Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.

Yours sincerely,

Vasco Azevedo

Vasco Azevedo, Editor-in-Chief
Integrative Omics and Applied Biotechnology
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ARTICLE

COMPARISON OF TWO GENOTYPING METHODS OF HCV- RT-PCR AND DIRECT SEQUENCING

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ABSTRACT

Hepatitis C virus (HCV) is an important human pathogen that can cause acute and chronic hepatitis, liver cirrhosis, and, hepatocellular carcinoma. HCV has been classified into 6 major genotypes and many subtypes. Several methods have been developed for HCV genotyping and direct sequencing is gold standard method. HCV was transmitted by different routes such as intravenous drug abuse (IVDA), tattooing, sexual, blood transfusion and other risk factors. In this study collected 180 samples of patients infected with HCV were referred to Reference laboratory in Kermanshah University of Medical Sciences, for HCV genotype testing and samples were genotyped by RT-PCR and direct sequencing of Core gene. Among them , 138 samples (76.6%) was 3a genotype, 35 (19.4%) was 1a genotype, 3 (1.7%) was 1b genotype and 4 (2.2%) was 3a and 1b. This study revealed that 3a is the most prevalent genotypes in Kermanshah province and IVDA is the main risk factor in this province and also Results of genotyping by RT-PCR was confirmed by direct sequencing .

INTRODUCTION

Chronic hepatitis, hepatocellular carcinoma and cirrhosis are most important liver diseases in the world [1]. Approximations 130–170 million persons, or 2–3% of the world's population, are infected with HCV. Prevalence estimates are 400 000 chronically infected subjects in Australia and Oceania, 14 million in the Americas, 16 million in the Middle East, 17.5 million in Europe, 28 million in Africa, and 83 million in Asia [2]. The researches show that the main factors responsible for the epidemic spread of HCV were the routes for transmission: blood transfusions or blood product administration, the medical use of unsterilized needles for injections and for large scale vaccination, intravenous drug use associated with sharing of injection equipment, heterosexual or homosexual activity with multiple sexual partners and tattooing[3]. Heavy alcohol consumption, particularly in females, age and HCV/HIV co-infection may be associated with more rapid progression of HCV liver disease, especially fibrosis. The HCV genome is a positive-sense, single-stranded RNA genome approximately 10 kb long. It has marked similarities to those of members of the genera and Flavivirus. Hepatitis C is containing one long open reading frame [ORF] that encodes for a non-functional polyprotein of approximately 3010 amino acids in length .This non-functional polyprotein is cleaved by cellular and viral proteases to yield at least ten different functional protein products. Structural proteins are the major are coded in the 5' quarter of the ORF and arranged as C ,E1 ,E2 and P7, while the nonstructural proteins are encoded in the 3' three-quarters of the ORF in the order NS2–NS3–NS4A/B–NS5A/B [4].

HCV has a high viral heterogeneity and different HCV isolates from around the world show substantial nucleotide sequence variability throughout the viral genome. Genotypes differ from each other by approximately 30% in amino acid sequence[5]. HCV genotypes have a striking geographical and epidemiological distribution and genotype identification is clinically important to tailor dosage and duration of the treatment [6]. Interestingly, not only do the HCV genotypes seem to differ in nucleotide sequence and geographical distribution, but there is also evidence of biological differences between the HCV genotypes[7]. Therefore, genotyping is an important tool for prognosis and follow-up of infected patients. Viral genotype determination before treatment is currently advised as a routine assay. Mode of transmission may also affect distribution of HCV genotypes [8]. Hepatitis C genotype is the most important virological factor predicting response to antiviral therapy[9]. Several regions of the HCV genome have been analyzed with the purpose of genotypic classification. The 5' NCR, Core, E1 and NS5B regions have been frequently amplified and studied for the purpose of genotypic classification. The NS5B region more often used for differentiation of subtypes and confirmation of genotyping results in research settings [10,11].

Several methods for genotyping HCV have been developed, including direct DNA sequencing [12], type specific PCR [13], restriction fragment length polymorphism, line probe assays, primer-specific and mispair extension analysis [14,15], heteroduplex mobility analysis by temperature gradient capillary electrophoresis [16], denaturing high preference liquid chromatography and gold standard method is direct DNA sequencing [17]. The aim of this study was to determine HCV genotype prevalence and comparison of two methods of HCV genotyping; RT-PCR and direct sequencing of Core gene in Kermanshah Province, the west of Iran. The distribution of HCV genotypes and their association with

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possible transmission routes [risk factors] in a group of HCV infected patients from Kermanshah province was investigated, as the data exclusively related to this area is limited.

Kermanshah province, located in the middle of the western part of Iran, has 1.95 million population which 69.7% reside in urban. While literacy of Kermanshah is 81.7%, this province has more drug abusers compared to other provinces of Iran [http://www.ar.org.ir/Default.aspx?tabid=133].

MATERIALS AND METHODS

In a cross-sectional study, a total of 180 people infected to HCV infection referred to the Central Medical Laboratory of Kermanshah from 2010 to 2013. Informed consent was signed by all participants. Data were stored in the database with no reference to the subjects' names. The study protocol and the consent forms were reviewed and approved by the Ethic and Research Committee of Kermanshah University of Medical Sciences. EDTA-anticoagulated specimens was centrifuged and plasma separated and the RNA was extracted by commercial QIAamp Viral RNA Mini kit [QIAGEN]. For purification of viral RNA from the collected serum samples, 140 µL of sera were used according to the manufacture instruction [QIAamp Viral RNA Mini-kit, QIAGEN, USA] To enhance binding of viral nucleic acids to the QIAamp Mini membrane, especially if there were very few target molecules in the sample, carrier RNA was also added to the reaction, as recommended. The addition of large amounts of carrier RNA reduces the chance of viral RNA degradation in the rare event that RNase molecules escape denaturation by the chaotropic salts and detergent in buffer. If carrier RNA is not added to buffer, this may lead to reduced viral RNA recovery. The HCV RNA in sera was measured by the commercially standardized quantitative reverse-transcription polymerase chain reaction [RT-PCR] artus HCV RG RT-PCR kit and ABI 7500 Real Time PCR instrument.The results are given in international units per milliliter [IU/ml] The following protocol in the instrument:

      45°C   for   10 min,   1 cycle;
      95°C   for   15 min,   1 cycle;
      95°C for 15 sec   and   60°C for 60sec   40 cycles.

Multiplex RT- PCR method with One Step-RT PCR Qiagen kit and specific primers for Core region of HCV genome were performed to determine viral genotype analysis. The primers that used in this study were purchased from metabion company [Table- 1].

Amplified PCR product run on 2.5% agarose gel. The viral load was estimated in the peripheral blood specimens using a commercial artus HCV RG RT-PCR kit [QIAGEN] for the detection of HCV RNA using Real-time PCR on ABI Prism 7500 instrument[Applied Biosystems, Foster City, CA].The amplification reaction was performed according to the manufacturer's instructions. HCV isolates were genotyped using universally accepted method of Ohno et al [17,18]. Briefly, based on this method for HCV genotypes manifestation, two rounds of nested PCR was done by one step RT PCR kit [QIAGEN]. Core specific primers, Sc2 and Ac2, were applied for the first- turn PCR and two mixtures of primers were used for the second-turn of Multiplex PCR. Primes of mixture A were specific for the detection of 1b, 2a, 2b, and 3b HCV genotypes [234 bp, 139 bp, 337 bp and 176 bp, respectively], while primers of mixture B were used for the detection of 1a, 3a, 4, 5a, and 6a HCV genotypes[208bp, 232bp, 99bp and 336bp, respectively]. Meanwhile, the PCR programs of these processes described by Ohno et al [1997] [18,19] and primer sequences are shown in Table- 1. The genotype specific band was visualized on a 2.5% agarose gel by ethidium bromide and UV light [Figure- 1].

Table 1: PCR, sequencing, and genotyping Oligonucleotide primers [modified from Ohno et al., 1997 13]

| Primer	Sequence [5'-3']	PCR round
Sc2	GGGAGGTCTCGTAGACCGTGCACCATG	1st round PCR
Ac2	GAG[AC]GG[GT]AT[AG]TACCCCATGAG[AG]TCGGC	1st round PCR
S7	AGACCGTGCACCATGAGCAC	2nd -round PCR for sequencing
A5	TACGCCGGGGGGTCA[TG]T[GA]GGGCCCCA	2nd -round PCR for sequencing
Mix 1		
S7	AGACCGTGCACCATGAGCAC	2nd -round PCR for genotyping
S2a	AACACTAACCGTCGCCCACAA	2nd -round PCR for genotyping
G1b	CCTGCCCTCGGGTTGGCTA[AG]	2nd -round PCR for genotyping
G2a	CACGTGGCTGGGATCGCTCC	2nd -round PCR for genotyping

G2b	GGCCCCAATTAGGACGAGAC	2nd -round PCR for genotyping
G3b	CGCTCGGAAGTCTTACGTAC	2nd -round PCR for genotyping
Mix2		
S7	AGACCGTGCACCATGAGCAC	2nd -round PCR for genotyping
G1a	GGATAGGCTGACGTCTACCT	2nd -round PCR for genotyping
G3a	GCCCAGGACCGGCCTTCGCT	2nd -round PCR for genotyping
G4	CCCGGGAACTTAACGTCCAT	2nd -round PCR for genotyping
G5a	GAACCTCGGGGGGAGAGCAA	2nd -round PCR for genotyping
G6a	GGTCATTGGGGCCCCAATGT	2nd -round PCR for genotyping

Direct Sequencing of HCV core gene was performed in both directions by ABI 3130 genetic Analyzer instrument[Applied Biosystem , Foster City, Calif].
The basic local alignment search
tool [BLAST] was used to search the public domain nucleotide database maintained by National Centerfor Biotechnology Information [NCBI] [20].
Data are presented as percentage [%] or number of patients. Chi-Square and Fisher's Exact tests were carried out by SPSS statistical package version 19.0 for windows. *P* values less than 0.05 were considered significant.
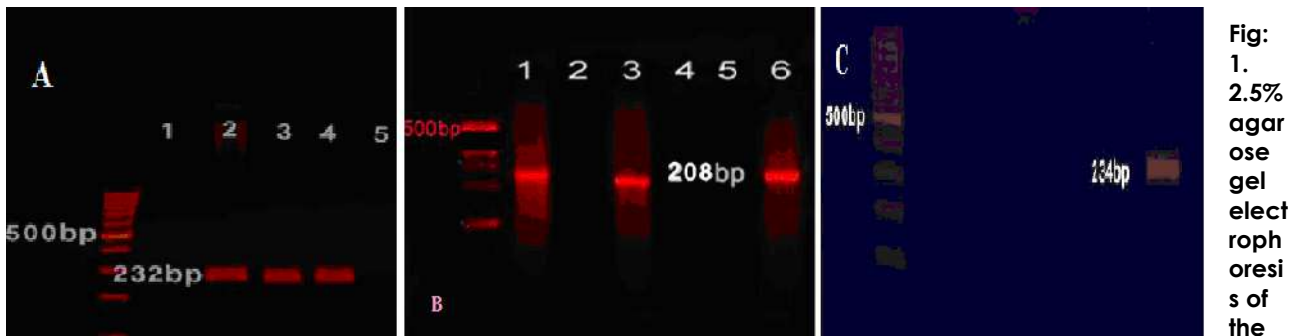
Fig: 1. 2.5% agarose gel electrophoresis of the PCR products of the HCV DNA from different genotypes. DNA marker 500 bp [lane M]; A: Genotype 3a; B: Genotype 1a; C: Genotype 1b.

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RESULTS

The distribution of the HCV genotypes in this study population is not in accord with the other known geographic distribution of HCV genotype in Iran. Viral load of all 180 cases was estimated using Real time PCR assay. Among these 180 HCV positive cases, 44 [24.4%] were female and 136 [75.6%] were male with an age range between 18 to 76 years old. There is a notable statistic; from 180 infected patients, 146 cases [81.1%] were connected with suspicious blood; 96 cases were addicted [IVDA], 43 cases had history of tattoo, 5 cases were health center staff, 2 cases had hemophilia and 34 cases [18.9%] have unknown risk factors [Fig. 2] that maybe is related to high risk sexual behavior that didn't declare because of cultural reasons. One important thing in the mentioned data is that 7 cases means 3.9% of total patients, 5 cases of Health staffs and 2 hemophilia cases, are infected due to fault in Health systems because frequency of HCV infection in normal population is 0.5-1% [21].
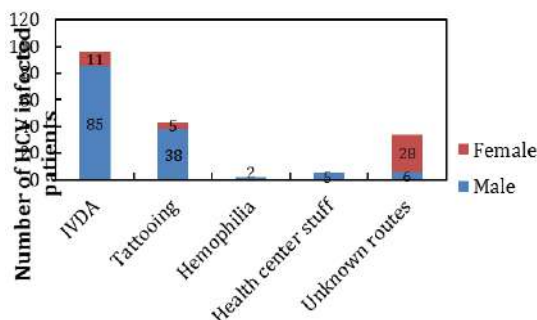

Fig: 2. Routes of infection in HCV infected patients

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This is an unfortunate report that could be a catastrophe for a Health system. Interestingly, 82.36% of unknown routes group that may referred to high risk sexual behavior cases comprises of women. These cases are not IVDA but almost have addicted spouse. As shown in Fig. 3, RT-PCR genotyping results for 180 people infected by HCV showed that 138 people [76.6%] had 3a genotype, 35 people [19.4%] had 1a genotype, 3 [1.7%] had 1b genotype and finally 4 [2.2%] had 3a and 1b genotype. Genotypes 2a, 2b and 3b were not detected in any samples. Classification of cases based on risk factors revealed that the predominant HCV genotype is 3a among drug abusers, tattooing and maybe high risk sexual behavior [unknown risk factors] groups [Table 2]. These groups comprise of <45 years old cases which consist with previous study that demonstrate usually IVDA cases have 3a genotype [22]. Prevalence of identified genotypes was determined for different age-groups of studied patients. The highest rate was observed in 31 to 50 yr old [Table 3]. Additionally, the results of direct sequencing strongly confirmed RT-PCR results [Figure 4].
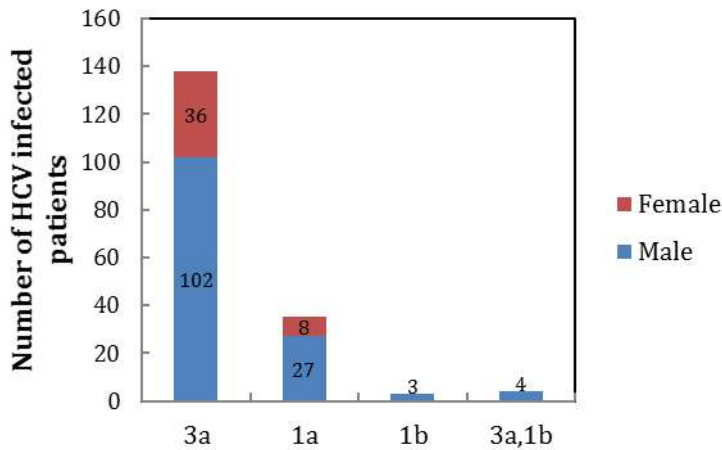
Fig: 3. HCV genotype distribution in Kermanshah province

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Table 2: Hepatitis C virus genotype subtypes prevalence within transmission route groups

Presentation of patients	HCV-genotype				Sig.
	3a [N = 138]	1a [N = 35]	1b [N = 3]	3a,1b [N = 4]	
Male/Female [%male]	102/36 [73.91]	27/8 [77.14]	3/0 [100]	4/0 [100]	
Transmission routes					
IVDA	77	16	0	3	0.05
Tattooing	29	10	3	1	ns
Hemophilia	0	2	0	0	ns
Health center stuff	4	1	0	0	ns
Unknown routes	28	6	3	0	ns

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Table 3: Frequency of HCV positive and there genotypes in different age groups

Age	Number of patients	Prevalence Genotype
0-10	0	-
11-20	2	1a
31 -40	98	3a
41- 50	46	3a
51-60	23	1a
61-70	8	1a
71-80	3	1a

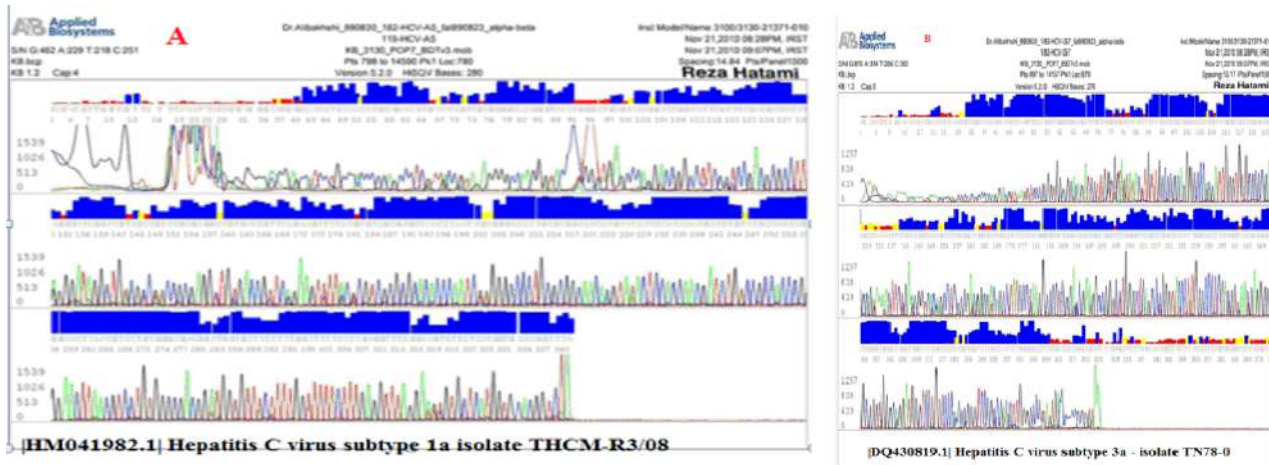**Fig. 4: Direct sequencing results of Core gene in HCV genotypes .** A: |HM041982.1| Hepatitis C virus **subtype 1a** - isolate THCM-R3/08. B: |DQ001257.1| Hepatitis C virus **subtype 3a** - isolate MOI257

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DISCUSSION AND CONCLUSION

Hepatitis C Virus [HCV] is one of the main causes of acute and chronic hepatitis that is distributed worldwide with prevalence varying between different countries from 0.2 up to 40% [2].

HCV genome is highly variable, leading to the classification of at least six genotypes, each with several subtypes. This heterogeneity is, at least partly, responsible for lack of availability of an effective vaccine [23,24].The importance of HCV genotyping as an epidemiological marker has been clearly shown, particularly in tracing the source of infection and elucidating the possible modes of transmission. Also The identification of genotypes is clinically important, and treatment protocols recommend that genotype information be obtained in order to delineate the duration and type of the medication to be used[14]. Based on sequence variation in both the coding and non-coding regions, several classification systems have been proposed. Ohno et al classified HCV to 1a, 1b, 1d, 2a, 2b, 3a, 3b, 4, 5a, and 6a isolates, based on the core region PCR with genotype-specific primers, that is widely acceptable [18,19].

Genotypes 1, 2, and 3 of HCV become manifest to have a worldwide distribution; however, their relative prevalence differs from one geographic region to another. In the United States and Europe, 1a and 1b subtypes are the predominant genotypes. HCV genotype 3a is more common among the abusers of intravenous drug in the United States and Europe [25]. In Middle East genotype 4 of HCV is common [24]. HCV genotype distribution in Tehran, located in the center of Iran, indicated that 3a genotype was the most frequent type [46.6%], and then type 1 [43.2%] is more common [26]. In comparison with other studies made in Iran ,it can be understood that the most common genotype of other provinces is type1. this genotype is uncommon in our region. The present study in Western Iran, indicate that type 3a of HCV is most prevalent in Kermanshah Province, Iran, which is different from other reports around the world. Moreover, it seems that there is a similarity between the pattern of genotype in Illegal drug users in kermanshah province when compared with other regions of IRAN. Interestingly, the results of RT-PCR in this study was confirmed by sequencing method. For this purpose, we were use of a sequencing technique for HCV genotyping consists of PCR amplification of core region of, ABI 3130 Genetic Analyser system..

