietary supplements showed that 13 percent of users of calcium dietary supplements, 11% iron supplement and 12 percent used zinc supplement during last year in which women were also used more dietary supplements than men [22].

Also in the present study, frequency of taking dietary supplements in married individuals was significantly higher than single people (74 percent vs. 21.7 percent). In study of Mirmiran et al., entitled "Tehran lipid and glucose study (TLGS)" using dietary supplements in most cases was higher in married men and women than singles [23]. But unlike the results of this study, during studies on Tai people [5] and America [24], no significant relationship was recognized between marital status and usage of dietary supplements

that this case can be influenced by cultural, economic and social factors of other societies in comparison with Iran's society that have different living arrangements.

In this study, the most usage of dietary supplements in women participated was related to nursing mothers and the least usage was related to pregnant women; the lack of some necessary supplements during the pregnancy in women of this study is noteworthy. In a study of Najmabadi et al., [17], the most usage of dietary supplements was related to pregnant women and nursing mothers. The study of Kholdi et al., in 2004 also showed that 76.6 - 78 % of mothers referred to health care centers in south of Tehran used regularly iron supplement and folic acid during their pregnancy [25]. The highest cases of taking dietary supplements during pregnancy and feeding among women indicated effective role of correct recommends by doctors, specialists of feeding and health care experts; on the other hand, it seems that due to low usage of necessary supplements in pregnancy women in this study, a special attention should be paid to education and its role in taking proper supplements during pregnancy.

In addition, in this study, taking dietary supplements in sportsmen was more than other men and also frequency of usage in people with a body mass index under 18 (thin people) was significantly higher than other participants in the study. The main concern is that in taking dietary supplements should pay attention to proper and scientific recommendations of trusted group of society because taking dietary supplements in sportsmen or for fixing thin should be based on the recommendations of feeding experts and consultants. What is confirmed by reports and studies is that taking dietary supplements helps to provide especial physiological needs like sportsmen or to supply nutrition uptake though diet, also taking dietary supplements for reducing shortfall risk and reducing uptake in old age and women in the age of menopause are recommended.

Findings of this study showed that 30.1% of people use different kinds of calcium supplements and vitamin D, if taking dietary supplements is based on physiological condition, on one hand, the issue caused happiness and it is a positive point of taking supplements and as under study population are referrals to health centers, this issue can indicate success of feeding education programs and improving mothers feeding in primary health care, on the other hand, it seems that programs of improving feeding and training for providing feeding needs in different ages should be developed and some approaches should be considered for elder people and prepares for prevention of vitamin deficiency problems in older age in order to prevent disorders like osteoporosis and other disorders which can be preventable by balance and proper feeding.

Among other findings of this study is high frequency of dietary supplements in ages 15-24 and 25-34 and also 45-54. In various studies, the most common age for taking dietary supplements was in children, middle-aged and aged people [20]. In this study, level of taking dietary supplements was the least in ages upper than 55 years (12%), in the study of Najmabadi, only 10 percent of under study population in age of 45-59 years and only 1.4 percent of 60 years old people and older take dietary supplements [17]. While, similar studies in other countries [20, 27] showed that middle-aged group take dietary supplements more than youth group.

In the present study, frequency of taking dietary supplements in people with chronic diseases or those that use drug because of chronic diseases are significantly higher than other participants. In Pietruszka et al. (1999) study in central and eastern regions of Poland; it is also shown that taking dietary supplements is more common and abundant in adults with healthy problem and also in adults in condition of drug usage [26].

In addition, reference for recommending dietary supplements in under study population was general and specialists doctors in 62.4% cases, feeding counselors and experts in 16.8% cases and willful usage and personal information in 6.9% cases. In Najmabadi et al., study, reference for recommendation was doctor or feeding in 75.5% cases and willful usage was 20.1% cases [17]. Fortunately, the findings of this study showed that taking dietary supplements is very little in willful cases; this can be resulted from under study population that this population was among those who refer to health care centers. Other results of this study indicate that the highest causes of tendency to take regular dietary supplements are preventive imagination of different kinds of diseases and health improvement, feeling of low dosage of these materials in daily food and consuming them for strengthening body security system and also as an aid in diet. In Najmabadi et al., study in Tehran, usage reasons were largely maintaining health and preventing mentioned shortages.