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ARTICLE

THE EFFECTS OF TEN-WEEK TRAINING FOLLOWED BY FOUR-WEEK DETRAINING ON SERUM RESISTIN OF NON-ATHLETE IRANIAN MALE STUDENTS

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ABSTRACT

*Given the importance of physical exercise in human health and prevention of cardiovascular disease among people, the purpose of this pretest-posttest quasi-experimental study is to evaluate the effect of physical exercise followed by a detraining period on serum resistin of non-athlete male students in Iran. 39 subjects were selected randomly from 339 non-athlete male students in Zahedan, Iran who had chosen physical education course volume one in 2013-2014 school year, divided into the three groups of 13 including endurance, sprint, and control. They exercised separately with a specified durations and intensity three times per week for ten weeks and then after 48 hours, they entered a four-week detraining period. To assess the effects of endurance and sprint training on serum resistin , blood sampling was carried out in 3 stages for 12-hour overnight fasting before starting and 48 hours after completion of training. In this respect, body mass index (BMI), Waist to hip ratio (WHR), subcutaneous fat percentage, and maximal oxygen uptake (vo2max) were measured. For analysis, statistical test were used: One-Way ANOVA for comparing within-group mean differences and Least Significant Difference (LSD) post hoc test for between-group mean differences. Obtained results showed that the serum resistin level in endurance and sprint groups were significantly reduced compared to control group (p<0.05), and after four-week detraining, its amount increased again. Also we found out that after ten-week training, BMI, WHR, weight, and subcutaneous fat of two endurance and sprint groups significantly decreased in comparison with control group (p<0.05), while Vo2max in endurance group was significantly increased. We concluded that ten-week endurance and sprint exercises can be a nonpharmacological method for the prevention of cardiovascular diseases.
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INTRODUCTION

Physical exercises are considered as one of the strategies to improve cardiovascular health. Regular exercises are often recommended, but the impact of these exercises on cardiovascular risk factors, such as resistin is not well known [1]. Resistin plays an important role in obesity-related diseases by regulating metabolic processes and the immune system Resistin was discovered in 2001 by Steppen et al. [2]. It was called "resistin" because of the observed insulin resistance in mice injected with resistin. The human resistin gene is located on chromosome 19p13 and with a molecular weight of 12.5 kDa, is formed of 108 amino acids as cysteine-rich pre-peptide, and its various isoforms has been reported [3,4]. Also, resistin, by impairing glucose metabolism, leads to an increased risk of atherosclerosis. Human studies have shown that because of low resistin expression in human adipocytes, its role as an important factor linking obesity with insulin resistance is not fully understood, because there are a lot of resistin protein in the human circulatory system, and may be released from these cells into serum [5]. It has been shown that the lack of physical activity increases the risk of obesity and Inactive lifestyle is also associated with increased risk of insulin resistance [6]. Given that the impact of physical exercise metabolism in obesity and insulin resistance has been a lot of attention; however, there are limited information about the effects of physical activity on insulin resistance-causing mechanisms and their relationship with each other. In addition, it is unclear whether changes in resistin may be due to changes in insulin resistance after aerobic exercise or not. The studies that have been done in the last decade about the relationship between resistin and insulin resistance have shown contradictory results. Some research have shown a direct association, some shown no association, and even some studies have shown an inverse association of these hormones with insulin resistance [7,8].Jones et al. [9] observed a significant decrease in serum resistin and no change in insulin resistance after eight- month exercise in overweight adolescents. Kadoglou et al. [10] and Liu et al. [11] showed a significant reduction in both resistin and insulin resistance after aerobic trainings. In contrast, Monzillo et al. [7] observed increased insulin sensitivity and no change in resistin after 6-month program consisting of combined hypocaloric diet and moderate physical activity. Thus, according to several studies and different results obtained in the past and given the importance of the resistin in the human body, and also since research on endurance and sprint training and detraining period in non-athletes is less, in the current study, our purpose is to investigate the effect of ten- week strength and sprint training and a 4-week detraining period on resistin levels of the non-athlete male students in Iran.

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PHYSIOLOGY-FITNESS

MATERIALS AND METHODS

In this pretest-posttest quasi-experimental study, 39 subjects were selected randomly from 339 non-athlete male students who had chosen physical education course volume 1 in school year 2013-2014, and divided into the three groups: endurance group (n=13), sprint group (n=13), and the control group (n=13). The inclusion criteria were: no regular physical activity, and no history of hospitalization and specific diseases and surgery. A written consent was taken from participants. One day before the start of training protocols and 12 hours of overnight fasting, in an upright position 10 cc blood was collected from antecubital vein in the right arm of subjects. After centrifugation for 5 min at 4000 rpm, serum was isolated from plasma and kept in micro-tubes in the laboratory at 20 ° C. Then endurance and sprint training protocol was administered for 10 weeks, according to [Table 1] [12, 13]. 48 hours after the last training session, in similar conditions blood sampling was repeated, and then participants entered a four-week detraining period. During this period, they had no physical activity and they just were doing their own chores. After 4 weeks, the final blood sampling was taken again in previous conditions.

Enzyme-linked immunosorbent assay (ELISA) technique was employed for measuring resistin by using the kit made by Boster Germany. The size of the anthropometric indices such as height, weight, body mass index (BMI), Waist to hip ratio (WHR), subcutaneous fat percentage, maximal oxygen uptake (vo2max) were measured in experimental conditions. Subcutaneous fat thickness of subjects was measured using a caliper (Yeongdeok-dong, Korea) at three points (below the scapula, abdominal and triceps) on the right side of the body. Measurement of each point was repeated three times, and then the average was obtained and compared by its formula. Vo2max also was measured by Bruce protocol on Runrace HC1200 treadmill.

To test the research hypotheses, first, In order to investigate the homogeneity of research data, the Kolmogorov-Smirnov test was used. Afterwards, One-Way ANOVA was used to evaluate the within-group mean differences, and Least Significant Difference (LSD) post hoc test was employed for between-group mean differences. All statistical analyzes were performed using SPSS version 17 and significance level was set as 0.05.

Table 1: Endurance and sprint training program

Training weeks		1	2	3	4	5	6	7	8	9	10
Endurance training	Time (min)	20	22	24	26	28	30	32	34	36	38
	Vo_2 max	50%	50%	55%	55%	60%	60%	65%	65%	70%	70%
Sprint training	Meter (M)	30	30	30	30	30	30	30	30	30	30
		60	60	60	60	60	60	60	60	60	60
		100	100	100	100	100	100	100	100	100	100
	Repeat	3	3	5	5	7	7	9	9	11	11
		2	2	3	3	4	4	5	5	6	6
		1	1	1	1	1	1	1	1	2	2
	Vo_2 max	95%	95%	100%	100%	100%	100%	100%	100%	100%	100%

RESULTS

[Table -2] shows the mean and standard deviation of the 39 subjects' age, height, weight, BMI, and WHR in three groups. Mean and standard deviation of resistin before and after training are presented in [Table 3]. As can be seen, changes in serum resistin values in endurance and sprint groups were significantly reduced after ten weeks of endurance (1.22 ± 0.08 ng/ ml) and sprint training (0.97± 0.06 mg/ ml) (P≤0.05). After four weeks of detraining, these values were significantly increased as 1.35±0.11 and 1.1 7 ± 0.06 ng/ml, respectively. The results of change in anthropometric factors [Table-4] showed that after ten-week training, BMI, WHR, weight, and subcutaneous fat of two endurance and sprint groups significantly decreased in comparison with control group (p≤0.05). After four weeks of detraining, these indices increased. Also, Vo2max in endurance group at the end of ten-week training was significantly increased (29.08 ± 0.97 ml/kg/min).

DISCUSSION

Many studies have shown that a regular physical activity has known cardiovascular benefits which reduce the prevalence of cardiovascular disease healthy people and patients [14], because physical exercise increases muscle mass and strength on the one hand, and on the other hand it will increase energy consumption and oxidation [15]. Regular endurance exercise with several mechanisms can increase blood

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volume, stroke volume, maximum energy consumption, and reduce the fat percentage and maximum energy consumption which directly affects the cardiovascular system. Increased plasma volume is one of the most important changes caused by the effect of endurance training. With the increase of plasma volume, blood volume also increases and as a result more blood enters the heart, and stroke volume and cardiac output is increased. These factors increase the maximum oxygen consumption. Endurance exercise increases the plasma volume through two processes: (a) with increasing secretion of antidiuretic hormone (ADH) and aldosterone; it causes water retention in the kidneys and hence, plasma volume increases, (b) With increasing plasma proteins, especially albumin, blood osmotic pressure increases and therefore more fluid stays in the blood. As a result of these two processes, the liquid part of blood (plasma) increases, and blood viscosity decreases [16-19]. In our study, endurance training reduced average body weight, BMI, WHR, and percentage of subcutaneous fat while Vo2max increased. Also, our results showed that endurance training reduced resistin level significantly. Our results are consistent with the results of [9-11], [20, 21], but is against the findings of [22]. Park et al. [21] demonstrated that twelve-week aerobic exercise can reduce serum resistin in obese middle-aged women. Jones et al. [9] after eight-month endurance training, observed the significant reduction of serum resistin but found no changes in insulin resistance of overweight adolescents. Kadoglou et al. [10] and Liu et al. [11] also showed that after endurance training resistin and insulin resistance reduces significantly. Rashidlamir et al. [20] examined the effects of eight weeks of aerobic exercise on the concentrations of fibrinogen and resistin in healthy overweight middle-aged men in Iran. They observed increased resistin due to endurance training and concluded that pro-inflammatory cytokines such as IL-1beta, TNF-alpha, and IL-6 stimulate resistin gene expression in peripheral blood mononuclear and increase the resistin. They attributed the increase in resistin after endurance exercise to the role of this hormone in Oxidative defense of the body, and stated that resistin in response to inflammatory stimuli acted as an antioxidant, is secreted from blood mononuclear cells in response to mild inflammation. On the other hand, Perseghin et al. [22] studied serum resistin in 23 elite athletes (sprinters, middle-distance and marathon runners) and in 72 sedentary men including lean and obese individuals and showed that endurance athletes had high resistin levels than sedentary men. Increased resistin was detected in the middle-distance and marathon runners, but not in the sprinters when compared with sedentary men.

There are also some studies that implied no change in serum resistin after endurance training. For example, in the study of Abbasi et al [23] conducted on obese middle-aged men in Iran, anthropometric parameters in the experimental group were significantly reduced following the intervention of three month exercise training, but insulin resistance and serum resistin remained unchanged. In this regard, they suggested that physical activity in the absence of diet has no effect on serum resistin and insulin resistance of these men. Sliwicka et al [24] in a study of ten young male triathletes, reported that the effect of systematic training on basal adipokine and resistin concentration was in a small extent, however, acute exercise may affect the response of these molecules Haghighi et al. [25] showed that that nine-week aerobic training has no significant effect on serum resistin and adiponectin in obese Iranian women; however, aerobic training significantly reduced BMI, WHR, and body fat percentage, and caused significant increase in lean body weight and Vo$_2$max.

With respect to serum resistin level in detraining period, our study showed significant difference and in this period resisitin was significantly increased. This is consistent with the findings of [26] and [27]. Zarifi et al. [26] suggested that after 8 weeks of detraining, resistin levels increase. Siahkohian et al. [27] concluded that detraining reduce performance and increase the level of resistin.

The results of the current study indicated that serum resistin level of non-athlete male students in pretest period and after ten-week sprint exercise followed by four weeks of detraining showed a significant difference ($p \leq 0.05$).). This level decreased significantly after ten-week sprint exercise and increased again in detraining period. In relation to this result of the research, due to the novelty of the subject, a similar or inconsistent study was not found.

Table 2: General characteristics of subjects

Group	Age (Year) (M ± SD)	Weight (kg) (M ± SD)	Height(cm) (M ± SD)	BMI(kg/m2) (M ± SD)	WHR (M ± SD)
Endurance	21.41 ± 2.15	69.45 ± 1.18	171 ± 4.27	23.16 ± 4.17	0.86 ± 0.06
Sprint	20.58 ± 1.56	68.45 ± 1.17	170 ± 5.21	22.66± 2.96	0.82 ±0.05
Control	20.66 ±1.82	69.58 ± 1.12	175 ±7.33	22.16 ± 2.28	0.81 ± 0.09

Table 3: The mean and standard deviation of resistin of subject in three groups and three stages

Variable/ group		Before training (M ± SD)	10-week training (M ± SD)	4-weeks detraining (M ± SD)
Resistin (ng/ ml)	Endurance	1.77 ± 0.15	1.22 ± 0.08	1.35 ± 0.11
	Sprint	1.53 ± 0.12	0.97 ± 0.06	1.17± 0.06
	Control	1.28± 0.02	1.30 ± 0.02	1.31 ± 0.03

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Table 4: Results of changes in anthropometric factors of subjects in three groups and three stages

Variable	Group	Before training (M ± SD)	10-week training (M ± SD)	4-week detraining M ± SD
Weight	Endurance	69.57 ±1.18	*67.05 ± 1.02	*67.67 ± 1.03
	Sprint	67.95 ± 1.10	**66.00 ± 1.01	*66.52 ± 1.03
	Control	69.58 ±1.12	69.33 ± 1.13	69.16 ± 1.13
BMI	Endurance	23.16± 4.17	**22.00 ± 3.66	*22.37 ± 3.84
	Sprint	22.66 ± 2.96	**22.00 ± 2.69	*22.39 ± 2.60
	Control	22.16 ± 2.28	21.75 ± 2.05	21.91 ± 2.23
WHR	Endurance	0.86 ± 0.01	*0.84 ± 0.01	*0.87 ± 0.01
	Sprint	o.87± 0.01	*0.86 ± 0.02	0.88 ± 0.01
	Control	0.81 ± 0.02	*0.81± 0.03	0.81 ± 0.02
Subcutaneous fat	Endurance	0.08 ± 0.01	*0.05 ± 0.01	0.07 ± 0.01
	Sprint	0.07 ± 0.009	*0.06 ± 0.006	0.07 ± 0.008
	Control	0.07 ± 0.005	*0.07 ± 0.005	0.07 ± 0.005
Vo$_2$max	endurance	24.33± 0.96	*29.08 ± 0.97	26.16 ± 0.96

* p$\leq$0.05 (within group), ** p$\leq$0.05 (between group)

CONCLUSION

The aim of this study was to investigate the effect of ten-week physical exercise on serum resistin of non-athlete male students (n=39, with mean weight of 69.45 ± 1.18, 68.45 ± 1.17, and 69.58 ± 1.12 kg in three endurance, sprint, and control groups, respectively). Results showed that the serum resistin level in

endurance and sprint groups were significantly reduced compared to control group (p$\leq$0.05), and after four-week detraining, its amount increased again. In this regard, we can conclude that ten-week endurance and sprint exercises can be a good, cost-effective and non-pharmacological method for the prevention of cardiovascular diseases.

CONFLICT OF INTEREST
There is no conflict of interest.

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ARTICLE

IMAGE ENHANCEMENT BASED ON DISCRETE WAVELET TRANSFORM

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ABSTRACT

In this paper, a new satellite image resolution enhancement technique based on the interpolation of the high frequency sub bands obtained by discrete wavelet transform (DWT) and the input image is proposed. The projected resolution improvement technique uses DWT to decay the input image into different sub bands. Then, the high frequency sub band images and the input low resolution image have been interpolated, followed by combining all these images to generate a new resolution enhanced image by using inverse DWT. In order to achieve a sharper image, an intermediate stage for estimating the high frequency sub bands has been proposed. The proposed technique has been tested on satellite benchmark images. The quantitative (peak signal to noise ratio and root mean square error) and visual results show the superiority of the proposed technique over the conventional and state of art image resolution enhancement techniques.
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INTRODUCTION

Satellite images are used in many applications such as geosciences studies, astronomy, and geographical information systems. One of the most important quality factors in images comes from its resolution. Interpolation in image processing is a well known method to increase the resolution of a digital image. Interpolation has been widely used in many image processing applications such as facial reconstruction, multiple descriptions coding, and resolution enhancement. A new framework for statistical signal processing based on wavelet-domain hidden Markov models (HMM's) that concisely models the statistical dependencies and non-Gaussian statistics encountered in real-world signals is developed in [1]. Efficient expectation maximization algorithms are developed for fitting the HMM's to observational signal data.

A wavelet domain image resolution enhancement algorithm is developed in [2]. A primary high-resolution approximation to the original image is obtained by means of WZP and is further processed using the CS methodology which reduces ringing. An efficient edge algorithm is used for the description of edge degradations such as blurring due to loss of resolution. Linear regression using a minimal training set of high-resolution originals is finally employed to rectify the degraded edges. A multiple description image coding scheme is proposed to facilitate the transmission of images over media with possible packet loss and is based on finding the optimal reconstruction filter coefficients that will be used to reconstruct lost descriptions in [3].

For this purpose initially, the original image is down sampled and each sub image is coded using standard JPEG. These decoded images are then mapped to the original image size using the optimal filters. Interpolation of the high-frequency sub band images obtained by dual-tree complex wavelet transform (DT-CWT) is proposed in [4]. DT-CWT is used to decompose an input low-resolution satellite image into different sub bands. Then, the high-frequency sub band images and the input image are interpolated, to generate a new HR image by using inverse DT-CWT. The resolution enhancement is achieved by using directional selectivity provided by the CWT, where the high-frequency sub bands in six different directions contribute to the sharpness of the high-frequency details such as edges. Super resolution is used for resolution enhancement of images or video sequences. Instead of super resolving frames globally, using localized motion based super resolution increases the quality of the enhanced frames. The super resolution on different sub bands of localized moving regions extracted from discrete wavelet transform (DWT) and composing the super resolved sub bands using inverse DWT (IDWT) to generate the respective enhanced high resolution frame in [5].

MATERIALS AND METHODS

The proposed Image Enhancement system is based on DWT. In this following section the theoretical background of all the approaches are introduced.

Discrete Wavelet Transform

Nowadays, wavelets have been used quite frequently in image processing and used for feature extraction, de-noising, compression, face recognition, and image super-resolution. The decomposition of images into different frequency ranges permits the isolation of the frequency components introduced by "intrinsic deformations" or "extrinsic factors" into certain sub-bands. This process results in isolating small changes in an image mainly in high frequency sub-band images.

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The 2-D wavelet decomposition of an image is performed by applying 1-D DWT along the rows of the image first, and, then, the results are decomposed along the columns. This operation results in four decomposed sub-band images referred to as low–low (LL), low–high (LH), high–low (HL), and high–high (HH). The frequency components of those sub-band images as shown in Figure 1 (b) cover the frequency components of the original image in Fig. 1 (a).
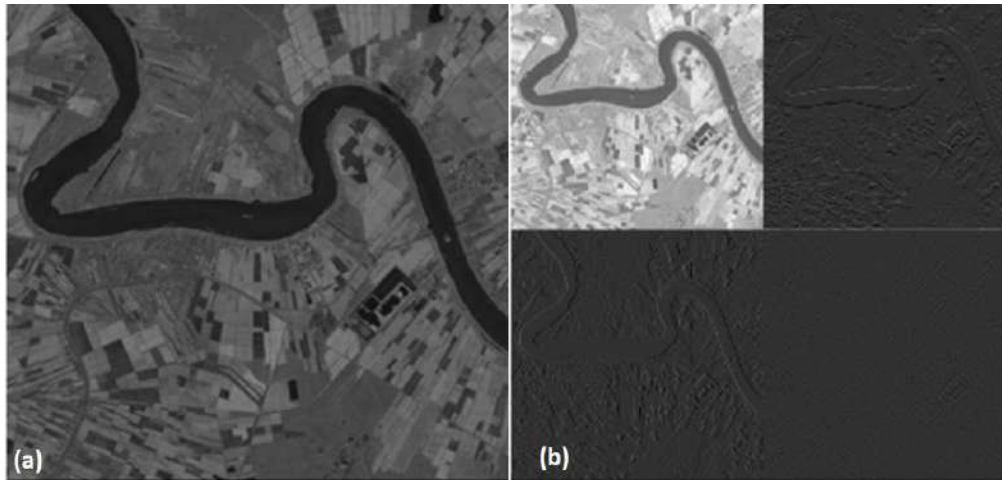

Fig. 1(a): Input Image **(b)** 2-D Wavelet decomposition

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Interpolation Techniques

Interpolation is the process of estimating the values of a continuous function from discrete samples. Image processing applications of interpolation include image magnification or reduction, sub pixel image registration, to correct spatial distortions, and image decompression, as well as others. Of the many image interpolation techniques available, nearest neighbor, bilinear and cubic convolution are the most common, and will be talked about here. Since, Interpolation provides a perfect reconstruction of a continuous function, provided that the data was obtained by uniform sampling at or above the Nyquist rate. Since Interpolation does not give good results within an image processing environment, since image data is generally acquired at a much lower sampling rate. The mapping between the unknown high-resolution image and the low-resolution image is not invertible, and thus a unique solution to the inverse problem cannot be computed. One of the essential aspects of interpolation is efficiency since the amount of data associated with digital images is large.

Bilinear Interpolation

Bilinear Interpolation determines the grey level value from the weighted average of the four closest pixels to the specified input coordinates, and assigns that value to the output coordinates. First, two linear interpolations are performed in one direction and then one more linear interpolation is performed in the perpendicular direction. For one-dimension Linear Interpolation, the number of grid points needed to evaluate the interpolation function is two. For Bilinear Interpolation (linear interpolation in two dimensions), the number of grid points needed to evaluate the interpolation function is four [6].
For linear interpolation, the interpolation kernel is:

$$u(s) = \{0 \mid s \mid > 1$$
$$\{1 - \mid s \mid \mid s \mid < 1$$

where, is the distance between the point to be interpolated and the grid point being considered. The interpolation coefficients

Bicubic Convolution Interpolation

Cubic Convolution Interpolation determines the grey level value from the weighted average of the 16 closest pixels to the specified input coordinates, and assigns that value to the output coordinates. The image is slightly sharper than that produced by Bilinear Interpolation, and it does not have the disjointed appearance produced by Nearest Neighbour Interpolation. First, four one-dimension cubic convolutions are performed in one direction and then one more one-dimension cubic convolution is performed in the perpendicular direction. This means that to implement a two-dimension cubic convolution, a one-dimension cubic convolution is all that is needed. For one-dimension Cubic Convolution Interpolation, the

number of grid points needed to evaluate the interpolation function is four, two grid points on either side of the point under consideration. For Bicubic Interpolation (cubic convolution interpolation in two dimensions), the number of grid points needed to evaluate the interpolation function is16, two grid points on either side of the point under consideration for both horizontal and vertical directions [7] .
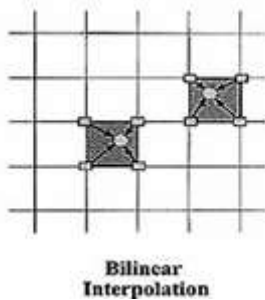

Fig. 2: Bilinear Interpolation

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RESULTS

DWT has been employed in order to preserve the high-frequency components of the image. DWT separates the image into different sub and images, namely, LL, LH, HL, and HH. High-frequency sub-bands contain the high frequency component of the image. The interpolation can be applied to these four sub band images. In the wavelet domain, the low-resolution image is obtained by low-pass filtering of the high-resolution image as in, and. The low resolution image (LL sub band), without quantization (i.e., with double-precision pixel values) is used as the input for the proposed resolution enhancement process.

In other words, low frequency sub band images are the low resolution of the original image. Therefore, instead of using low-frequency sub band images, which contains less information than the original input image, we are using this input image through the interpolation process. Hence, the input low-resolution image is interpolated with the half of the interpolation factor, α/2, used to interpolate the high-frequency sub bands. In order to preserve more edge information, i.e., obtaining a sharper enhanced image, we have proposed an intermediate stage in high frequency sub band interpolation process. As shown in Fig. 5, the low-resolution input satellite image and the interpolated LL image with factor 2 are highly correlated. The difference between the LL sub band image and the low-resolution input image are in their high-frequency components.

Hence, this difference image can be use in the intermediate process to correct the estimated high-frequency components. This estimation is performed by interpolating the high-frequency sub bands by factor 2 and then including the difference image (which is high-frequency components on low-resolution input image) into the estimated high-frequency images, followed by another interpolation with factor α/2 in order to reach the required size for IDWT process.

The intermediate process of adding the difference image, containing high-frequency components, generates significantly sharper and clearer final image. This sharpness is boosted by the fact that, the interpolation of isolated high-frequency components in HH, HL, and LH will preserve more high-frequency components than interpolating the low-resolution image directly. Certain improvement in the paper is also made actually the paper speaks about the gray scale image but here we use the same principle to the RGB image.

DISCUSSION

The quantitative (peak signal to noise ratio and root mean square error) and visual results show the superiority of the proposed technique over the conventional and state of art image resolution enhancement techniques.

Table 1: Relationship between MSE and PSNR values

Name of the image	MSE	PSNR(db)
Sample.jpg	0.47514	51.3626
Sample1.jpg	0.2817	53.633
Sample2.jpg	0.3544	52.525
Sample3.jpg	0.45554	50.875

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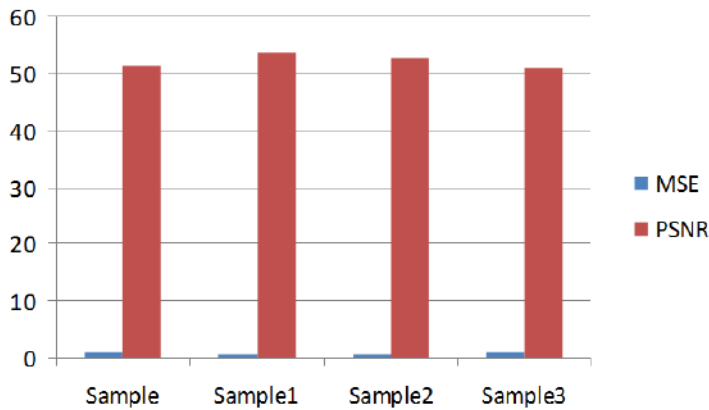From the Table 1, it is clear that lower the MSE value, higher the PSNR value.

Fig. 3: Graphical Representation of MSE and PSNR

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CONCLUSION

This paper has proposed a new resolution enhancement technique based on the interpolation of the high-frequency sub band images obtained by DWT and the input image. The proposed technique has been tested on well-known benchmark images, where their PSNR and MSE and visual results show the superiority of the proposed technique over the conventional and state-of-art image resolution enhancement techniques

CONFLICT OF INTEREST
There is no conflict of interest.

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ARTICLE

EFFECT OF STRESS ON THE NURSES' WORK LIFE QUALITY IN PSYCHIATRIC WARD

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ABSTRACT

Background: One of the factors influencing the quality of nurses' work life is stress. Therefore, the use of preventive and efficient practices to manage stress seems essential. So the purpose of this study is to investigate the effect of Stress Inoculation Program on the quality of the nurses' work life in the psych ward department. **Methods:** This study was a clinical trial type whose research units were constituted by 60 nurses and the healthcare working in Ibn Sina Hospital of Mashhad city who were divided into 2 groups based on random allocation. Data collection tool included a questionnaire of work life quality completed in three stages: before the intervention, at the end of the second session and one month after the intervention. The intervention included Stress Inoculation Program, consisting of workshop for 2 days and 8 hours each day with one week interval that was conducted for the intervention group. **Results:** The findings showed that the average score of work life quality of the " Stress Inoculation" group changed from $80/01 \pm 13/18$ at the pre-intervention stage to $86/71 \pm 18/72$ after the intervention and $88/32 \pm 17/26$ in follow up stage of one month later, which significantly is more than the control group ($p<0.005$). In the control group, the average score of work life quality also significantly reduced at the post-intervention stage and follow up one month later comparing with that of at the pre-intervention stage ($p=0.000$). **Conclusion:** Since Stress Inoculation Program is a cheap, safe, and effective way to improve the quality of work life for nurses, it is suggested to be used in order to improve the quality of cares.

INTRODUCTION

The main key to success of managers in achieving organizational goals and giving life back to the organization is to improve the work life quality of employees [1]. Moorhead and Griffin defined quality of working life as "the employees' ability to satisfy their own important personal needs using the experiences that have gained in the organization" [2]. In fact, the work life quality means that the organization provides a work atmosphere in which there is excellent performance, full participation, personal, professional, and organizational development and professional satisfaction [3].

In any organization, high quality of work life is essential to attract and maintain the employees but this issue has a particular importance in sustainability of the healthcare system particularly in attracting and maintaining nursing staff [4]. Kettle also states that any health system should focus on work life quality of nurses of that organization [5]. However, health care organizations have rarely investigated methods to increase efficiency in personnel levels and lots of efforts by managers to create the latest changes have been dysfunctional; so that the results of some research suggests that work life quality of nurses has acutely been disrupted and the increased medication errors have led to morbidity, mortality and dissatisfaction of patients [6]. Work life Quality of nurses is under the influence of demographic, social, cultural, environmental and field features [7]. In the meantime, another factor affecting the quality of life about which many studies have been done is stress [8]. A review of stress in workers of different health care wards in 17 countries showed that nurses experience more stress in most countries, and though they lack appropriate coping resources, they are at risk of occupational burnout more than other people and this can lead to physical and mental illness and ultimately reduce their quality of their life [9,10]. According to various studies, there is an inverse relationship between stress and quality of life [11].

Psychiatric nurses are encountered with more stressful factors due to the lack of organized structure in psyche wards, inadequate professional training and unpleasant psychological environment governing the interaction among therapeutic team members [12]. Researchers believe that the quality of work life has significant impact on nurses' reactions such as organizational identity, job satisfaction, work participation, effort and professional performance [13].

Given the key role of nurses in taking care of patients with mental disorders, paying attention to their work life quality and efforts to improve it are essential; Because nurses can favorably provide care to patients if they themselves are at a desirable level in terms of different aspects of life and work [14]. On the other hand, since the stress affects the nurses' work life quality, stress management programs can probably be useful to improve their life quality and thus maintain and improve quality of care

Recently, researchers have found in medical fields that it is better for intervention to be conducted in a preventive approach for stress management. These types of interventions to reduce stress are based on the important role of cognitive intermediaries in the development and maintenance of stress [15]. One of

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the preventive methods of management is Stress Inoculation Program, which has been proposed by Michenbum [16]. Stress inoculation as medical immunization is a cognitive-behavioral application created based on a person's exposure to stressful conditions in order to form psychological antibodies or skills to deal with stress [17]. The program includes three stages: conceptualization, acquisition and practicing the skills. At the first stage, the main focus is on making relation based on cooperation with the individuals and helping them better understand the nature of stress and its effects on excitement and operation. At the second stage, the person's skills of dealing are reinforced and new skills are also taught to him. At the third stage, the individual uses his skills to increase the ability to deal with any kind of stress as well as stronger stresses [18]. The principles of Stress Inoculation Program are that the individuals should change their beliefs about stressful behaviors as well as their self-talk about the ways of dealing with stress [19]. So far, many studies have shown the effectiveness of this method on reduction of pain, agitation, and depression and on development of professional operation [20]; it has also been reported that Stress Inoculation is more effective than methods such as cognitive behavioral therapy and support groups in controlling stress [21].

Despite the extensive search carried out, studies that measure the effect of inoculation on life quality of nurses were not found the researchers are planning to investigate whether stress inoculation program is effective on the work life quality of the nurses in psych ward?

MATERIALS AND METHODS

This clinical trial study was conducted in 1390 on the nurses and paramedics working in Avicenna psychiatric hospital in Mashhad. The study inclusion criteria were possessing an associate, undergraduate, or graduate degree in nursing, or a diploma in medic works employment at Avicenna hospital at least from of 6 months ago, lack of consuming effective psychiatric drugs, no days off more than two weeks in the past month and exclusion criteria were absence during more than 10% of the time in the workshop, participating in other stress management workshops, discharge or transfer to other hospitals and exposure major to stress (death of someone close, divorce, etc.) when running the study.

The hypothesis of this study: Stress Inoculation Program increases work life quality of psych ward nurses. The sample size of this study achieved using the proportions formula

$$\left(n = \frac{\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2 \left(s_1^2 + s_2^2\right)}{\left(\bar{x}_1 - \bar{x}_2\right)^2} \right.$$

and with confidence coefficient of 95%, accuracy of 05% and test power of 80%, and 25 patients in each group which given the possibility of research units loss, 32 patients were considered for each group (64 people total). After coordination with the educational supervisor for conduction of the intervention, ads to register for the Stress Inoculation workshop were installed in different wards of the hospital; From among 72 nurses and 32 hospital paramedics, 64 eligible people for the study voluntarily registered to participate in the research. After homogenization in terms of work shift and responsibilities, the participants were randomly divided into two groups of test (n = 32) and control (n = 32) on the basis of registration number. Finally, with the loss of research units, 60 people (30 in the test group and 30 subjects in the control group) participated in the study.

The tools used in this study included demographic characteristic form and work life quality questionnaire of Dargahi and colleagues. Demographic characteristics questionnaire included information 16 questions about individual and occupational characteristics that was provided according to the objectives of the research and studying the latest resources and related articles. Work life quality questionnaire contains 30 questions and each question was regarded as a component related to the quality of work life. Scoring each phrase was as completely dissatisfied phrases (1), dissatisfied (2), no comment (3), satisfied (4) or quite satisfied (5). In this scale, the minimum score for the satisfaction of life quality is 30 and the maximum is 150. The higher score obtained from this questionnaire indicates more satisfaction with the quality of work life. Also, the score of work life quality was classified in three levels of less than 90 as unfavorable, 120-90 as relatively favorable and more than 120 as favorable. The validity of these questionnaires was certified by 10 expert professors of Mashhad University of Medical Sciences. The reliability of the questionnaire of work life quality has been calculated by the port and colleagues test-retest method and its internal consistency, and Alpha Cronbach correlation coefficient was obtained as 0/7 and 0/88 respectively. In this study, the reliability of this questionnaire was evaluated in the internal consistency method in that the Cronbach alpha coefficient was 0/81. Data collection was conducted in both groups simultaneously in three stages, before the intervention, the end of the second session and one month after intervention. After the adoption of the University Research Council and approval by the ethics committee, the intervention was held according to "stress inoculation" stages by a researcher and clinical psychologist cooperation through group discussion and mental imagery for intervention group during two sessions (each session lasting 4 hours) within a week time pace of each other. The first session included an explanation about the nature and consequences of stress, identifying common coping skills among the participants and relaxation training. At the end of the first session, participants were given practical

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assignments to do during the week and recorded the stressful factors if encountered during the week, also practice relaxation technique daily. In the second session, time management and cognitive restructuring techniques were taught to the participants. Then, using mental imagery, the participants were confronted with stressful factor. Research units were followed for one month after the intervention. During this time, the researchers also contacted with the participants of the intervention group by telephone once every two weeks were and carried out necessary strengthening and Follow-up in relation to following the training program. In order to comply with ethical considerations and take advantage of the content of the workshop and the privilege of re-education by the control group, inoculation program was also performed for the control group two months after the end of the study in the same manner for the intervention group.

SPSS software version 11.5 was used to analyze the data. The results of Kolmogorov - Smirnov and Shapiro Wilke tests showed that quantitative variables in this study had normal distribution; to describe demographic information and personal characteristics, the mean, standard deviation, and frequency distribution table (relative, absolute) were used. To study the homogeneity of the two groups in terms of qualitative variables, chi-square and independent T tests were used. Pre- and post-intervention of control and test group (intergroup) in terms of work life quality was carried out using test of variance analysis with repeated measures and the quality of work life between the two groups was compared by independent T test. The relationship between the impact of inoculation program on the quality of work life and personal characteristics was performed using bilateral variance analysis test. In the tests conducted, the confidence coefficient and significance level were considered as 95 percent a = 0.05 respectively.

RESULTS

The majority of research units in both groups (50% in the intervention group and 73/31% in the control group) were male and their mean age was 36 years. The research units in both inoculation and the control group were married. 96/71% of the research units in each group had a bachelor's degree. The average work experience of participants in the psych wards was 90/9 years. In terms of employment status, the majority of research units in both groups (61/58% in inoculation and 32/63% in the control group) were contract employed. In terms of all field variables, there was no significant difference between the two groups and they were homogeneous [Table 1].

Table 1: Compare the Background variables in the intervention and control groups

Background variables	Groups		Test result	
	control	intervention		
	number (percentage)	number (percentage)		
Gender	22 (73.3)	15 (50)	male	Chi-square 0.63
	8 (26.7)	15 (50)	female	
Age (mean ± standard deviation)	6.42±35.41	7.65±36.50		independent T 0.567
marital status	4 (12.33)	3 (10.0)	single	Chi-square 0.688
	26 86.7	27 (90.0)	married	
Employment Status	12 (43.3)	11 (36.7)	Official	Chi-square 0.713
	18 (56.7)	19 (63.3)	Contractual	
Work experience in psych ward (mean ± standard deviation)	5.21±4.65	7.12±6.61	independent T 0.816	

In terms of work life quality at the stage before intervention, there was no statistically significant difference between the two groups and both groups were homogenous in this respect. This average got increased by a rate of 6/7 score (5/6%) in the inoculation group at pos-intervention stage and by the rate of 8/3 score (6/9%) at follow-up stage one month later. As well, in the control group, the average score of work life quality decreased from 83/41 ± 11/31 score before intervention to 83/40 ± 11/50 score after the intervention and 82/72 ± 10/91 score in the fellow-up stage of one-month later. Independent t-test results (comparison between groups) showed that the score of work life quality in the intervention group had increased significantly compared to the control group after the intervention stage (p = 0.002), as well as the stage of follow-up one month later (p = 0.000). Also comparison of the mean score of work life quality in the stages before and after intervention and follow-up one month later in the inoculation group (within group comparison) by variance analysis test and with repeated measures showed that there is a significant difference between the three stages (p = 0.000). The same test showed the difference between pre-intervention and post-intervention stages as (p = 0.000), between post-intervention and one month after the intervention as (p= 0.000) and also between pre-intervention and follow-up one month later

stage as (p= 0.000) that had an upward movement. In the control group, the results of variance analysis test with repeated measures (within group comparison) also showed that the average score of work life quality in the stages before and after intervention and follow-up one month later, has a significant difference (p= 0.000). The same test showed this difference between post-intervention and follow-up one month later stages as (p = 0.000), between pre-intervention and one month after the intervention as (p= 0.000) that had a downward movement [Table 2].

In terms of life quality levels, 26/7% group members of inoculation program in the pre-intervention, 36/7% in post-intervention and follow-up one month after intervention stages had a relatively good life quality. Also, 23/3% members of control group in the pre-intervention and post-intervention stages, 20/7% in follow-up one month later stage had a relatively good life quality. None of the subjects participating in this study were at the optimum level in terms of work life quality.

Bilateral ANOVA statistical test results showed that none of the background variables studied had significant effect on variation in quality score of work life in the pre-test and follow-up a month later stages.

Table 2: Compare the mean score of work life quality of nurses in psych ward, studied in the stress inoculation program and control groups, at the stages pre-intervention, post-intervention, and follow-up one month later

P-Value[#]	Group						Stage
	Total		Inoculation program		control		
	number	mean ± standard deviation	number	mean ± standard deviation	number	mean ± standard deviation	
0.359	60	12.6 ±81.7	30	13.8 ±80.0	30	11.3 ±83.4	pre-intervention
*0.002	60	15.3 ±85.1	30	18.7 ±86.7	30	11.0 ±83.3	post-intervention
*0.000	60	14.8 ±85.5	30	17.6 ±88.3	30	10.9 ±82.7	follow-up one month later
			0.000=p		0.000=p		Test result Repeated Measures

#: Independent t-test*: p<0.05 was considered significant.

DISCUSSION

Findings of this research showed that the average of work life quality in the intervention group increased significantly after intervention and these changes was preserved till after one month. This indicates the impact of stress inoculation program on the work life quality of nurses in psych wards. Given that, despite an extensive search, no studies were found that have measured the impact of the inoculation program or other stress management techniques on the work life quality, the findings of this study were compared with the studies which have measured other interventions on the quality of work life or the effect of stress management interventions on quality of life. The study findings by Dargahi and colleagues (2006) showed that one of the factors affecting the work life quality of nurses is occupational stress and lack of transparency about their job prospect [22]. In this study, the implementation of stress inoculation program also helped increase the work life quality of nurses. Stress inoculation program has a flexible and proactive approach. In this program, patients are taught coping skills and the duration and mode of implementation of the program would vary based on positioning and living condition of individuals. The program teaches the person how to prevent from exposure to stressful conditions as a result of his interaction with the environment by controlling his emotions, behavior, and performance [21].