CONCLUSION

Finally, it is mentioned that this study faced with some limitations; in fact, as under study population was formed by those who refer to health care centers, thus, people was randomly selected through those who refer to different parts of health care centers to receive different services; therefore, different physiological conditions like young sportsmen and loss weight was less often seen that this case was among limitations of researchers in this study. Other limitation of this study is type of this study because cross-sectional studies are not so strong to determine causality and by doing this kind of studies, determinant effect of effective factors on taking dietary supplements cannot be proved. In the end, according to the fact that tendency to take dietary supplements was more in lower ages, therefore it is necessary to provide training and nutritional strategies appropriate for different age groups about the proper use of nutritional supplements.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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FINANCIAL DISCLOSURE

The authors report no financial interests or potential conflicts of interest.

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ARTICLE

COMBINED EFFECT OF IRANIAN PROPOLIS AND HONEY ON HEALING OF INDUCED INCISIONAL WOUND IN RAT

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ABSTRACT

Background: Surgical site infections (SSI), are problematic for the patients. The beehive products can improve wound healing, but whether it can improve healing surgical wounds, has not been well investigated. This experiment was conducted to compare Iranian beehive products with cold cream for healing of incisional cutaneous wound in rats as a model for surgery wounds. **Materials and methods:** 50 male Wistar albino rats were randomly allocated into five equivalent groups, as control, cold cream, propolis cream, honey and a mixture of honey and propolis cream (MHP). A skin incised wound model was induced on the back of all animals. The incision area in the five groups were covered twice-daily with cold cream, propolis cream, honey and a MHP respectively. The percentage reduction of wound size was measured at the start of experiments, some specific days and at day 22. The wound tensile strength was also measured at day 30. **Results:** The results showed that, there were statistically significant differences between the percentage reduction of wound size in treated groups by beehive products compared to the cold cream group on most days of the experiment. Also, the differences between the percentage reduction of wound size in MHP group, compared to honey and propolis groups were statistically significant on days 13 and 19. The differences between the wound tensile strength of treatment groups, compared to cold cream were also significant. **Conclusion:** We concluded that, there may be a synergistic therapeutic effect between propolis cream and honey in the healing of wound in rat. These results provide a rationale for studying the topical application of this mixture in a clinical setting.

INTRODUCTION

The surgery wounds infection with numerous organisms, with additional problems of drug resistance, determines the drug strategy program by the physician for patients. Recently, a summary of data from several hospitals in Europe, Asia, Africa and America found an overall 2.3% incidence of Surgical site infections (SSI) [1, 2]. Besides the morbidity and mortality associated with SSI, there are noteworthy cost implications. A research found that it cost over USD 3000 more to treat a patient with a SSI compare to a non-infected patient [3]. In Britain, a research over a 2-year period found the additional cost attributed to the SSI was £5,239 [4]. So, prevention of SSI has become a priority for health care facilities. The latest studies shows that the remedies of biogenic source with their analgesic, anti-micro-organic and healing properties are becoming more and more important in wound healing [5].

Honey is an ancient remedy that has regained popularity and is widely used as an antimicrobial for treating wounds, ulcers, and burns [6]. On the other hand, propolis has been used in folk medicine for centuries [7]. It has some roles such as immune enhancing [8], antitumor [9], cytostatic [10], anticarcinogenic [11], anti-inflammatory [12], oxygen radical scavenging [13] and antimicrobial activities [14]. Some researchers believe that propolis is the antibiotic of the 21st century for the reason that its synergic action with other antibiotics. In vitro synergy between propolis and antimicrobial drugs has been examined, and preparations combining propolis with antifungal agents and antibiotics are of potential medical interest [15]. Topical application of propolis to several kinds of wounds has been found to be operative in making a fresh granulating bed and controlling infection [16-18].

Skin incisional wounds seem to be an ideal model for drugs antimicrobial properties and laboratory testing of healing and clinical assessment [19, 20] in traumatic wound and surgical procedures [21]. Wound tensile strength is also one of the most significant factors in healing of wound and is a helpful assessment that shows the dermal collagen fibers organization in the recently composed collagen [22]. The goal of the present research was to study the wound healing rate, regarding wound closure time and tensile strength, treated with topically applied 60 % propolis cream, honey and MHP compared to cold cream group in rat.

EXPERIMENTAL DESIGN AND ANIMAL GROUPS

Animals

The trials were done on 50 male Wistar rats, weighing between 200 and 250 g. They were individually housed in a room with natural light cycle and constant temperature ($24 \pm 2^\circ\text{C}$), where they had free access to water and standard feed.

KEY WORDS

Propolis, Honey, Cutaneous, Wound, Rat

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Instrumentation and surgical procedure

Experiments were done in the animal house of the Semnan faculty of medical sciences in IRAN in accordance with usual guidelines. The rats were anesthetized by intraperitoneal injection of pentobarbital (75 mg/kg). A linear 3cm incision was made over the skin of animal back, after shaving the back, as described by Okada et al with some modifications [23].

Experimental design

The rats were randomly separated into 5 groups, as control, cold cream, propolis cream, honey and the mixture of honey and propolis. Control group did not take any treatment. Cold cream group received topical cold cream two times a day from the start of experiments to the end of wound closure. The topical treatment was done by 60 % propolis cream, honey and the MHP at the same time. Both researchers and subjects were kept blind about all trials.

60% propolis cream preparation

Propolis and honey sample used in this study was collected by hand from colonies of honeybees located in the northwest area of Mashhad in Iran. The propolis sample was kept at 4 °C and stored in a dry place. The sample was ground to a fine powder and extracted with 80% ethanol in a shaker at room temperature for 48 h. After that, the extract solution was filtered through Whatman No. 4 filter paper, and concentrated in a rotating evaporator to obtain the crude extract in paste form, then kept in a dark and dry place.

Ethanolic extracts of propolis were prepared and applied as described by ISLA et al [24] with slight changes. The crude extract was frozen at -20 °C, and crushed in a chilled mortar. After that, the prepared powder was pull out with ethanol (15 ml of 80 % ethanol/g of propolis). Three days later, the supernatant of suspension was concentrated in an evaporator and the remainder was added to the same volume of 60 % cold cream (19 % distilled water, cold cream, 12.5 % spermaceti +0.5 % borate of soda + 12 % white wax + 56 % liquid paraffin + Botafarma). The MHP was prepared by adding the round powder of propolis to the same amount of honey.

Measurement and evaluation of wound healing

The size of the wound area was measured on the 1st, 2nd, 4th, 10th, 13th, 16th, 19th and 22nd days from the start of the experiment in all groups, by using a permanent marker and transparencies. The wound sizes of all animals were measured as described previously [25]. After calculating the wound area, wound healing percentages were calculated by walker formula [26]. Wound healing percentages were computed for 4th, 10th, 13th, 16th, 19th and 22nd days after the start of the experiment in all groups as follows:

Percentage of wound area = (Wound area in day X / Wound area on the first day) × 100

Percentage of wound healing = 100 - Percentage of wound area

Tensile strength of the wound, which determines the force per unit of cross-sectional area required to discontinuity the wound, was determined on the 30th day by continues, constant water flow technique [27] with some modifications.

Statistical analysis

Data analysis was done blind to avoid bias. All values were presented as Mean ± SEM. The normality of data was checked by the Shapiro–Wilk test via using the SPSS software. All data had a normal distribution. Thus, the obtained data were analyzed by one-way ANOVA followed by analysis of the Tukey test post hoc. If the p-values are less than 0.05, the differences can be considered significant.

RESULTS

The combined effect of Iranian propolis and honey mixture (MHP) on the tensile strength of incisional wound measured in this study, are shown in Table 1. A statistically significant difference was seen between the mean of wound tensile strength of control and honey groups ($F_{4,45}=1.004$, $P=0.005$), cold cream and the MHP groups ($F_{4,45}=21.004$, $P=0.000$), and between honey and the MHP groups ($F_{4,45}=21.004$, $P=0.008$) on the 30th day of the experiment.