Results of the study by Hamidi et al (2013) also showed that the work life quality and perceived stress have a negative relationship with each other, that's quality of working life comes down by increasing the perceived stress, and reduction of the level of perceived stress increases the quality of working life and this is consistent with the findings of our study [23]. Konnert and et al (2009) also suggest that the implementation of cognitive-behavioral group therapy among nurses is cost effective and stress coping program, decrease in negative thoughts and increase in happy and breezy events caused a significant improvement in the group of nurses who were exposed to these programs [24]. Stressinoculation program will not only help the person deal with the problems and stressors advantageously, but also have a more positive cognitive assessment of themselves due to successful behave in dealing with these factors, and will consequently experience more positive excitements and affections and their self-confidence will increase [25]. In the study by Elizur titled as "work life quality and its relation to life quality", the systematic investigation of these two concepts showed that psychological factors such as perceived stress of individuals influence on their coherent and adaptive performance and ultimately affect their quality of work life and quality of life [26]. Since one of the causes of stress is the assessment of situations as threatening that is caused by the person's negative automatic thoughts, stress inoculation pattern by identifying and challenging negative automatic thoughts, provides an opportunity for the individual to re-evaluate these thoughts and be able to change them [26].

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In the research by Khaghanizade and colleagues (1387) titled as "study the relationship between job stress and work life quality among nurses", it was found that nurses with high QWL have less job stress [27]. A study was conducted by Yamazaki and Takeuchi titled as the relationship between work-family conflict and positive perception of the content (a type of stress management) in Japan. This study was performed as a self-executive questionnaire among 388 female Japanese nurses working in three hospitals in Tokyo. Based on the findings of this study, one of the most important variables affecting nurses' work-life conflicts is one's ability to cope with stress [28]. By learning stress inoculation training methods, the individual's ability to deal effectively with incidents increases and encouraging the person to use these methods in everyday life would lead to receive positive feedback in him; this reduces the individual's avoid from situations and he feels more control over the environment [29].

The findings of this study are not consistent with the results of the research by Dehghan Niri and colleagues who measured the effect of body relaxation on stress and life quality of the students inhabiting in student dormitories [30]. This difference is probably due to differences in the type of intervention, different work and life situations of nurses with those of students as well as their differences in the perception and expectations of life quality. Due to the different situations, factors affecting the relationship between stress and life quality of nurses and students have been different which have also affected final results as well. The study of Dehghan Niri and colleagues has been conducted at the end of semester during which the mental stress caused by exams and assignments of the semester end has caused that relaxation alone cannot significantly improve their life quality. In the current study, at the followed-up a month later stage as well, work life quality of the intervention group has significantly improved compared to pre-intervention stage as well as post-intervention stage; And it could be due to repetitive and optimal use of inoculation program by nurses during the course of pursuing in their daily functioning, as well as changing the quality of working life with the passage of time.

Field variables examined in this study have not significantly affected the quality of work life. Of course lack of revealing a significant relationship does not mean lack of real relationship; because the sample size of this study was not set to evaluate this relationship and investigating these relationships requires greater sample size.

This study had limitations of which the most important are: 1. because all subjects were selected from the hospital and the sample size was small the results of this study are only applicable to the same environment. 2. Individual differences of nurses in perception of the experiences and aspects of life quality and the groups were attempted to be relatively homogeneous by random allocation of intervention and control groups from this point of view; 3. Lack of full control over the exchange of information between the intervention and control groups that the test group was also asked to refrain from disseminating information by the end of the study for reducing the dissemination of information; The control group was also assured about holding workshops of stress inoculation program after the intervention, and the exchange of information was controlled as possible by this way.

CONCLUSION

Paying attention to the quality of work life and emphasis on ways which enhance the quality of work life increase nurses' job satisfaction and productivity and as a result their participation and satisfaction of working becomes more and the stress of work gets reduced.

Given that the findings of this study showed that conducting Stress Inoculation Program makes the nurses' work life quality increase, this method can be used as an effective and practical strategy for better and more healthy stress management and promoting the nurses' work life quality so that so that thereby, both quality of nursing services enhance and patients satisfaction increases.

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PHYSIOLOGY

ARTICLE

AN EFFICIENT STEGANOGRAPHY ALGORITHM USING VISUAL CRYPTOGRAPHY AND AES ENCRYPTION

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ABSTRACT

In this paper, an efficient approach for securing information is presented by the combination of steganography, cryptography, and visual cryptography. Steganography is a process by which the transmitting information is secured in a defined manner. At first, the secret information is divided into two shares by random grid techniques as similar in visual cryptography. Then, one of the shares is embedded by Least Significant Bit (LSB) into the cover image. Finally, Advanced Encryption Standard (AES) is used for encrypting the embedded image. The cipher key for encrypting the data is generated by the use of Discrete Cosine Transform (DCT) on the cover image. To recover the secret image, both cipher key, and the remaining share are required which increases the level of security.

INTRODUCTION

Steganography is data hiding method used to hide the secret information into the cover information. The cover data can be in any form such as documents and media files. Many studies have been done in connection with steganography. The secret information is hidden in random pixels [1]. The selection of random pixels is based on location, shape, and colour of the pixels in the cover image. After selecting the random pixels, LSB approach is used to hide the secret information. A modified LSB image steganography technique is described in [2]. Braille method is used for representing secret data by six bits only. Among the six bits, three bits are hidden in the red layer; two bits are on the green layer and one bit in the blue.

Genetic algorithm and visual cryptography based algorithm is designed [3] to transmit over networks. At first, LSB approach is used for hiding secret into cover. The security of the secret data is increased by modifying the location of pixels based on genetic algorithm. This data is split by visual cryptography into two shares. In order to retrieve the secret data, the receiver has to apply the reverse process using the secret key. An approach for multiple secret sharing schemes is discussed [4]. From the two secret data, only two shares are created by XOR operations and then, they are encrypted. While decoding, the secret data cannot be validated if decryption is not possible.

A simple approach for better security is achieved [4,5] by using LSB based steganography. The Advanced Encryption Standard (AES) cryptography will change the secret message into cipher text and to ensure two layer security of the message. The LSB bit is used for storing the status bit and not the message bit. An AES based secured steganography system is implemented in [6] which uses 128 bit for both plain text and secrete key. The state-of-art steganography approaches are reviewed [7]. The different types of steganography such as image steganography, audio steganography, and video steganography are focused.

A reliable steganography approach both in a gray and colour image is described [8]. The secret data is embedded in randomly scattered pixels which increase the security of the data. A robust audio steganography is designed in the temporal domain [9]. For additional security, AES is used. The embedded sequence is changed dynamically and randomly. A combination of cryptography and steganography is used for security [10]. The security level is increased in the key level by SHA-1. LSB is used for embedding process, and AES is used for encrypting the cover data.

In this paper, an efficient approach for hiding data is presented using visual cryptography, AES encryption, and LSB embedding process. The paper is arranged in the following order. In section 2, the methods and materials used by the proposed approach are discussed. The results obtained by the proposed system are discussed in section 3, and finally, the conclusion is given in section 4.

MATERIALS AND METHODS

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The proposed steganography algorithm is built by the use of two well-known techniques namely LSB embedding process and AES encryption. The level of security of the secret image is increased based on these two algorithms. Steganography is a data hiding method used to hide the secret information into the cover information. The proposed work mainly focuses on the LSB technique to hide the secret messages into the cover image. Advanced Encryption Standard (AES) is used for encrypting the embedded image. The main feature of the proposed work is two level securities with DCT based cipher key generation. The proposed steganography algorithm using visual cryptography and AES encryption is shown in [Fig.1].
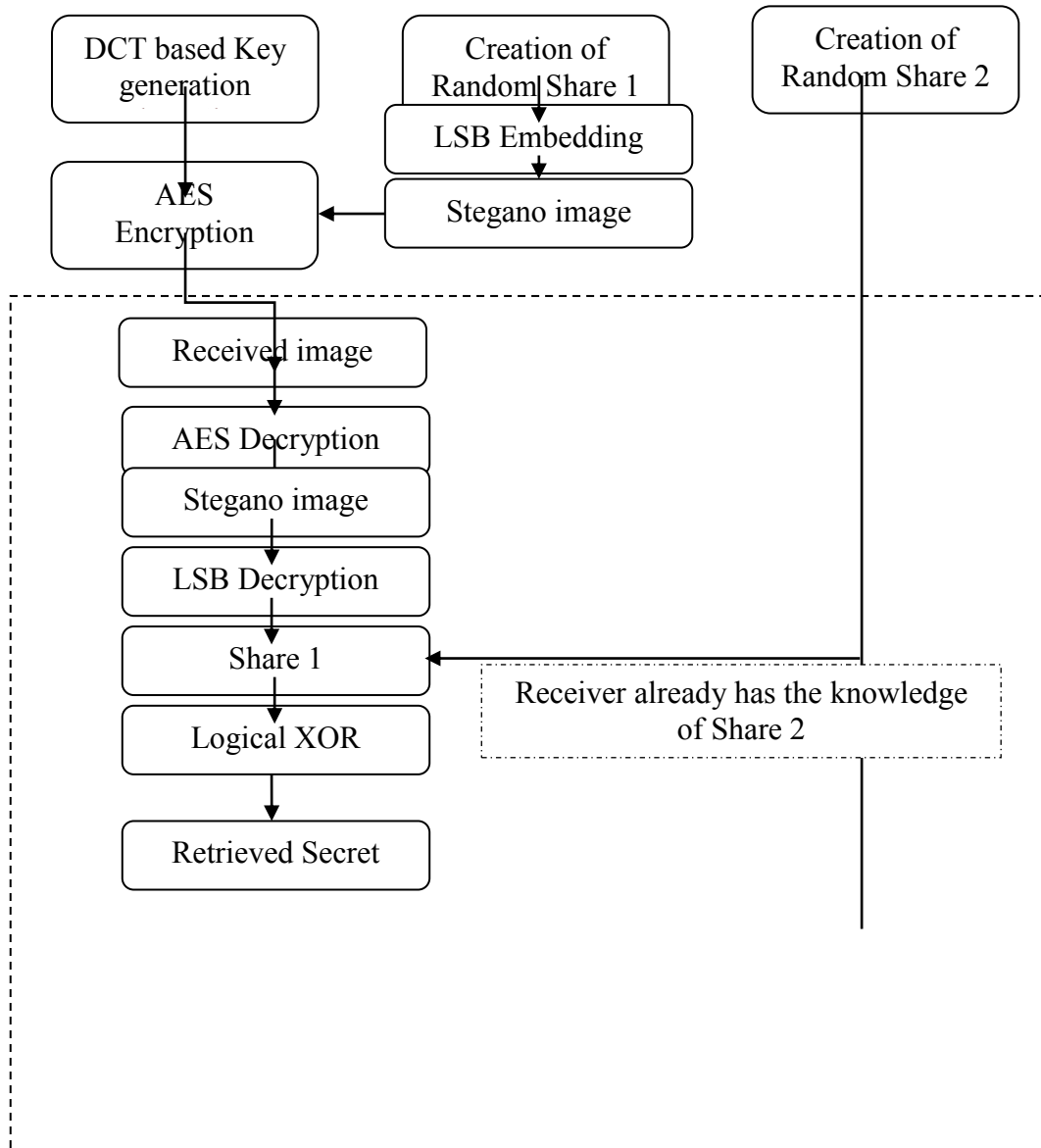
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Fig. 1: Block diagram of the proposed steganography algorithm using visual cryptography and AES

Visual Cryptography

Three algorithms for encryption are discussed in [11] by random grids. The given binary secret image is encrypted into two cipher grids of the same size. The first cipher grid is created by assigning each and every pixel with either 0 (black) or 1(white). By the use of secret image and the first cipher grid, the second cipher grid is created. Both cipher girds are required to recover the secret image. Thus, one of the two random grids is given to the receiver for verification. If the random grids are stacked with logical XOR operation, the secret image is revealed. The proposed approach uses the same technique to generate two shares from the secret image. Then one of the shares is embedded into the cover image for first level security.

LSB embedding process

To hide the cipher grid into the digital cover image or else another media is based on Least Significant Bit modification technique. LSB is one of the spatial domain methods. The LSB of the binary sequence of each pixel is replaced with the binary of the secret message. It provides high security for transmitting much information and easy to implement and to combine with other hiding techniques. For an 8-bit grayscale image, each pixel is represented by 8 bits. The last bit in a pixel is called as Least Significant bit as its value will affect the pixel value only by "1".

AES Encryption and Decryption

In December 2001, the NIST published a symmetric key block cipher is called as AES [12]. AES encrypts and decrypts a data block of 128-bits. It depends on some rounds. The input to the algorithm is a single 128-bit block. The block is represented as a row of the matrix of 16 bytes. The process of converting the original data to ciphertext is called encryption. It is achieved by applying mathematical transformations. These transformations are called as encryption algorithms and require an encryption key. The proposed system uses DCT [13] for generating the key. Decryption is the reverse process which outputs the original data from the cipher text using the same key.

RESULTS AND DISCUSSION

This section gives the simulation results of the proposed system described in the above section. [Fig. 2] shows the secret image and their corresponding two shares created by random grid techniques. In random grid techniques, one share is created by generating random numbers of 0's and 1's. The second share is created using XOR approach by the use of the first share.

Fig. 2: (a) Secret image **(b)** Share 1 **(c)** Share 2

After share creation, the second share is given to the receiver to verify the data. The next step is the application of embedding technique. The first share is embedded into the cover image by LSB process. [Fig. 3] shows the cover image and the embedded image.
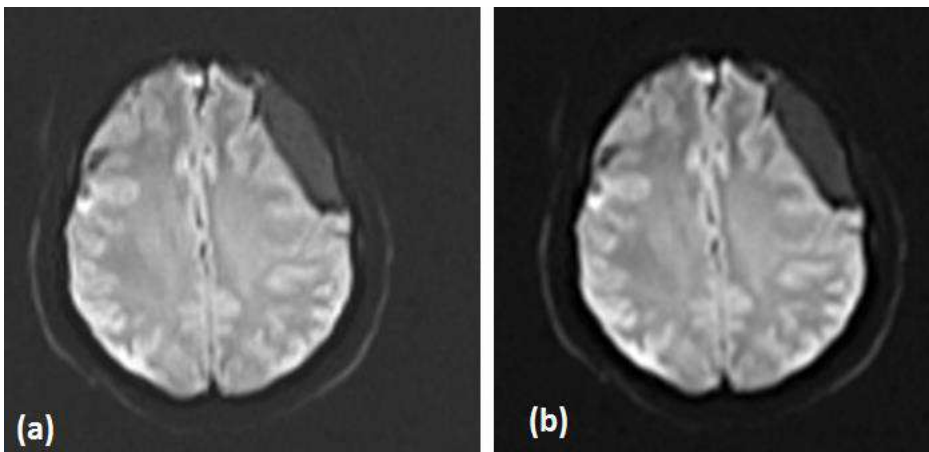
Fig. 3: (a) Cover image **(b)** Embedded Image

From the [Fig. 3], it is observed that there is no difference between the cover image and embedded image. Thus, no body has the knowledge that the secret image is hidden into that cover image. Further, the security of the secret image is increased by the use of AES encryption. The key for encryption is obtained by DCT decomposition of the cover image. From the AC and DC coefficients, 126-bit key is generated and then the embedded image is encrypted using it. This encrypted image is transmitted. At the receiver side, the receiver decrypts the image at first. Then, the first share is retrieved by the reverse LSB process.

Finally, the secret image is obtained by the XOR operation of retrieved first share and the second share in the receiver hands. Fig. 4 shows the AES encryption and decryption images along with retrieved secret image.

Fig. 4: (a) Encrypted Image **(b)** AES Decrypted Image **(c)** Retrieved Secret image
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CONCLUSION

In this paper, an efficient steganography algorithm using visual cryptography and AES encryption is presented. Visual cryptography technique is applied to generate two shares from the secret image. Then, one of the shares is embedded into the cover image using LSB procedure. The cipher key is generated using DCT transform 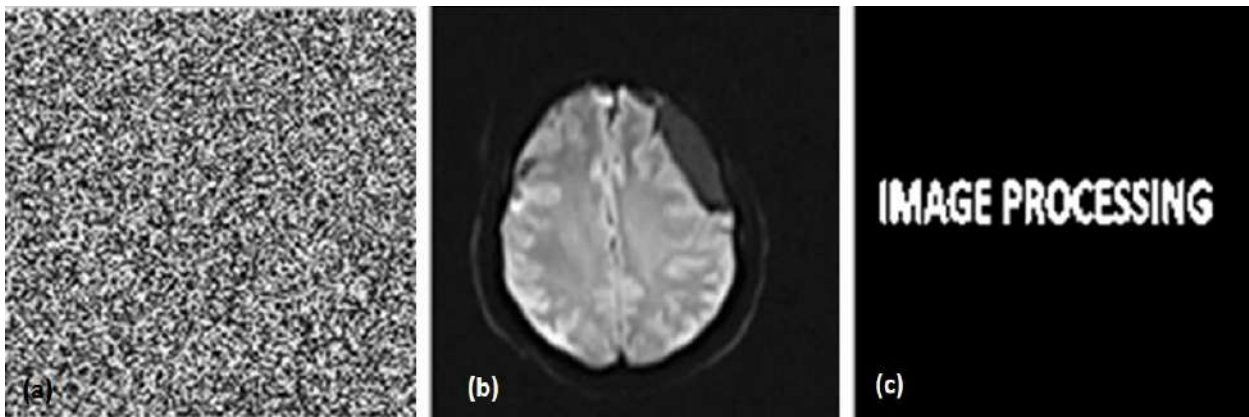and used with AES algorithm to encrypt the embedded data. The level of security of the secret image is higher than the standard steganography and encryption algorithm as the proposed system combines the visual cryptography into them. Hence, without the knowledge of the second share, no one can recover the secret information. In future, the proposed system can be extended to identify the tampered regions if anyone attempts to recover the secret and modify the information.

CONFLICT OF INTEREST
There is no conflict of interest.

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None.

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COMPUTER SCIENCE

ARTICLE

SECURING INTERNET BANKING USING MULTIPLE ATTRIBUTES SCHEME AND OTP

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ABSTRACT

In the latest cryptographic trends, attributes play important role in increasing security level. In online banking, users are relocated to the banking domain with secure medium. In this paper attributes are used as measure components to form secrete key. Attributes of website data, trusted authority & user attributes from banking domain are considered as parameters to form key. Based on these attributes, one time password is generated. After one time password verification, same key will be formed which will be matched with the newly formed key by one time password. In this approach, key will be generated temporarily and it will be matched with newly formed key by one time password method. This multiple approaches will increase the security level to make online transaction safely.

INTRODUCTION

In the recent networking approaches, network security is prioritized thing which can't be avoided. Even its level is important. Through cryptographic approaches are applied to increase the level in the network security, unauthorized user act as the level breaker. Hence, we need to improve the security level in online transaction. When the term money transaction comes, it shows data sensitivity and its importance. Online banking transactions show extreme need to improve its secure mechanism in current and in future point of view. In previous system of online banking one time password (OTP) scheme has been used as an authentication mechanism in dynamic structure where it overcame the server database compromising problem at one time by adding the breakpoints in hash chain of OTP [1]. It has used the computational calculations of Deffie Hellman problem so as to prove the security against attacks in insecure network [2].OTP actually carried out in numbering format and changed its state in every 30-40 seconds to another format. Hence it has dynamic feature of using over the traditional network [3]. To make it more effective in privacy we are further adding flavor of OTP in attribute based encryption in which multiple attributes will be taken in considerations so that we can possibly achieve highly secure transaction with reduced complexity [4].

MATERIALS AND METHODS

After the traditional digital signature system in cryptography, new systems has stepped out and proposed with new and innovative techniques. Some alternative way is made to signature scheme called as identity based encryption (IBE) [5]. In this particular concept keys are generated based on users credentials after the authentication. Person transferring the messages over the network doesn't needs to know the public key of receiver [2]. Receiver just has to authenticate himself as to get so as to access to message. Online Email system is the first demonstration example given which was based on identity based encryption technique [6].

Attribute Based Encryption

In this era, attributes are considered as measure factor to make encrypted form [7,8]. Attributes are considered as user attributes. User attributes are considered as set of user component details like user name, date of birth, city and so on. User attributes are uniquely applied where their values are predefined with respect to attribute value [4].