The combined effect of Iranian beehive products on wound healing percentages measured in this study are shown in Fig 1. A statistically significant difference was seen between the mean of wound healing percentages of control and cold cream groups on day 7 ($F_{4,45}=12.944$, $P=0.047$). A statistically significant difference was also shown between the mean of wound healing percentages of cold cream and propolis groups on day 7 ($F_{4,45}=12.944$, $P=0.017$). A significant difference was also seen between the mean of wound healing percentages of cold cream and the MHP groups on days 10 ($F_{4,45}=8.452$, $P=0.003$) and 19 ($F_{4,45}=8.452$, $P=0.003$). There was also statistically significant difference between the mean of wound healing percentages of control and honey groups on day 10 ($F_{4,45}=8.452$, $P=0.006$). The

mean of wound healing percentages difference between propolis and the MHP groups was statistically significant on day 13 ($F_{4,45}=6.783, P=0.001$) and a significant difference was seen between the mean of wound healing percentages of honey and the MHP groups on days 7 ($F_{4,45}=12.944, P=0.003$) and 13 ($F_{4,45}=6.783, P=0.044$).

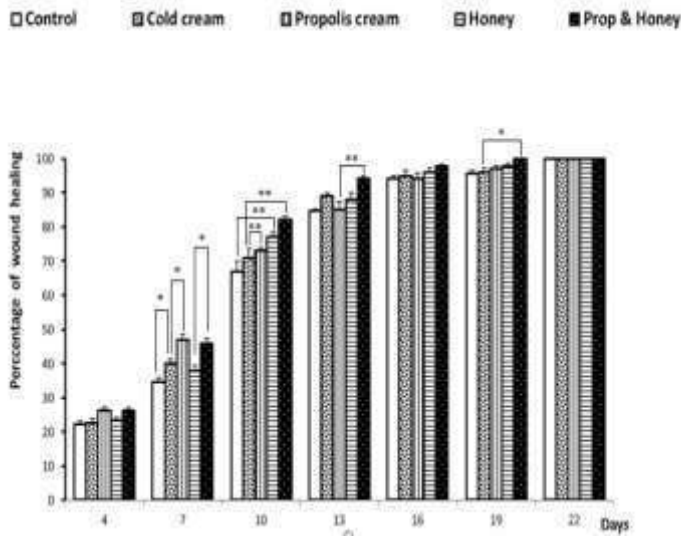


Fig. 1: Changes in wound healing percentage (Mean \pm SEM) of the rats following the induction of wound at the time of wounding (day 0) and at termination (day 22).

* P <0.05 as compared with the control group

** P <0.01 as compared with the cold cream group

Table1: Effect of honey bee products on the tensile strength of incisional wound model on rats compared to cold cream treatment

Groups	Treatment twice a day	Tensile strength
1	No	615.9 \pm 1.3
2	Cold cream	622.8 \pm 2.4
3	60 % propolis cream	635.7 \pm 7.1*
4	Honey	663.5 \pm 7.4 ^{\$}
5	Mixture of honey and propolis	702.4 \pm 13.5* [#]

*P<0.01 vs cold cream group
#P<0.001 vs propolis cream group
\$P<0.01 vs honey group
&P<0.01 vs control group

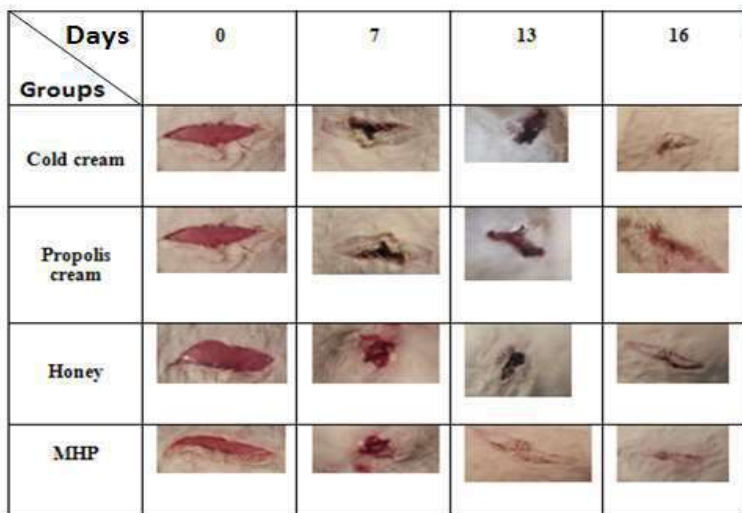


Fig. 2: Representative photos of each treatment on rats subjected to skin incision wounds, during experiments. Wounds were generated at day 0 and photos were taken as on **Fig. 2:** Each row shows the same wound as it progresses for 16 days. **Notes:** The animals received 1 g of local applied cream consisting of cold cream, 60 % propolis cream, honey and a mixture of honey and propolis cream twice a day.