Attribute set = A_1 = {$a1_1, a1_2, a1_3, a1_4, ..., a1_n$}= {'Name' ,'Surname' ,'City' ,'Date of Birth',.....,'Country'}
Attribute set = A_2 = {$a2_1, a2_2, a2_3, ..., a2_n$}= {'Time of registration' , 'place of registration' ,.....,'agent'}

These attributes are used to form a secrete key [10]. After applying logarithmic approach and probability, main attribute set is formed which is combination of these attributes. Further cryptographic algorithm is applied and secrete key is generated. Attribute based encryption is technique is widely used technique in the online security approach. Even cloud services are made more secured by applying multiple security approaches using attributes [9].

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Cipher text Policy

In networking users able to access the data only if a user proves themselves with set of credentials or attributes to authority. Cipher text policy provides the confidentiality of data over the compromised server and provides security against the collision attack [7]. This method is closer to KP-ABE (Key Policy) access control [10] in which cipher text has been dealt with set of attributes. Unlike this, CP-ABE (cipher text policy) has used the user's secret key with number of attributes. Before decrypting data any message has to pass through an attribute based access structure. Suppose we have different users with attributes set $\{S1, S2,...., S_n\}$.
The combination gives

$$A \subseteq 2^{\{S1,S2,S2,...Sn\}} \quad \text{If } \forall\, \alpha, \beta$$
Where, $\alpha \in A, \alpha \in \beta \text{ then } \beta \in A$

In this structure access structure is non empty subset structure of $\{S1, S2,...., S_n\}$ in which if all sets appeared inside A are said to an authorized set where as the sets outside the A are not authorized sets [7]. Hence the roles of different parties are given by all attributes and that are associated with the keys in cipher text policy attribute based encryption. On the other hand when we are using CP-ABE, there are four fundamental steps to use it for secure data transmission [10]. First is the setup to generate the public parameter and creating secret master key. Second is the encryption where message, public parameter and cipher policy access structure is used. Key generation is the next step which takes master secret key and attributes sets which provides private key of user in output. Last step of decryption has been done using cipher text having access policy, master key, public parameter and decrypts the message.
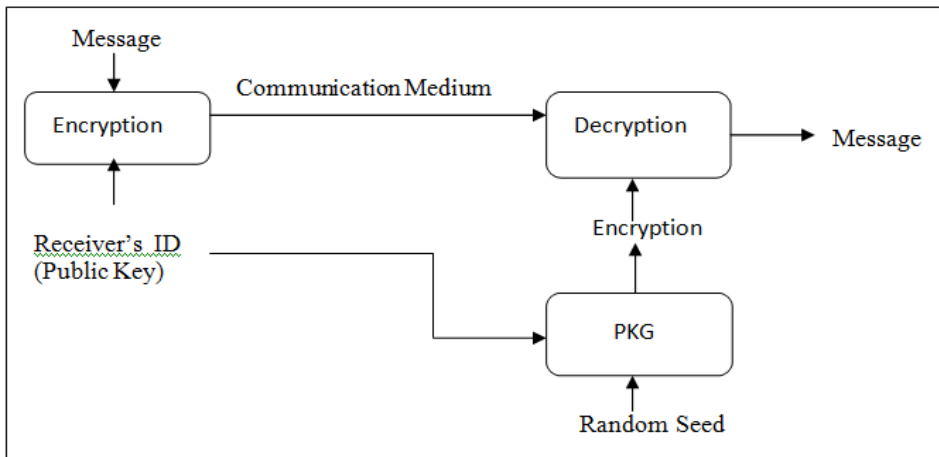
Fig. 1: Identity Based Encryption [4]

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RESULTS

User uses secure online banking to do online transactions [11]. User visits merchant's website directly or indirectly and connects to the banking domain to complete online financial transaction [12]. In this paper, three main components of the system are considered. Each system has its own policy. This policy is applied when particular request is generated. All systems are installed at different locations which are unknown to the users and merchants.

Policy M-S (Merchant System)

Policy M-S (Merchant's Policy) has its own components like attributes and data validation. Merchant makes request to the trusted authority and bank domain simultaneously. These requests carry current data attributes and co ordination request. Attributes are carried towards bank domain are current data attributes including hosting site name, day, date, month, time in hour, minute and second. Request carried towards trusted authority is coordination request which means to poke the trusted authority to contribute its attributes in encryption technique. Current data attributes are considered as a secrete data which changes according to transactions done by the user. This is the new term added to the existing approach. Here current data attribute set is considered as D_A.

D_A = { 'Hostname','Day','Date','Month','Time1:Hours','Time2:Minutes','Time3:Seconds'}.
D_A = $\{D_1, D_2, D_3, D_4, D_5, D_6, D_7\}$

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Above attributes are considered as attribute set of current attribute set which is generated by the registered merchant at the time of transaction [7]. These attributes are delivered to the bank domain.

Policy T-S (Trusted System)

After receiving request from merchant, trusted authority sends its own attribute set to the bank domain to form main attribute set [12]. Trusted Authority Attribute (TAA) is formed from the policy T-S. This policy checks for merchant and user validity. Also it applies random logic while generating TAA. Trusted authority acts as third party trusted authority [12]. While doing official registration of trusted authority with bank, some secrete attributes set is shared between bank domain and trusted authority. While delivering attributes to the bank domain for encryption, random selection is done. These randomly selected attributes are delivered which are identified by Policy B-S of the bank domain.
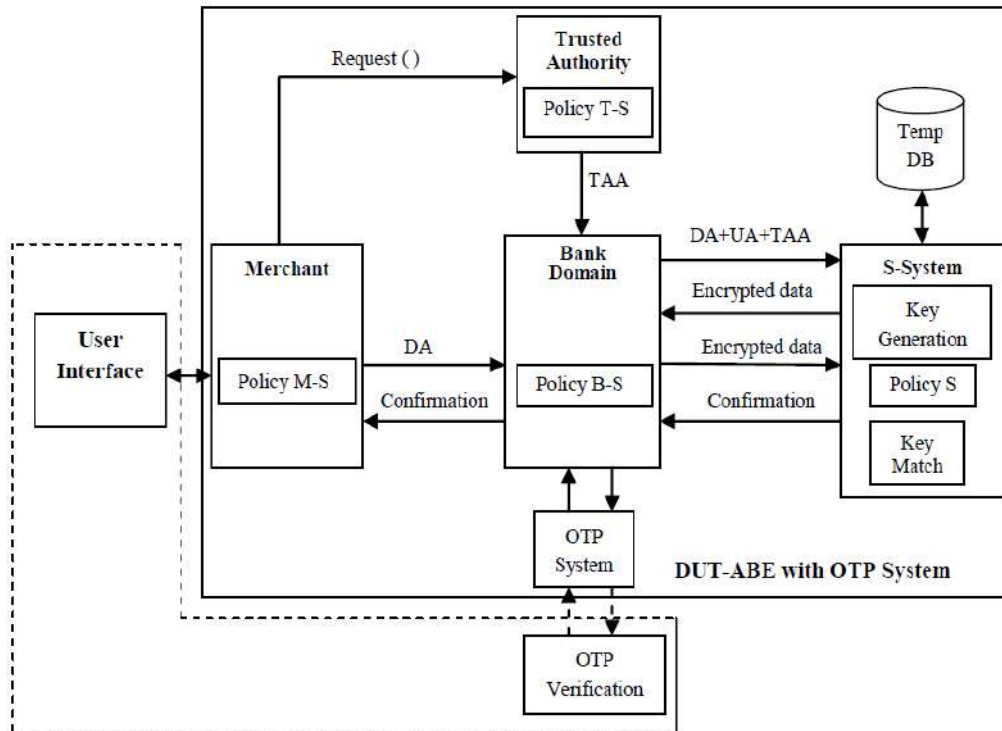
Fig. 2: DUT-ABE with OTP System

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Trusted attribute set is considered as T_A.

T_A = {'Attribute1',' Attribute2',' Attribute3',' Attribute4',' Attribute5',.....,' Attribute N'}
T_A = {T_1,T_2,T_3,T_4,.....,T_N}
T_{AA} = {T_2,T_4,T_5,T_9}

From this attribute set, some attributes are selected randomly which are delivered to the bank domain. Delivered attribute set is considered as T_{AA}.

Policy B-S (Bank System)

Bank domain already has user attributes which are taken from user details. Policy B-S (Bank's Policy) waits for TAA & DA. After receiving it, it adds User Attribute (UA) set to the received attributes. This policy forms main attribute set from DA, UA &TAA [4][12]. Main attribute set is transferred to the S-System. When user creates bank account, his or her user details are considered as bank or user attributes like name, date of birth, city and so on. Here we have considered user attribute set as U_A.

U_A = {'Name ,'Surname' ,'City' ,'Date of Birth',.....,'Country'}
U_A = {U_1,U_2,U_3,U_4,.....,U_N}

Bank system receives the attribute sets from trusted authority and merchant. Bank system delivers these attribute sets including its own user attribute sets to the S-System

Policy S (Secure System)

S-System (Secure System) is neither directly connected to any other system except bank domain. Retrieved attribute set is converted into encrypted format. S-System receives all attributes and forms main attribute set considered as M_A.

$$M_A = D_A \; U \; T_{AA} \; U \; U_A \tag{1}$$
$$M_A = \{D_1, D_2, D_3, D_4, \ldots, D_N, T_1, T_2, T_3, T_4, \ldots, T_N, U1, U2, U3, U4, \ldots, U_N \} \tag{2}$$

(1)&(2) shows that M_A is a union set of current data attributes, trusted authority attributes and user attributes [4] [12]. On these attributes, randomized selection method is applied. These Randomized Selection (RS) algorithm is applied on this data where selected components are stored in the array set [13]. As shown in (3), random elements are selected by applying randomized selection algorithm. It is stored in array set. This array set is applied with RSA algorithm to form encrypted format of data as shown in (4) [14]. This data is considered as E_D.

$$M_A \xrightarrow{\text{RS}} \{D_2, T_3, T_4, D_4, U1, U_4\} \tag{3}$$
$$M_A \xrightarrow{\text{RSA-E}} E_D \tag{4}$$

RSA algorithm based application is installed on both sides where RSA-encryption (RSA-E) and decryption(RSA-D) is done [15]. In similar way, normal data is nothing but decrypted data M_A. When decryption occurs at both S-System and bank domain, same decryption technique is applied [15].

$$E_D \xrightarrow{\text{RSA-D}} M_A \tag{5}$$

In (4) and (5), represented data is similar. Decrypted data is in the form of user understandable format which is in the form of combination of text and numbers. Policy B-S delivers this data to the user through one time password for versification purpose. One password is generated through this formatted data which is unique. One time password generated will be received either through email or through SMS(Short Message Service) [1]. It will be based on user's option.

After entering one time password manually by user, information will be delivered back to the Policy B-S. Policy B-S will convert this data into encrypted format and revert it to the S-system through communication channel which is present at other or unknown location to verify entered one time password information. Policy S (Secure) will decrypt the information first. Decrypted data will be checked for data validation and regarding confirmation will be delivered to the Policy B-S. This confirmation will be delivered to the merchant will be further transferred to the user for acknowledgement purpose. After successful delivery of the acknowledgement, all data stored in the temporary database will be deleted automatically. When next time new transaction will occur, new data attributes based data will be used which shows more secured approach in recent cryptographic trend.

CONCLUSION

In online banking, security techniques applied is important factor. Communication channel is sensitive component of secure online banking. It is not necessary that communication channel is always secured while doing online transactions. In existing security approach, communication channels and security resources are not secure as required. In proposed approach, security provider resources are unknown to the system. Combination of multiple attributes including user, current data and trusted authority makes authentication data more secured. Different policies are applied in different levels to enhance the encryption level. One time password is also additional security method used for user identification. Code travelled through the communication medium is delivered in secured form which is hard enough to decrypt. Attributes are not secured in the existing approaches which are overcome in proposed approach because there is possibility of leaking user details retrieving attributes. Respective attribute data is created for temporary purpose only which shows there is no possibility of data piracy to do next transactions. Each time new attribute based data is generated to complete online transaction. By applying multiple attribute scheme, security in online banking can be enhanced and user details can be securely stored.

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ARTICLE

RELATIONSHIP BETWEEN THE PERSONALITY TRAITS, DEFENSE MECHANISMS AND MARITAL SATISFACTION OF ELEMENTARY SCHOOL STUDENTS' PARENTS

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ABSTRACT

The purpose of this study was the relation between personality traits, defense mechanisms and marital satisfaction of the parents of the elementary school students. Research sample includes of 300 (150men and 150women) from parents of the elementary school of Qazvin city that sampling with multistage. Questionnaires in this study were NEO–Five Factor Inventory, Defense Styles Questionnaire and Enrich marital satisfaction scale. For the analysis of the data used multiple regression, ANOVA and Pierson correlation. The results showed that there wasa meaningful relationship between such personality traits as neuroticism, extraversion, agreeableness, conscientiousness, mature defense mechanisms, immature defense mechanisms and marital satisfaction. Neuroticism, conscientiousness and immature defense mechanisms had a significant effect on anticipated marital satisfaction. Marital satisfaction without personality traits such as top extraversion, agreeableness, conscientiousness and below neuroticism had no duration even with a starting balance. Couples who most use matures defense mechanisms have more marital satisfaction.

INTRODUCTION

The marriage is to enter a new phase of life which covers an important part of personal and social life [1]. In the meantime, what seems to be more important than the marriage itself and formation of family is the marital satisfaction. The marital satisfaction is one of the deepest and the most fundamental human pleasures and its absence is one of the greatest and the most profound source of suffering [2]. The marital satisfaction is the couple agrees about problems and struggles, common interests and activities, frequent and public displays of affection, mutual trust, a few complaints and a few complaints about loneliness, misery and suffering [3]. Soleimanian believe that the marital satisfaction is the conformity between the current situation and the expected situation. According to this definition, the marital satisfaction exists when there is conformity between the current situation of an individual in marital relations and his expected situation [4].

Sherbafalso believe that three general areas of satisfaction, including the individual's satisfaction with marriage, satisfaction with family life and general life satisfaction are overlapping and interdependent. In fact, the marital satisfaction has a significant impact on other aspects of couples' life. The concept of marital satisfaction is one of the most common concepts to show happiness, sustainability and quality of marriage, but this cannot be easily achieved [5]. Many factors can influence the marital satisfaction. Some people consider the intellectual and emotional maturity of couples, similar cultural class and similar interest and ideology to be among factors affecting marital satisfaction [6]. Others consider religion [7-9], children [10-12], duration of marriage [13], education [14-16], sexual issues [17, 18] and economic issues [19, 20] as important factors in marital satisfaction.

In the meantime, the factor which can affect the marital satisfaction more consistently in the long term is the personality characteristics. Personally, as a challenging concept among researchers, represents durable and unique features which indicate a person's behavior in all fields. Raymond Cattell has a definite personality and said "personality provides the ability to predict what a person will do in a particular situation" [21]. All port considers personality traits as the readiness to present the same or similar answers to various stimuli. In the other words, traits are stable and durable responses to the environment [22]. Therefore, since the personality is relatively stable and predictable, it plays an important role in family life and marital satisfaction. Research findings show that the personality factors and aspects can predict marital quality better than calendar variables such as age, education and experience of divorce in the past [23].

Some researchers who have investigated the relationship between personality traits and marital satisfaction have indicated the importance of personality traits in satisfaction and dissatisfaction of couples. For example, Stroud et al. consider the marital dissatisfaction to be the result of the mutual influences of personality traits, stressful life experiences and compliance process [24]. They believe that the personality traits affect couple's dissatisfaction indirectly through the process of couple's compliance. Lazarideset al. point out the mediator role of personality as a regulatory factor in the manner of communication and stability among couples [25]. The results of their studies, Strode et al. found that marital satisfaction and personality traits are linked together and duration of marital relationship influences marital satisfaction as an important factor. The most widely used models in the study of

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personality traits is the five-factor personality model. These five factors include neuroticism, extraversion, openness, agreeableness and conscientiousness [24].

Various studies suggest that these five personality factors are useful in assessing the relationship between personality and marital adjustment and they are commonly used in the study of marital relationships [26]. Therefore, since marriage is the union of two different characters, investigating the relationship between personality traits and marital satisfaction of couples will bring useful and productive outcomes. Life satisfaction, happiness, development and public health, reducing the conjugal, marital discords reduction, divorce prevention and finding valid criteria in mate selection are among these outcomes [27]. Therefore, this study aims to examine the role of personality factors in marital satisfaction from the perspective of pathology.

Defense mechanisms are among the factors influencing the marital satisfaction, but few studies have been focused on it. Defense mechanisms are an automatic setting process which acts to reduce cognitive dissonance and to minimize the sudden changes in internal and external reality by influencing the manner of perception of threatening events [28]. Defense mechanisms include mature defense mechanisms, immature defense mechanisms and neurotic defense mechanisms [29]. Defense mechanisms which are responsible for protection of ego in dealing with various forms of anxiety may be normal or abnormal and efficient or inefficient depending on their function. The mature defense mechanisms are regarded as the normal and efficient methods of adaptive confrontation. The neurotic defense mechanisms and immature defense mechanisms are considered to be the maladaptive and ineffective methods of confrontation [30].

These defense mechanisms are used by all human beings since their existence is valuable to show the failures unimportant and to protect excitement and anxiety. For this reason, they are called "the normal adaptive response". If they are used excessively, all the defensive positions which were created to protect the values of the person will cause disruptions. Therefore, despite the necessity of psychological defense, it brings some losses. First, in all of them, two important issues of "self-deception" and "escape from reality" have a vital role. It makes the access to "real self" very difficult. The constant and unreasonable use of it will lead to a situation that the person cannot properly solve the problems by the use of reason and logic and adapt himself to the environment [31].

Consequently, defense mechanisms also influence the relations between spouses and marital satisfaction. While the habit of using the abnormal defense mechanisms may bring compromise problems due to ignoring the facts and issues, the effective use of normal and adaptive defense mechanisms can help the couples to have a more realistic view of each other and to pay more attention to the needs of each other. While denial, rationalization, suppression, identification and projection may separate the couples, bring some problems in understanding each other's needs and make the disputes permanently, the use of some defense mechanisms such as introjection, displacement and sublimation can lead them in the process of empathy and supplementation. With reduced the challenging topic, they can help the couples to solve the specific problems of each other. Therefore, due to the abundant use of this mechanism in daily life and their unconscious nature, the investigation of their effects on the couple's interactions with each other and marital satisfaction in the form of research is very important.

Although marriage is pleasant, statistical data suggest that couple's consent is not obtained easily. Therefore, the most powerful human link which is marriage is in serious danger [32]. The rising rate of divorce and the existence of conflicts and turmoil in relations between spouses confirm this matter. The studies also concluded that divorce in marital life has a negative impact on physical and mental health of couples. These negative effects are also evident in their children [26]. Family is the first social institution where people grow and establishes other social institutions. These social institutions will be healthy only if the family is healthy. Therefore, understanding the underlying factors to identify and explain the factors affecting marital satisfaction are among the topics considered in this study, which can lead to the realization of successful marriages and mutual consent as well as the continuation of healthy life. As it was stated, the personality traits and defensive styles are among the factors which affect the marital satisfaction. With regard to the research literature, there are insufficient researches in this area in our country. For this reason, the aim of this study is to investigate the relationship between the personality traits and the defense mechanisms and marital satisfaction of elementary school students' parents in Qazvin.

MATERIALS AND METHODS

This study was a descriptive (non-experimental) and co-relational research. In this study, the relationship between the independent or predictor variables (personality traits and defense mechanisms) and criterion or dependent variable (marital satisfaction) were investigated.

The statistical population of this study was all parents of elementary school students in Qazvin in academic year of 2012-2013. The research sample was 300 parents (150 men and 150 women) of elementary school students in Qazvin. Given the nature of the subject and marital satisfaction questionnaire, there was the possibility of subjects drop. To achieve the desired sample, 100 subjects (50 men and 50 women) were added to the sample size to be substituted for the respondents who present incomplete and missing questionnaires. Due to some limitations, both parents did not take part in this study. Therefore, one of the parents (father or mother)

participated in this study and completed the questionnaire. The selected parents should live with their spouse and have a common life. They should also have a sufficient level of literacy to enable them to complete the questionnaire. The sampling method used in this study was the multi-stage random sampling. Eight public and private schools (4 girls' schools and 4 boys' schools) were selected randomly out of two regions of education. Several classes were selected out of these eight schools and some students were randomly selected through the list of students. Their parents (mother or father) came to school to obtain and complete the questionnaire.