Abbreviation: MHP, mixture of honey and propolis cream

DISCUSSION

On recent years, the development of antibiotic-resistant bacteria is a great challenge facing human and veterinary medicine concerning to the infection of surgery wounds. Indeed, studies have been focusing on the last few decades on an alternative therapy and natural medicinal products that can potentially cease and/or remove the threat of microbial resistance, and stimulate tissue repair for the wound local treatment, due to the high costs of traditional treatments [28, 29].

The goal of this experimental research was to assess the wound curing activity of honey bee products on a rodent incisional skin wound as a suitable model for clinical surgery wounds. In this study we used Wistar rats to assess the cold cream, propolis cream, honey and a MHP on a randomized controlled study.

As the wound tensile strength indicates the organization of subdermal collagen fibers on the recently composed collagen, it is a critical measure [30]. It shows how much the healed tissue resists to rupture under strain and may partly show the quality of the repaired tissue [31]. Clinical and experimental trials designed to effect wound healing rates or strength have been showed with varied success [32].

According to our results, wound tensile strength measurements showed that 60 % propolis cream significantly increased treatment of wounds compared to cold cream group on day 30 of experiment [Table 1]. Also, the honey treatment showed better healing of wounds significantly compared to control group and the healing effect of MHP on regard to wound tensile strength, was better than propolis cream or honey group separately [Table 1].

Wound healing percentage measurements showed that cold cream significantly increased treatment of wounds compared to control group on day 7 of the experiment [Fig. 1], which was reported and discussed by the author previously [33]. Also 60 % propolis cream increased treatment of wounds significantly compared to cold cream on day 7 of the experiment [Fig. 2]. As the solvent applied for the extraction of propolis may influence the potency of its antimicrobial activity, we prepared ethanolic extract and the results of propolis ethanolic extract healing effect were consistent with the earlier studies [17, 34]. Despite the propolis extract on this study supplied via ethanol showed a high percentage of wound closure time, the propolis aqueous extract used by author significantly hadn't improved wound healing previously [35]. So we suggested that on addition to the kind of preparation, the inconsistency seen between studies may be due to the dosage, form and geographic origin of propolis. Although the adverse reactions related to the usage of propolis on wounds are poorly documented on the literature, a well-documented allergic reaction to propolis is contact dermatitis [36]. It has been found that if very large quantities of propolis are administered, it is not toxic to mammals or humans [37]. As we expected, no mortality was seen on all five groups of our study and the rats gained weight on a normal way (data not shown), which confirmed that the doses of honey bee product used, were not toxic.

Propolis is a complex mixture consisting of at least 230 different natural substances including aminoacids, flavanoids, phenolic acids esters, phenolic acids, cinnamic acid, terpens, caffeic acid, and trace minerals such as iron and zinc [38]. As the antioxidant, anti-inflammatory and antimicrobial activities of the natural product constituents are the most important properties for wound healing and different natural substances on propolis may have all these actions, the wound healing effect of propolis is explicable.

One special feature of propolis is its anti-inflammatory properties. Flavonoids and caffeic acid decrease the inflammatory response by inhibiting the production of prostaglandins via blocking the lipoygenase activity leading to phagocytes and immune cells stimulation and make effective analgesic and anti-inflammatory mechanisms similar to aspirin and with fewer side effects [39, 40]. Antibacterial effect of propolis is related to its flavonoids, esters, and circular acids [39]. Bioflavonoids on propolis stop an exodus of inflammatory mediators from mast cells and thus inhibit the allergic reaction and inflammation [41]. Flavonoids also have antioxidant [42] and antimicrobial effects [43], while coumaric acids, lignan and diterpen have antibacterial effects [44].