The tools used in this research were the five-factor personality questionnaire (NEO-FFI-60), defensive styles questionnaire (DSQ-40) and ENRICH (evaluation and nurturing relationship issues, communication, and happiness) Marital Satisfaction Scale. At each school, a specific date was determined for presence of parents (mother or father) in the school. On the specified date, the necessary explanations were given to parents about the purposes of the study, its importance and sincerity in responding. Then, the parents filled out questionnaires. The one-way analysis of variance, multivariate linear regression and Pearson correlation were used to analyze the data. SPSS Statistics V21 was used for statistical analysis.

RESULTS AND DISCUSSION

In [Table 1], the descriptive indicators related to defense style subscales (mature, immature, and neurotic) were presented.

Table 1: The statistical indicators related to defensive styles variable's subscales

Defensive Styles	Females		Males		Total	
	M	SD	M	SD	M	SD
Mature	44.99	10.69	47.63	9.43	46.31	1.26
Immature	113.2	24.59	109.38	26.23	111.29	1.06
Neurotic	46.7	9.83	47.92	9.29	47.31	1.19

[Table 2] provides the mean and standard deviation of the 5 subscales of personality traits included neuroticism, extraversion, openness, agreeableness and conscientiousness.

Table 2: The statistical indicators related to personality traits variable's subscales

personality traits	Females		Males		Total	
	M	SD	M	SD	M	SD
Neuroticism N	23.04	8.2	19.33	7.11	21.18	7.88
Extraversion E	29.48	6.2	31.09	4.88	30.28	5.63
Openness O	23.81	4.54	23.98	4.39	23.89	4.46
Agreeableness A	32.6	5.99	31.06	5.51	31.83	5.79
Conscientiousness C	37.55	5.62	37.11	5.9	37.33	5.75

Regarding the level of marital satisfaction, the results in [Table 3] indicated that the average of marital satisfaction of men (177.37) were higher than women's (163.64). The difference was close to 14 scores.

Table 3: The statistical indicators related to marital satisfaction variable

Gender	M	SD
Females	163.64	30.04
Males	177.33	25.17
Total	170.51	28.51

In [Table 4], the results of comparing the scores average of marital satisfaction in three groups with mature, immature and neurotic defensive styles were provided. With respect to the amount of F (F=16.75) (Table 5), there was a significant difference between these three groups in terms of marital satisfaction (p< 0.01).

Table 4: The descriptive indicators related to marital satisfaction in three groups of defensive styles

Defensive Styles	Number	M	SD	SE
Mature	101	179.71	27.72	2.75
Immature	93	157.63	25.96	2.69
Neurotic	106	173.03	27.53	2.67

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Table 5: The results of the one-way analysis of variance (ANOVA) to compare defensive styles and marital satisfaction

Source of variations	Sum of square	Degree of freedom	Mean Of Square	F	Sig.
Between Group	24643.82	2	12321.91	16.75	0.0001
Within The Group	218487.15	297	735.64	-	-
Total	243130.98	299	-	-	-

In [Table 6], the results of the correlation coefficient between each pair of variables were given. Based on the results, it can be said that there was a significant relationship between the marital satisfaction and the personality traits including neuroticism (r=-0.49), extraversion (r=0.36), agreeableness(r=0.37) and conscientiousness (r=0.39), mature defensive style (r=0.13) and immature defensive style (r= -0.38).

Table 6: Correlation between marital satisfaction variable (criterion) and variables of personality traits and defensive styles (predictor)

Variables	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. Marital Satisfaction	-								
2. Neuroticism	-.49**	-							
3. Extroversion	.36**	-.52**	-						
4. Openness	.10	-.11*	.11*	-					
5. Agreeableness	.37**	-.45**	.32**	.03	-				
6. Conscientiousness	.39**	-.36**	.4**	.08	.42**	-			
7. Mature Defensive Style	.13*	.13**	.36**	.04	.03	.12*	-		
8. Immature Defensive Style	-.38**	.51**	-.20**	-.04	-.48**	-.3**	.23**	-	
9. Neurotic Defensive Style	-.10	.2**	.05	.01	.06	-.02	.41**	0.32**	-

*p < .05. **p < .01.

[Table 7] shows the results of the analysis of variance. It indicated that the regression of criterion variable scores toward the scores average of predictor variables was statistically significant. In other words, the used model was an appropriate model.

Table 7: Analysis of variance of the scores of criterion variable and predictor

Source of variations	Sum of square	Degree of freedom	Mean Of Square	F	Sig.
Regression	78214.36	8	9776.79	17.25	0.0001
Residual	164916.62	291	566.72		
Total	243130.98	299			

The standardized Beta coefficients to assess the contribution of each predictor variable were presented in [Table 8]. The results show the great effect of the three predictor variables of neuroticism, conscientiousness and immature defensive style in anticipation of the criterion variable (marital satisfaction).

Table 8: Standardized Beta values and coefficients

Predictive variables	Non-Standardized coefficients		Standardized coefficients	t	Sig.
	B	SE	β		
Constant	143.39	19.22		7.45	0.0001
Neuroticism	-0.88	0.24	-0.24	-3.65	0.0001
Extroversion	0.29	0.31	0.05	0.93	0.35
Openness	0.21	0.31	0.03	0.7	0.48
Agreeableness	0.39	0.29	0.08	1.34	0.17
Conscientiousness	0.85	0.28	0.17	3.07	0.002
Mature Defensive Style	0.26	0.16	0.09	1.6	0.11
Immature Defensive Style	-0.19	0.07	-0.17	-2.7	0.007
Neurotic Defensive Style				-0.46	0.64

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The aim of this study was to investigate the relationship between the personality traits and the defense mechanisms and marital satisfaction of elementary school students' parents in Qazvin. The results showed that there was a significant negative relationship between the marital satisfaction and personality traits of neuroticism and there was a significant positive relationship between the marital satisfaction and personality traits of extraversion, agreeableness and conscientiousness. There was a significant negative relationship between the marital satisfaction and immature defense mechanisms. These findings were consistent with the research results ofWatson and Clark [33], Abdollahzadeh [27], Razzaghi et al.[34], Mehrabizadeh et al.[35], Malaf et al. [36], Shakerian et al.[37],Botwin et al. [38], Bass [39], Ahadi [40], Rasoulzadeh and Abbasi [41].

The significant negative relationship between the marital satisfaction and neuroticism is due to the association between the high scores of neuroticism and emotional instability, depression, hostility and the experience of negative emotions such as fear, sadness, confusion, anger, guilt and hatred. People who have such features are more prone to impulsiveness, aggression and vulnerability. They are also more likely to have compatibility problems and irrational beliefs. The mentioned features can hurt any communication and interaction, especially between spouses. It will most likely lead to general or partial dissatisfaction with marital life. In this situation, couples will experience lower satisfaction in their interpersonal relationships.

People with personality trait of extraversion have positive emotions like intimacy, being active, gregariousness, optimism and positive excitements such as joy, happiness and love. These people are full of energy and prefer to be in public and create intimacy with others. These features are most relevant to the quality of interpersonal relationships. These positive features improve the quality of relations between spouses and increase the intimacy between them. They increase not only the satisfaction of their marital life, but also the happiness and life satisfaction.

The significant positive relationship between the marital satisfaction and the personality trait of agreeableness can be explained on the basis that a person with this feature is basically altruist and has sympathy for others. He is eager to help others and believes that others are reliable. People who receive a high score in this feature respect others, hide their aggression, forgive and forget. They are humble, gentle and compassionate and always consider others. Like extraversion, the agreeableness is another aspect of interpersonal tendencies. It can affect couples' marital satisfaction in a positive way since the marital life is based on altruism, sympathy, helping each other, mutual trust, respect, forgiveness and humility. Lack of these features will damage the durability and strength of marital life severely even if it doesn't have an unbalance start.

People who have conscientious or dutiful personality traits are purposeful, strong-willed and determined. They adhere to ethical principles and do their moral obligations accurately. They work very hard to achieve their goals. These people are entirely responsible and have no conflict in doing their tasks. They have a definite purpose and planning for their life. They are mainly responsible and reliable. The results of the present study showed that the conscientiousness variable and the personality trait of neuroticism are good predictors of marital satisfaction and there is a positive and significant relationship between the personality trait of conscientiousness and marital satisfaction. The present study did not find any relationship between openness to new experiences and marital satisfaction. Based on the above theories and researches, individuals' openness to new experiences confirms their flexibility and tolerance in the field of communication. Therefore, it seems logical thatthe relationship between the spouses which is one of the closest and most intimate interpersonal relationships and (according to Ericsson) requires the highest interactive capacities and communication tolerance to maintain intimacy to be affected by such a feature on both sides of marital life. Based on these descriptions, the researcher has doubt about finding no positive association between openness to experience and marital satisfaction just in terms of method and administration. He suggests the repetition of such a research with more accuracy.

There is a significant negative relationship between the immature defense mechanisms and marital satisfaction. It means that, by increasing the use of immature defense mechanisms, the marital satisfaction decreases. There is a significant positive relationship between the mature defense mechanisms and marital satisfaction. It means that, by increasing the use of mature defense mechanisms, the marital satisfaction increases. The present research did not find any relationship between the neurotic defense mechanisms and marital satisfaction. In fact, the couples who mostly use the mature defense mechanisms compared to the immature and neurotic defense mechanisms have more marital satisfaction. The finding could also be justified on the basis that the use of immature and neurotic defense mechanisms and engaging in the defense mechanisms of denial, rationalization, revocation will bring two issues of escape from reality and self-deception. These issues make the couples not to have efficient, adaptive and effective confrontation with life problems. These couples will have negative interactions in their marital relations due to the use of immature and neurotic defense mechanisms.

These issues also make the couples not to accept your weaknesses and to have a dynamic and rationalized defense. These couples are insecure, worried and anxious about being attacked, threatened and reprimanded. They will create a kind of unbalance behavior in their relations. The couples who use the mature defense mechanisms to reduce the anxiety caused by their instincts' unconscious desires which are morally unacceptable are far more consistent in dealing with issues of their marital life. On a theoretical assessment of the research findings, the characteristics of each defense style and personality trait can be specifically focused. The extroverts have more adaptive and social power. The agreeableness will bring social conformity. The openness to experience disappears any prejudice, rigidity and dogmatism in interpersonal relationships. It creates a flexible and tolerant

person who doesn't ignore others' experiences and wants to interact with them. Responsibility also prevents people from confrontation with others without any norms, values and inward frameworks. By comparing each of these personal traits which are designed and built entirely out of social and interpersonal view with mature defense mechanisms, understanding their positive correlation and high convergence will not be difficult.

Basically, the maturity of some defense mechanisms is due to their social and adaptive functional power. The mechanisms which prevent the person from being isolated and strengthen his social relations are in contrast to the negative mechanisms which do not show any successful adaptive and social action, make the person involved in secrecy and covering the inner conflict and stop his social relations.

In addition to benefits, all researches have some limitations. In order to overcome these limitations, it is suggested to conduct further researches on defense mechanisms and marital satisfaction in different samples. Due to the unconscious nature of the defense mechanisms, some researches must be done on the constructive or destructive role of these mechanisms in interpersonal relationships and their effects. Arranging some meetings to inform the couples about the unequivocal effects of personality traits on marital life is also suggested to help the couples to change the personality traits which lead to marital dissatisfaction.

CONFLICT OF INTEREST
There is no conflict of interest.

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FINANCIAL DISCLOSURE
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ARTICLE

HIGH PIEZORESISTIVE BEHAVIOR OF VERTICALLY ALIGNED MULTI-WALL CARBON NANOTUBES ARRAY

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ABSTRACT

Due to their unique electrical, mechanical, and electromechanical properties, vertically aligned carbon nanotube (VACNT) films are promising to be used as piezoresistive based sensors. When VACNT is subjected to mechanical deformations, its resistance changes dramatically. This effect can be utilized for strain sensing, pressure sensing, and also for nanoelectromechanical transducers in micro electromechanical systems (MEMS). In this work, at first a simple method was employed to fabricate VACNT films within the cylindrical pores anodic aluminum oxide (AAO) substrate. Then, electronic and mechanical properties of VACNTs were obtained after removing AAO substrate. Finally, by using VACNT films, which were attached to a paper substrate, a mechanically flexible load sensor was fabricated. Experimental results show that using VACNT film instead of random orientation carbon nanotube film increases gauge factor of the sensor, which is an important designing factor.

INTRODUCTION

Unique structural of Carbon nanotubes (CNTs) such as electromechanical features, thermal properties, and high aspect ratio, introduce them as an ideal sensing component for piezoresistive sensors [1]. The piezoresistive effect in CNTs has been investigated for the first time by Tombler et al. [2]. For instance, the gauge factor of CNTs was reported to be about1000, which is five times larger than that of single crystal silicon. Other measurement presented by Cao et al. [3] illustrated a gauge factor up to 3000 for individual single wall carbon nanotubes (SWCNTs). In another research,Obitayo and Liu [4], have presented some important features of CNTs for strain sensing. Dharap et al. [5] have used pure SWCNT films (bulky paper) as a sensing component in strain sensors. Further studies were presented by Li et al. [6] using thin film of nanotubes as strain sensor. The film was made of randomly orientated bundles of SWCNTs and was attached to a rubber strip using epoxy. Another concept with multi walled carbon nanotubes (MWCNTs) film was reported recently by Li et al. [7] and Song et al. [8].

For practical application, Pham et al. [9] have fabricated poly (methyl methacrylate) (PMMA)/MWCNT composite films based sensor. Also, a new type of polyisoprene/MWCNT composite strain sensor was reported by Knite et al. [10]. Kang et al. [11] and Loh et al. [12] have cited the use of CNT/polymer composites as strain sensors for structural health monitoring. Piezoresistive behavior of CNTs has also been applied for pressure sensors [13, 14] and force sensors [15]. Due to CNTs random orientation, the gauge factor was found to be rather small for CNT films [16-17] that may hinder the potential application of CNTs. To overcome the above mentioned limitation of CNTs, using vertically aligned carbon nanotube has been recently investigated [18-22].

This paper presents the concept and demonstration of nano electromechanical sensors based on vertically aligned carbon nanotubes (VACNTs). At first, the VACNT films were synthesized on the porous anodic aluminum oxide (AAO) substrate by using chemical vapor deposition (CVD). Here, the AAO is used as a substrate for growing VACNTs. The proposed method possesses numerous advantages such as fast alignment, facile array design, mass production, high level of structural diversity, and complexity. The electronic and mechanical properties of VACNTs were then obtained after removing AAO. Finally, by using VACNT films, which were attached to a paper substrate, a mechanically flexible load sensor was fabricated. Electromechanical measurements reveal that the VACNT films can be utilized as an exceptional piezoresistive electromechanical transducer with higher gauge factor values in comparison with the state-of-the-art strain gauges.

MATERIALS AND METHOD

The process of preparing AAO substrate contains two steps, as follow: in the first step, bath ultrasonic was used for aluminum foil cleaning. After that, it was electrochemically brushed by ethanol and perchloric acid (HClO4) solution under constant voltage of 20 V for 2 min to achieve a mirror-like surface. Anodization

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process was carried out in an electrochemical cell at the temperature of 10 ℃. The first anodization process was carried out using 0.2 M oxalic acid under 40 V for 2 h. The formed porous oxide film was chemically removed using a solution consisting of the following weight percent: 6% phosphoric acid (H3PO4) and 1.8% chromic acid (H2CrO4). This process was carried out at 65 ℃ for 2 h. Second anodization was carried out under the same conditions of the first anodization for 58 h. Then, in order to achieve through-hole membrane, one side of AAO was covered by preserving polymer.. This sample was submerged in 5% (wt) phosphoric acid (H_3PO_4) for 3 h, and the temperature was kept at 50 ℃. Afterwards, the sample was placed in saturated mercuric chloride ($HgCl_2$) solution to separate aluminum, the preserving polymer was also mechanically separated. Then, the barrier layer was removed using 5% (wt) of phosphoric acid (H_3PO_4) at the temperature of 30 ℃ for 60 min. Finally, anodic alumina substrate was preheated by being pressed between two α-alumina disks at 800 ℃ for 1 h to enhance its stability. This process is necessary to avoid fracturing and curling of substrate which usually occurs during phase transition of the amorphous alumina. The FESEM surface images of AAO synthesized are shown in [Fig. 1a].

Preparation of CNTs within the AAO substrate was carried out using chemical vapor deposition (CVD) method in a tubular electrical furnace (DI=50 and L=1100 mm). The controlled flow of C2H2 and Ar was injected into the reactor as the carbon precursor and carrier gas, respectively. The AAO substrate was vertically placed in the furnace, and temperature increased with a rate of 5 ℃/min from room temperature to 650 ℃ under Ar flow (200 ml/min). The ratio of C_2H_2/Ar was adjusted to be about 0.01 when the reactor temperature reached to 650 ℃. Afterwards, the acetylene (C_2H_2) flow was removed and the reactor was cooled to room temperature under the atmosphere of Ar. CNTs membranes were also put in 200 ml/min flow of O2 at 400 ℃ for 1 h under smooth oxidative treatment. Finally, all samples were washed by ethanol (C_2H_6O) and dried in 60 ℃ for 4 h under vacuum condition. [Fig. 1b] shows the image of VACNTs after removing AAO and the TEM images of fabricated CNTs are presented in [Fig. 1c].
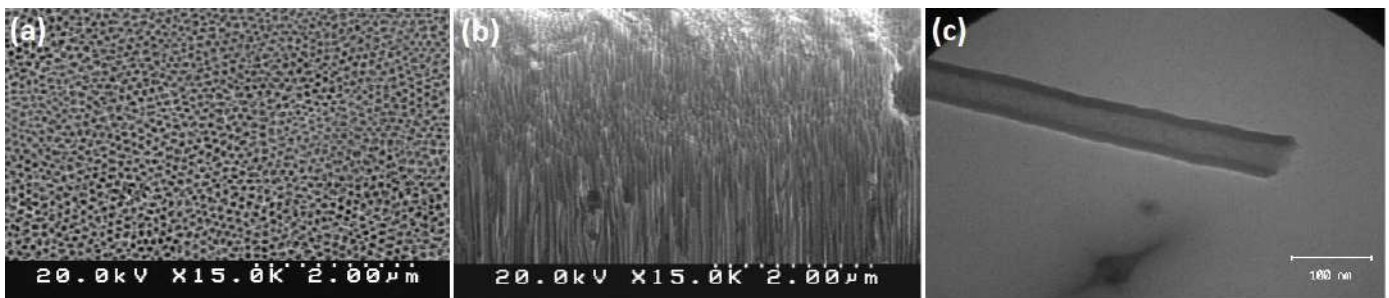

Fig. 1(a): FESEM image of AAO substrate prepared by anodization process. (b) FESEM image of VACNTs after removing AAO (c) TEM image of VACNTs

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In order to prepare the VACNTs/paper-based load sensor, an A4 paper with a thickness of 340 μm was chosen as the substrate, and it was patterned to the required dimensions (80 mm × 8 mm) using a laser cut with a precision of 0.12 mm. In the next step, the electrical contact pads were printed using a silver ink. Then VACNTs film (5×5 mm) was attached to the paper substrate. In order to certify the connections between the printed contact pads and sensing components (VACNTs film) a small drop of silver ink was placed on the connections using a painting brush. Moreover, in order to protect the connections a nylon film was placed on the top and bottom of the sensor using a paste. At the end, by connecting the contact pads to the multimeter (Keithley 2400 source meter), it was possible to read the resistance change of the system. A simple illustration of the VACNTs/paper-based load sensor can be observed in [Fig. 2].

RESULTS AND DISCUSSION

All the measured voltage-current (V-I) characteristics of VACNTs film showed a linear ohmic V-I behavior [Fig.3]. This matter indicates the correlation between the piezoresistivity of VACNTs film and the deflection caused by the loaded force. The results determined a resistance of 2.8 kΩ and 115 kΩ corresponding to parallel and perpendicular CNT directions.

In order to perform the relation between the applied compressive strain on the VACNTs film (input) and resistance change (output) we have calibrated the VACNTs/paper-based sensor by using a multimeter. [Fig. 4] shows the resistance of the VACNTs at each measurement step. When the paper-based sensor is unloaded, VACNTs' resistance has returned to its initial value. This confirms that the original VACNTs – silver contact was a firm contact, i.e. slipping between VACNTs –silver contact did not occur during the experiments.
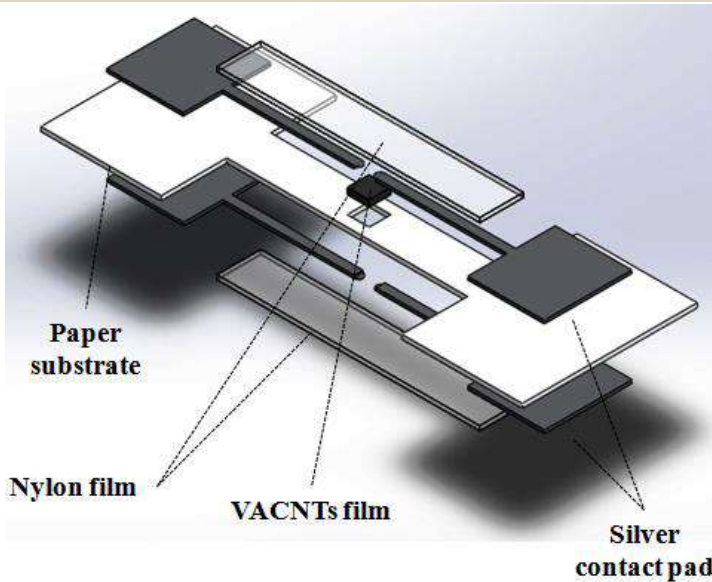
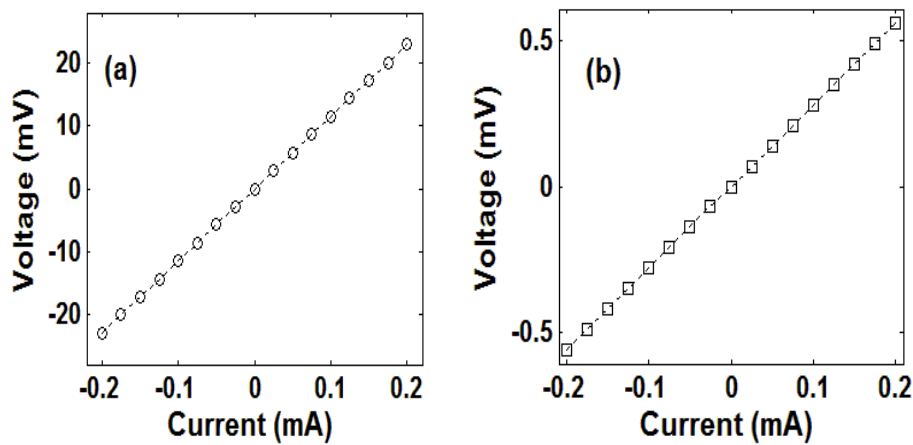**Fig. 2:** Schematic view of a VACNTs/paper-based load sensor

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Fig. 3: Linear current–voltage curves for VACNTs film (a) perpendicular and (b) parallel

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Since the VACNTs are strongly attached to the surface of the sample, and the dimension of testing element is ignorable compared to the paper substrate, it is reasonable to assume that the maximum strain in the middle of the beam is completely transferred to the VACNTs. The strain in the sample can be written as below

$$(1) \quad \varepsilon = \frac{3FL}{4EWH^2}$$

where the young's modulus is shown by E, the applied force is presented by F, and the length, width, and thickness of the paper beam are shown by L, W, and H, respectively.

In order to compare the results from VACNTs/paper-based sensor and literature, the gauge factor G can be calculated as:

$$(2) \quad G = \frac{\Delta R / R_0}{\varepsilon}$$

Where ε and ΔR are the strain and relative change of resistance, respectively.
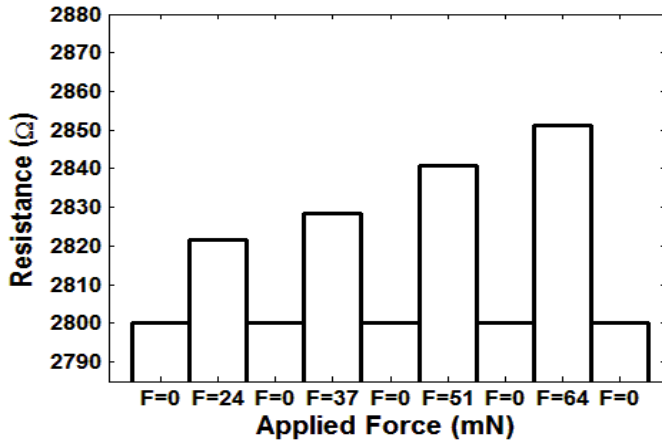
Fig. 4: The electrical resistivity variation of longitudinal VACNTs against applied force (Unload condition indicated when F=0).

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[Fig. 5] shows the relation between the relative resistances (ΔR/R) versus applied strain of VACNTs element. The longitudinal (GL) and transverse (GT) gauge factors, measured when the VACNTs are respectively aligned with longitudinal stress direction, are GL= 9.07 and GT = 0.76. The results presented a linear resistance change with the applied forces. The sensitivity of the VACNT film was found out to be 1.9 mVmN-1. Moreover, by studying [Fig. 5] the range of linearity and force resolution were found to be 110 mN and 50 µN, respectively.
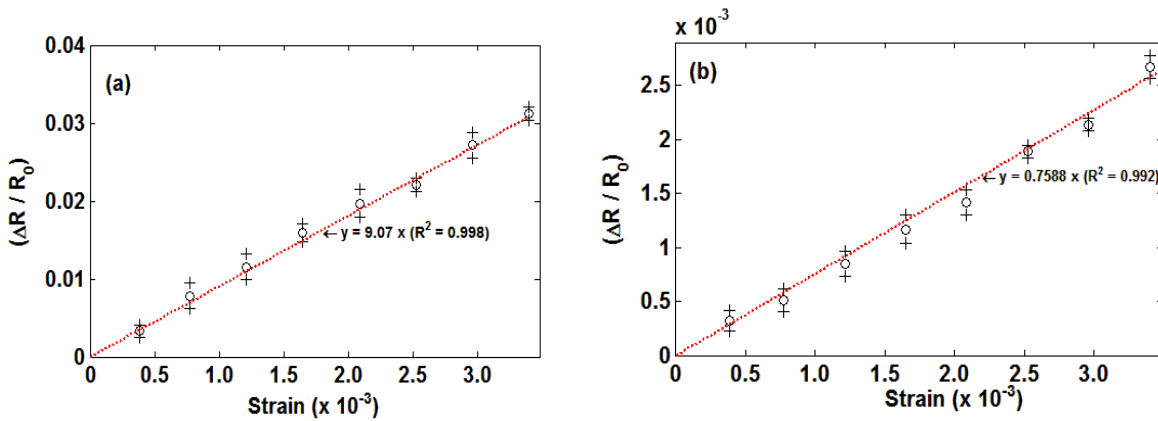

Fig. 5: Relative change of resistance against the applied mechanical strain of VACNTs (a) longitudinaly and (b) transversely aligned directions.

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As it can be observed in [Table 1], the measured gauge factor values of VACNTs are about 3 times higher than the gauge factor of CNTs film [7 and 8] and bundle [17], which have been previously reported in literature. The higher gauge factor of VACNTs indicates higher sensitivity of this sensor, in comparison with other reports.

[Table 1]: Measured gauge factors value of CNTs.

Work	gauge factor
This work (VACNTs)	9.07 (longitudinal)
	0.76 (transverse)
X Li et al. [7] (MWCNTs film)	2–3.76
Song et al. [8] (MWCNTs film)	0.01–1.25
Sickert et al. [17] (SWCNTs bundle)	2

On the other hand, the presented gauge factors are far away from that individual CNT reported by Tombler et al. [2] and Cao et al. [3].

In this work, the calculated gauge factor is the result of the integration of CNTs instead of single CNTs, leading to a lower gauge factor in comparison with published works with much higher gauge factor. Due to the imperfect alignment of the nanotubes in the forest, nanotubes might form small bundles. This imperfection provided the essential lateral interconnection for mechanical cohesiveness, but deteriorated the principal orientation of the sensing element.

CONCLUSION

In this paper, an attempt has been made to use the piezoresistive effect of vertically aligned carbon nanotubes in the sensing applications. We have developed a paper-based sensor based on piezoresistive property of VACNTs for measuring the magnitude of applied load. We have prepared anodized aluminum oxide substrate, and VACNTs film through the aforementioned process. The results showed that using VACNTs film increased the gauge factor (GL= 9.07 and GT = 0.76) and subsequently improved the sensitivity of the system. VACNTs exhibited the following characteristics: range of linearity of 110 mN, force resolution of 50 µN, and the sensitivity of 1.9mV.mN-1.

CONFLICT OF INTEREST
There is no conflict of interest.

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ARTICLE

EXPLAINING THE UNDERSTANDING OF MEDICAL CENTERS STAFF OF THE CAPACITY OF RADIOLOGY INFORMATION-SYSTEM (RIS): A QUALITATIVE STUDY

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ABSTRACT

Introduction: Radiology Information System impacts on improving the performance of doctors and other radiology personnel and more importantly improving treatment-management status of patients trough better management of radiology information. It seems that evaluating the effectiveness of the system is important from the perspective of radiology employees. **Methods:** In this qualitative study, data were collected through semi-structured and in-depth interviews and analyzed by content analysis and constant comparison method. Participants were 12 employees of radiology departments of affiliated hospitals of Semnan University of Medical Sciences, Iran. Purposeful sampling method was used and continued until data saturation. **Results:** The two themes that emerged from the interviews were divided into data recovery capacity and system capacity in information input and output. The themes were included access to patient's previous information, search features in the system, providing the desired output and input data. **Conclusion:** This study showed that hospital staff of training hospitals of Semnan has limitedly understood the capabilities of Radiology Information System. This was because of the limited capacity of this information system in information retrieval, image archiving, and limited data entry methods that had led the employees not to well understand the benefits of this information system.

INTRODUCTION

Radiology Information Systems (RIS) is one of the sub-systems of hospital information system (HIS) formed for sharing information, contributing to the effectiveness of clinical processes and reducing errors [1,2]. It is now more than seven years that Semnan University of Medical Sciences has launched RIS in the radiology wards of the subsidiary hospitals. During the initial examination of this system, issues such as demographic, insurance, financial, and administrative information of the patients with information about the x-ray type done for clients is recorded on the computer- all the parts of this system are not yet fully active yet. Considering that a systematic evaluation of the efficacy of this information system is not available, it seems that investigating the views of the users and beneficiaries of this system about the efficiency and functionality of it can provide valuable information about the evaluation of this system. In addition, what meaning the efficiency of RIS has from the perspective of the staff and has this system been able to provide the appropriate functionality.

Given the importance of RIS in improving the performance of doctors and other radiology personnel and more importantly improving treatment-management status of patients trough better management of radiology information, and understanding users' expectations of an information system are critical in its evaluation, it seems that evaluating the effectiveness of the system is important from the perspective of radiology employees.

This is so because in Iran, no studies have been conducted on the effectiveness of this information system. RIS in the form of HIS runs in most hospitals affiliated to Semnan University of Medical Sciences, and this information system can have multiple segments. However, considering the social, economic, and cultural contexts in Iran, perhaps enabling all relevant facilities related to RIS is not possible in Semnan University of Medical Sciences. Thus, in this respect, numerous questions are raised to the researchers, such as whether this information system is efficient from the perspective of personnel. Moreover, in what fields has RIS been efficient?

MATERIALS AND METHOD

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This study has used the conventional content analysis method. Conventional content analysis is an appropriate method for obtaining valid and reliable results from textual data to create knowledge, new insights, report facts and suggest ways for practical solutions [3]. Some of the questions that this qualitative method can answer include: what the users know about the efficiency of the radiology information system and what is their view about it, the current complexities with regard to the system and what kind of effects to expect from it, which function of the information system could be more important for the users and what factors affect the efficiency of the radiology information system. Accordingly, the present study is a qualitative one because it addresses the issue of the nature and effect of information systems and inquires about the efficiency and different aspects of information systems.

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The case studies include the radiology departments in affiliated hospitals of Semnan University of medical sciences. The research sample included the administrations and personnel of the radiology department, physicians, nursing staffs and medical students. Therefore, the users who were willing to cooperate and share their experiences with regard to the radiology information systems were interviewed.

The inclusion criteria were the willingness of the participants and having at least three months of work experience. The participants were chosen by purposeful sampling from the people who had the sufficient experience about the topic and willingness to participate in the study. In light of the principle of maximum variation in sampling, the participants were chosen from the users with different age and work experience. The personal information of the participants, including, age, gender, education and experience was recorded. Sampling continued until data and category saturation [4].

12 people participated in this study. Having obtained the approval and written consent of the participants, the sample people were interviewed individually in a detailed manner. A structured interview, field notes and manuscripts were used for data collection. The core question in the interview concerned the efficiency of the radiology information system. The interviews began with an open question, namely, "What is your definition of the radiology information system?" The follow-up questions were asked based on the responses of the participants to further investigate the topic, including: what uses does the radiology information system have for you in a single shift? How helpful is the system in carrying out your duties? Is the system in your ward efficient enough? Why or why not? What criteria do you have for the efficiency or inefficiency of the system? What could increase the efficiency of the system in your ward? What are some of the advantages of using the system? Some exploratory questions such as, Could you explain more? or Please, make an example" were used to encourage the participants and get more accurate data. Each interview lasted between 30 to 45 minutes. The interviews were done in the radiology departments of the hospitals. The interview conditions, such as location, temperature, light and air conditioning were appropriate. The data collection and analysis were done simultaneously. The data analysis was done by using the conventional content analysis method and was based on the responses of the personnel.

The Lincoln and Guba criteria were used to determine the rigor of the data. Prolonged engagement of the researchers, sufficient participation, appropriate interaction with the participants, integration of the data and member check increased the credibility of the data [5]. On time transcription, external check and rereading the whole data ensured the dependability of the data. Time triangulation and sample variety increased the credibility and conformability of the data. The determining-confirming of the data was proven by the impartiality of the researchers and agreement on the codes and themes. Transferability and fittingness were observed by including direct quotations and examples and the consultation of experts in the field [6].

Ethical considerations

For this study, the approval of the committee of ethics of Semnan University of Medical Sciences was obtained. The permission of the administrative was obtained prior to the start of the study. The participants were informed about the aim of the study and the way to answer to the questions. The confidentiality of the data and the right to exit from the study were explained to the participants. Also, a written consent was received from the participants for voice recording and interview.

RESULTS

Table 1 shows the participants' demographic characteristics.

Table 1: Subjects study's demographic characteristics

Characteristics	Groups	N	%
job	Radiologist	1	8
	Nurse	1	8
	Worker	8	66
	Student	2	18
Sex	Male	8	66
	Female	4	34
Education	Technician	5	42
	Bachelor degree	3	25
	Master degree	1	8
	Doctor	3	25
Job experience (Year)	< 10	6	48
	10-14	4	34
	15=<	2	18

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After determination of the basic concepts, 29 initial codes were extracted from the interviews. These codes were summarized after reviewing and classified based on similarities and fit, and after further reviews and comparing classes, their internal meanings were identified as primary themes. The primary themes were given names according to their nature conceptually and in an abstract form. Themes and their formation process are shown in Table 2 and approved by the statements of participants.

Table 2: Codes, Classifications and Themes

Codes	Classifications	Themes
Maintenance and filing	Access to Patient's Pervious Data	The capacity of information retrieval
Data Archive		
Storage of Data		
Access to Pictures		
Limitation of Maintenance Capacity		
Data Research	Possibility of Researching in System	
Research Possibility		
Easy of Research		
Errors Report	Providing the desired output	The capacity of system in inputting and outputting of data
Practices Report		
Incomes Report		
Data Obtain Duration		
Statistics		
Practice Assessment		
Record of Patient's Identifications		
Data Input	Inputted Data	
The possibility of Data Input		
The accuracy in data entering		
Mistake in Data Input		
Unavailability of patient's Picture		
Time –consuming in data entering		
Data entering is time-consuming		
Possible of fast data entry		
Data entering via online		
Manually and Automatically Entering of Data		
Possibility of Data Editing		
Inability to Edit the Recorded Data		
Completely Entering of Data		
Awareness of Patient's History		

A: Data recovery capacity

Access to patient's previous information

Participants expressed that RIS should be such that patient records, information, characteristics of patients, hospitalization history, and other demographic data can easily be determined and used. Participants believed RIS should have the ability to archive patient information, so that it can be used at whenever necessary. Based on their responsibility, the staff should be able to have access to radiology images of the patient whenever needed, and if they have additional information about previous diseases and disease history, they should be able to view them without any problems. The following two quotes exist in line with this concept.

"All information is stored in a server. Images of years are saved, and it is a very good archive." "Another advantage of this system is that you can go and see the image of your patient even when he is not in the hospital because it keeps and saves it and has a memory. You can see the image of everyone who has come to the hospital from the first day."

Search features in the system

They believed that RIS should have good search facilities, so that they can easily see patient's radiology images based on the characteristics of the patient. Alternatively, if they are supposed to look for a particular word, they could easily receive supplemental features by searching through entering a word, so that they register the intended word as soon as possible. The following is examples of statements of the

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participants. "The great help that the system has is that I can search the information I get from the patients in other parts and can review the patients' file. If it is not used properly, it may cause trouble. If I enter patient data wrongly, the images can be wrong. If I enter the patient's name wrongly, you cannot find the patient no matter how much you search. Another issue is searching. Sometimes if you do not have the patient's code, you can hardly find him."

B: system capacity in information input and output

Providing the desired output

Participants believed that RIS should have appropriate capacity in data entry and providing output. In other words, patient's information and records should enter the system easily and without spending a lot of time and with enough precision. Systems should have the ability to connect to other systems and receive information from them. One of the participants stated, "With this system, deduction, personnel errors, everything is recorded and is available. I can get daily information of exposes, income, and all the information monthly or daily." Another participant stated, "With this system, the number of exposes, number of images, the exact number of patients, and patients' records all are recorded."

Input data

Participants believed that entering patient's data to RIS is time-consuming currently, and because of problems in some cases, some problems are created in the image search that cause delay in following patient's problems. Two following two quotes are in line with this concept. "Sometimes, the patient's image in the system is not available- either the name of the patient or admit code is entered wrongly. I do not know what has happened, but sometimes, the patient's image is not in the PACS (picture archiving and communication system), and this is a problem." "Working with it is a little bit harder because we must enter everything, father's name, date of birth, National ID, and it takes a bit of time."

DISCUSSION

The results of this study showed that one of the key components of the efficiency of RIS is high capacity of data recovery by the system. RIS should provide the possibility of access to patient's previous information and provide adequate facilities to search information in the system. In their study about information systems in the radiology department, Nani et al. state that in clinical and organizational terms, the primary goal of RIS, before anything else, is to improve service delivery. In radiology department, planning for evaluation or archiving images are two important issues that HIS can help [7].

In archiving images, it is essential that the information be properly archived and retrieved when needed. In their study on the structure of the RIS, Pereira et al. stated the system's ability to store and retrieve information an essential part of the system [8]. This information includes demographic data, information created by experts, and information generated by the system itself.

Demographic data of the patients is obtained through the connection of this system with HIS, which shows the importance of the relationship between this system and its integration with other hospital information systems. Clinical information includes information that is created by an expert and used for diagnosis. Some information is also caused by the system itself that is the result of the analysis, processing, and statistical analysis [8]. This is while in the present study, RIS does not have a good relationship with other HISs, and the participants note that the specialized information about the disease is given to the patients on the CD or as images to be able to be used by professional or other colleagues. After all, although creating RIS at Kosar Hospital has not been able to save all information in an integrated way, so that it can be retrieved by the departments concerned, its weak relationship with the HIS has somewhat provided the access to specialized images. It should be noted that specialized radiology images could correctly be used only when reported by the radiologist that unfortunately, the specialized information regarding the reporting of radiologist is not recorded in RIS and only images are recoverable. Iwata et al. suggest that data integration in HIS is by forming an electronic file for each patient, where all hospital activities from admission to discharge are done [9]. It seems that, due to limitations, the possibility of integrating all data in HIS does not exist, and this change takes place gradually. The integration of this information enables recovery of all demographic, expert, and systemic information, but now there is only the possibility of retrieving images and demographic data in incomplete manner, and this can reduce the efficiency of RIS in Kosar Hospital. Adding patient demographic information completely along with the background, and the symptoms from admission systems and ward to RIS, and adding specialized information about diagnosis in RIS, so that they are easily retrievable could increase the efficiency of this system in the hospitals of Semnan University of Medical Sciences and have the advantages mentioned about the use of RIS.