Wound healing includes inflammatory, proliferative and remodeling phases. An earlier work has shown that anti-inflammatory action of propolis is more effective than dexamethasone [45]. So, it is probable that the most important mechanism of propolis action on wound healing is through its anti-inflammatory properties and its contribution to the production of collagen.

Our results also showed that honey increased wound closure time significantly compared to the control group on day 10 of the experiment. Surprisingly the MHP not only could accelerate wound healing significantly compared to cold cream on more days of the experiment but also its healing effect was significant compared to propolis cream on day 13 and compared to honey on days 7 and 13 of the experiment.

Honey contains moisture absorption properties that can decrease edema of the wound. Our results on faster wound closure time by honey treatment is consistent with the other's works [5, 46]. Honey causes hydrogen peroxide production with its insulin-like effects and induces stimulation, cell proliferation and angiogenesis on the wound area. Moreover, it has an essential role on the removal of microbial agents [47]. Additionally, some researchers have shown that honey can accelerate granulation tissue formation

and angiogenesis on the wound region [48]. Honey supplies oxygen and available nutrient to fibroblasts via increasing angiogenesis, and oxygen supplied from hemoglobin for its acidic PH and leads to improved activity of fibroblasts and collagen development. As angiogenesis is accelerated, recovery of wound occurs earlier [48, 49].

Our study concern to the combined effect of honey and propolis on wound healing had some similarities to the work of Takzaree et al and the results had consistency. They proposed that as angiogenesis is an essential factor on wound healing process, the blood vessels growth on propolis and honey combined groups on comparison with control group had an increasing trend to nutrition, oxygenation, cell proliferation and ultimately accelerate wound healing [49]. Better wound healing acceleration on MHP compared to propolis or honey groups was shown on our study as we expected. On spite of the difference between the models of wound, the origin of honey bee products and different kinds of applied methods, the novelty of our work compare with the work of Takzaree et al was assessing wound healing on regard to wound tensile strength on addition to wound closure time measuring.

Propolis also has the potential to establish synergic effects with synthetic antibiotics, leading to a development on its antimicrobial effects on both in vitro [15] and in vivo [50]. Flavonoids on honey and propolis stimulate the immune system and enhance its antibiotic properties, reducing the wound size and restoring it, caused to decrease inflammation, infection and stimulation of fibroblasts to produce collagen fibers. This finding is consistent with the hypothesis that the antimicrobial properties of MHP may have prevented the wound healing delay that would otherwise happen on the wounds infection.

So, reduction of wound size and increasing of wound tensile strength on our MHP treatment group [Fig. 3 and Table 1] may be due to a synergistic effect that increased collagen fiber due to promoted fibroblasts and has consistency with the other's works [47, 51] [Fig. 3 and Table 1]. It suggests that the MHP group may be able to increase fixation and compaction of the collagen fibers. Nevertheless, on the future, more researches require to be done to approve it.

CONCLUSION

This short experimental study concludes that the combined propolis honey (MHP) has a significant incisional wound curing activity on the animal model. This synergism between honey and propolis may contribute to the decreasing the of synthetic drugs administration and the management of antibiotic resistant microorganisms' production, may open new windows for the synthesis of novel drugs. Patients management with cutaneous wound continue to struggle surgeons and physicians on dermatology and surgery area [22] and some medications such as a combined propolis honey preparation may be usable to accelerate the healing of full thickness skin wounds. More works are also essential to be done on combined propolis and honey component stability on different preparations, safe, and bioavailability and effective doses for the management of diseases. So, we suggest further studies of combined propolis honey preparations to be used on man whom surgical wounds show an increasing therapeutic clinical and clinical challenge.

LIMITATIONS OF THIS STUDY

We request the reader to bear with us on this study as we focus on the macroscopic results, i.e., the end outcome of wound healing, rather than the details of the healing mechanism. Although there is much to be done, our work has found important results on the field of the effect of Iran's honey bee product on wound healing. Our wound healing research was also done only on regard to wound closure time and tensile strength measurement and samples for this study were collected from only one geographical site.

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COMPETING INTEREST

The authors confirm that the study wasn't done on any financial or commercial associations that could be explained as a possible competing interest.

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