Due to limitations listed in RIS of the hospitals affiliate of Semnan University of Medical Sciences, some of the functionalities of the system have been understandable for users and some benefits of it have not.

This was so that some users were able to understand features such as access to images, the possibility of archiving information, and search for information in information system understand, while some were dissatisfied with these capabilities of the system.

One of the users was a ward nurse stated, "All information is stored in a server. Images are saved for years, and it is a very good archive." Another staff also stated, "Information system has enabled me to see the images taken a few days ago, but if it were radiology image, we could not see the image after discharge."

Thus, to gain access to patients' images and disease records, it is necessary that the information system be capable of storing and archiving information, because this information integration causes the user to be able to have access to image data and disease records at any time from any part of the department. The results indicate that this information system has been successful in one part of the management of patient information, because the availability of information about the processes of storage, retrieval, and notification (distribution of information) are, in fact, among the characteristics of quality management.

Data should be readily available and usable whenever needed (for all clinical, administrative, and organizational purposes), otherwise their documenting and detailed recording are lost [7]. In their study in America with the aim of "Studying the effects of RIS / PACS system on professional performance of radiologists," Fredell et al. concluded that the use of this technology accelerates doctors' access to diagnostic images, increases specialized skills, and satisfaction [10]. Their study also showed that launching RIS / PACS is a factor to improve access to diagnostic images, provide better interpretation, increase efficiency, and ultimately user satisfaction [11].

In relation to the theme of system capacity at the entry and exit of information, the users were able to understand reporting capabilities, the patient's profile, and entering the required information. This was to an extent that many radiology staff said, "In this system everything is precise and can be checked. In this system, when a medicine is returned, I should check it. In the previous system, sometimes three or four days later, the accountant would say, for example, your paper does not have date, and it has problems, but now investigating is easier. The same goes for images. Moreover, in this system, I enter the patient's information services, accurate patient information with respect to the type of insurance, the service that should be done as directed by the doctor, and so on, everything is entered and exists."

Capacity of the system at the input and the obtaining output is one of the important points in RIS efficiency. High capacity in entering accurate and adequate information can increase satisfaction of information output. The study by Yaghmaei et al. regarding the involvement of the staff in attitudes towards computers showed that, computer systems and HIS have brought about easier access to patient's information. This issue is largely related to the proper and comprehensive insertion of information and the possibility to provide suitable and comprehensive output [12].

The results show that the presence of information systems in radiology has led the users to believe that RIS is more of a health care support than a hindrance, because this system is designed to support professional processes in the radiology department. This system has the responsibility for functions like registration and patient's follow-up, examination scheduling, reporting the results, film archive management, database management, auditing and accounting, as well as automatic fax of the reports to the related physician [13]. These capabilities of RIS work to improve hospital performance, as the findings of Yucel et al. about the criteria used to evaluate HIS showed 63 percent performance improvement as one of the management benefits [14]. Moreover, in the study by Alipour et al. it was shown that by using this system, a significant decrease in the use of processes affecting the patient length of stay took place and improved hospital performance [15]. The findings of these studies are consistent with our study.

CONCLUSION

This study showed that hospital staff of training hospitals of Semnan has limitedly understood the capabilities of RIS. This was because of the limited capacity of this information system in information retrieval, image archiving, and limited data entry methods that had led the employees not to well understand the benefits of this information system.

FINANCIAL DISCLOSURE
None

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ARTICLE

REMOVAL OF METHYLENE BLUE DYE FROM SOLUTIONS USING GRANULATED FLY ASH

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ABSTRACT

About 80,000 tons of dyestuff is produced in India annually for different industrial applications. Dyes enter the environment through wastewater released from dye manufacturing and dye consuming industries. It is estimated that 10–15% of the dyes are lost in the effluent during the dyeing process. The effluents are highly polluted and are toxic to the aquatic life present in receiving waters. Disposal of fly ash emanating from thermal power stations is another problem which is causing serious global concerns. Utilization of this material as an economical and low-cost product for different applications such as building material, filler and as an adsorbent has been researched. In the present studies, fly ash was utilized as a potential low-cost adsorbent for the removal of methylene blue dye from aqueous solutions. Adsorption studies were carried out in batch experiments with different concentrations of the dye and adsorbent and different exposure times. In order to facilitate the removal of the fine particulate adsorbent after use, the fly ash was granulated in calcium alginate gel. The granules could be used for removal of methylene blue from solutions, albeit with a lower efficiency. The removal of methylene blue varied from 0.0034 to 0.2, mgg-1 respectively when the initial dye concentration was raised from 1 to 20 mgL-1. The dye removal by fly ash granules followed Langmuir and Freundlich adsorption isotherms. The massive amounts of waste fly ash may serve as a valuable resource to reduce water pollution and recover the dyes from wastewaters. The fly ash, being a stable inorganic material can be subsequently put to end-use as filler or a building material.

INTRODUCTION

Combustion of coal in modern power plants produces some bottom ash while most of the burnt minerals escape with the flue gasses in the form of fly ash that is subsequently removed from the gas by electrostatic precipitation. About 125 million tons of fly ash is produced in India annually, of which only about 2% is used [1]. The remaining huge amount has raised serious concerns about disposal and/or use. In India about 80,000 tons of dyestuff is produced, of which a large portion is lost in effluents during dyeing process causing water pollution [2]. The present work describes attempts to utilize fly ash as an adsorbent for removal of dyes from solutions. Fly ash is well known as a cost-effective adsorbent and has been used for removal of metals and dyes [3-5]. A variety of cheap and abundantly available materials have been used for the removal of pollutants, but these may require costly processing to generate adsorbents. Use of fly ash, on the other hand, eliminates the time and cost of any further elaborate processing and it is available in huge amounts as a waste material.

MATERIALS AND METHOD

The Fly ash sample- the fly ash sample was obtained from Eklahara Thermal Power Station located in Nashik, India. The as obtained sample was washed thoroughly with deionized distilled water, dried in an oven at 800 °C for 2 h, sieved to desired particle size (150 μm) and finally stored until use. The morphology of fly ash was studied by scanning electron microscopy (SEM).

Preparation of fly ash granules

Alginate-fly ash composite granules were prepared by admixing fly ash in sodium alginate solution (1 % w/v, 100ml) followed by dropwise extrusion of the solution into a cold solution of $CaCl_2$ (0.1 M) through a 5ml syringe. The granules were allowed to harden by leaving them in $CaCl_2$ solution for 24 h at ambient temperature followed by washing with deionized double distilled water.

Dye sample

A Stock solution of methylene blue dye (100mg.l-1) was prepared by dissolving an appropriate amount of the dye in deionized double distilled water. Working solutions were prepared by suitably diluting the freshly prepared stock solution with deionized double distilled water.

Adsorption studies

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Dye removal studies were carried out at ambient temperature, c.a. 25°C±2°C by admixing suitable amounts of the fly ash sample with methylene blue solutions (1-20 mg.dm-3, 5ml) adjusted to pH 7 ± 0.1.in test tubes. The tubes were shaken at 100 rpm using an orbital shaker (Orbitek, India) for up to 2 hr

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in order to facilitate contact of dye molecules with the adsorbent. After exposure for appropriate time intervals, the fly ash was separated by filtration using a Whatman No.1 filter paper and the solutions analyzed for residual dye content by absorbance measurements using a UV-VIS spectrophotometer at 665nm. Appropriate negative controls of dye samples without fly ash were kept in order to eliminate error due to adsorption of dyes to the filter paper and/or the test tubes. Activated carbon was used as the positive control in the experiments. Similar experiments were carried out using granulated fly ash with activated carbon granules as positive control. Efficiency (%) of dye removal was calculated in order to determine the optimum parameters according to equation 1 as follows:

$$E (\%) = (C_i - C_f / C_i) \times 100 \qquad (1)$$

Where, E is the dye uptake efficiency, C_i is the initial dye concentration and C_f is the final dye concentration in mg L-1.

The uptake capacity (Qe) in batch experiments was calculated as follows (equation 2):

$$Q_e = V(C_i - C_e)/1000W \qquad (2)$$

Where, Q_e is the adsorption capacity (mg g-1), C_i is initial dye concentration (mg L-1), C_e is equilibrium dye (mg L-1),W is the amount of fly ash (g) and V is the volume of dye solution (mL).

Data emanating from adsorption studies was plotted to construct isotherms according to the Freundlich and Langmuir models. The linearized form of Freundlich isotherm is represented by equation 3 as follows:

$$\text{Log } Q_e = \log K + (\log C_e)/n \qquad (3)$$

Where, C_e is the equilibrium dye concentration (mg L-1), Q_e is the amount of dye adsorbed (mg g-1), K is adsorption capacity and 1/n is the adsorption intensity. A plot of log Qe versus log Ce gives a straight line of slope 1/n and intercept K.

The capacity of dye uptake was determined by plotting Ce/Qe against Ce, using the Langmuir equation. The linear equation of Langmuir represented as equation 4. The Plot of specific sorption Ce/Qe against equilibrium concentration Ce gave the linear isotherm parameters Qmax and b.

$$C_e/Q_e = (1/Q_{max} b) + (C_e/Q_{max}) \qquad (4)$$

Where, C_e is the metal concentration in the solution at equilibrium (mg L-1), Q_{max} (adsorption capacity) and b (energy of adsorption) are the Langmuir constants.

RESULTS AND DISCUSSION

Fly ash sample- the fly ash sample consisted of near homogeneous particulate matter. The loss in weight after washing with deionized water was negligible. Characterization of fly ash by scanning electron microscopy (Figure 1) revealed characteristic spherical particles of <5μ diameter. Chemically, fly ash consists of oxides of metals that play the vital role in the adsorption behavior [1]. Nalawade and co-workers reported the occurrence of metals in soil in the vicinity of thermal power stations and fly ash dumping grounds providing evidence for the inorganic constituents of fly ash [6-7].

Preparation of fly ash granules- Different methods have been reported for entrapment of biomass and other materials in polymeric matrices. Polymeric material derived from poultry waste was used in the preparation of beads for silver and gold biosorption [8-9]. In the present study, alginate was used as the matrix for preparation of fly ash granules because alginate is a natural, biodegradable, nontoxic and hydrophilic polymer that produces thermostable gel by association with divalent cations [10]. Carboxyl and hydroxyl groups of alginate play an important role in cation exchange capacity and affinity of alginates towards metal ions [10, 11] although the exact mechanism of sorption is not understood. Due to the swelling behavior of alginates, the granules are highly porous with good water retention capacity and thereby display good mass transfer kinetics for sorption of solutes from aqueous media [12-13]. Alginate gels are also used for encapsulation of macromolecular agents and low molecular weight therapeutic agents [14]. The alginate-fly ash composite granules used in present studies were spherical (3.4 mm average diameter), grey in color and had good swelling property and water retention capacity as evidenced from a 40% increase in diameter when soaked in water.
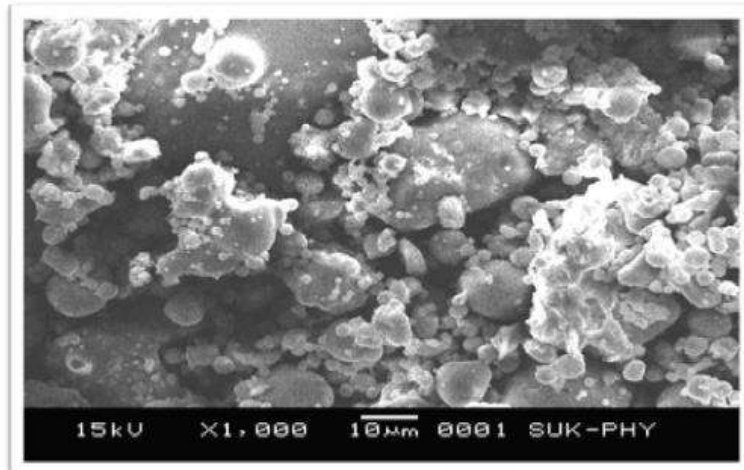
Fig. 1: SEM image of fly ash sample

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Adsorption studies- Adsorption occurs due to unbalanced residual forces at the solid or liquid surfaces and is a surface phenomenon in contrast to absorption which is a bulk phenomenon. It was observed that dye uptake was rapid in the initial stages of the contact period (5 to 120 min) and subsequently became slow as the system attained equilibrium [Table-1]. This could be a result of the progressive reduction in the number of available binding sites for the dye with time and the increasing repulsion between the bound and unbound dye molecules. Pre-soaked granules displayed more rapid adsorption than the dry granules as earlier reported for metal biosorption by *C. cladosporioides* biomass beads [9].

Table 1: Effect of time on removal of MB by fly ash, fly ash granules and activated carbon

Time (min)	Dye removal Efficiency (%)		
	Activated carbon (powdered)	Fly ash (powdered)	Fly ash beads
5	68.45	4.7	7.5
10	70.12	7.93	8.78
15	74.80	28.65	34.33
30	85.96	56.43	50.34
60	>99	56.55	50.40
120	>99	56.55	50.81

The simplicity of the molecular structure of methylene blue and the presence of silica, iron and calcium oxides in fly ash indicates that surface adsorption by physico-chemical processes (hydrogen bonding and Van der Waal's forces) is possible without much stearic hindrance. Interestingly, it was observed that the efficiency of dye sorption with activated carbon was very high in the initial 5 minute contact (>95%) while it was low (<10%) in case of fly ash and fly ash granules. The equilibrium values were similar (>50%) for activated carbon and fly ash justifying the use of fly ash as a cost-effective adsorbent for dyes. According to Khan et al. (2009), the hydroxyl groups associated with fly ash play a role in binding to charged pollutants in the wastewater. The adsorbent acquires positive charge below the pHzpc value and negative charge above this value that aid in binding [1]. Weber and Morris demonstrated increase in adsorption capacity with the increase in molecular weight of solutes [16].

Table 2: Effect of dye concentration on removal of MB by fly ash and activated carbon

MB Conc. (mgL⁻¹)	Activated carbon		Fly ash powder		Fly ash beads	
	Efficiency (%)	Specific dye uptake (Q)	Efficiency (%)	Specific dye uptake (Q)	Efficiency (%)	Specific dye uptake (Q)
1	92.3	0.0046	63	0.0031	69.03	0.0034
2	93.02	0.0419	54.3	0.0123	55.00	0.0143
5	90.96	0.1074	55.11	0.0691	55.35	0.0692
10	97.56	0.4883	10.02	0.0582	13.41	0.0610
15	93.22	0.9582	9.04	0.1432	10.42	0.1562
20	87.03	1.14	3.09	0.2	5.00	0.21

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Table- 2 shows the increase in the dye uptake capacity in terms of mg dye bound per gram of the adsorbent on a dry weight basis as a function of initial dye concentration. Increase in dye concentration from 1 to 20 mg L^{-1} resulted in a 60-fold increase in the dye uptake capacity of granulated fly ash from 0.0035 to 0.21 mg g^{-1}. The increase in dye uptake capacity in a case of activated carbon was to the tune of 250-fold. However, the efficiency of dye sorption reduced at higher concentration of the dye, particularly in the case of fly ash indicating that the fly ash can be used as a polishing treatment to remove residual traces of dye remaining in wastewaters after physicochemical treatments. The studies indicate that it might be necessary to undertake efforts to improve the performance of fly ash by physical and/or chemical treatments.

The process of adsorption is usually studied by plotting the well-known adsorption isotherms, viz. the Langmuir and Freundlich isotherms that provide insight into the sorption mechanism, surface properties and affinity of adsorbent for the adsorbate. Modeling of equilibrium data allows comparison among different adsorbents under different operational conditions, designing and optimizing the processes [10] and also for the comparison of data from different laboratories. In the present studies, it was found that the methylene blue adsorption by fly ash granules obeyed both Freundlich [Figure-2] and Langmuir [Figure-3] adsorption isotherms with R^2 values exceeding 0.95. The data pointed to monolayer coverage with constant adsorption energy according to the Langmuir model and the involvement of heterogeneous surfaces according to the Freundlich model [1, 8, 10, 15].
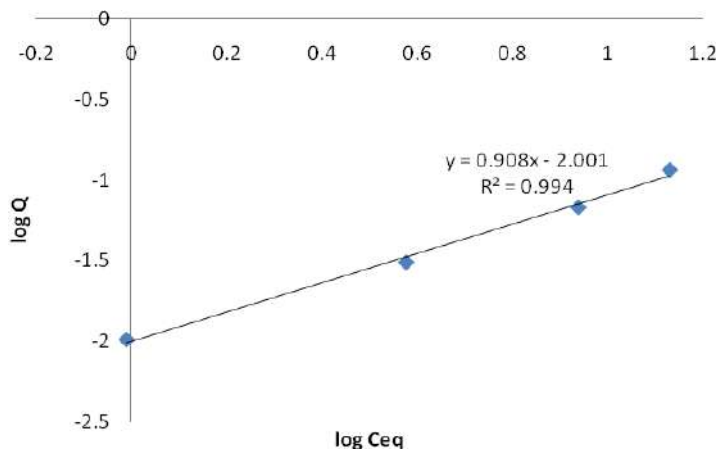

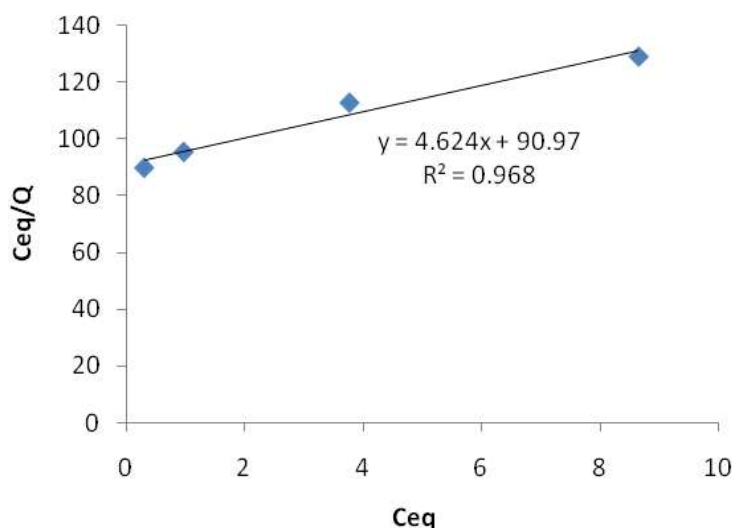**Fig. 2:** Freundlich isotherm for methylene blue uptake by fly ash granules

Fig. 2: Langmuir isotherm for methylene blue uptake by fly ash granules

One important characteristic of Langmuir isotherm is a dimensionless constant R$_L$ given by Equation 2 as follows:

$$R_L = \frac{1}{1 + bC_0}$$

(2)

Where C_0 (mg dm^{-3}) is the initial concentration of adsorbate and b (dm^{-3} mg^{-1}) is the Langmuir constant. There are several probabilities for R_L value: for linear sorption $R_L = 1$; for favorable sorption $0 < R_L < 1$; for irreversible sorption $R_L = 0$ and for unfavorable sorption $R_L > 1$ [10]. The dye uptake data in the present investigations pointed to favorable sorption kinetics with R_L value of 0.51.

The values of both Langmuir and Freundlich isotherm parameters are given in **Table- 3.**

Table 3: Parameters for Langmuir and Freundlich adsorption

	Langmuir Parameters		Freundlich Parameters	
Q_{max}(mgg^{-1})	4.62	K		0.09
b (L.mg^{-1})	0.19	1/n		0.79
R^2	0.967	R^2		0.994

It is worth mentioning that in contrast to most of the attempts towards degradation of dyes, the present work points towards the possibility of recovery of dyes from wastes using waste fly ash. Both the recovered dye and regenerated fly ash beads can be recycled for further dye removal process. The possibility of selective dye adsorption and/or desorption from the fly ash granules needs to be investigated in order to make the process applicable on a large scale for real industrial effluent containing a mixture of dyes.

CONCLUSION

This work indicates that alginate-fly ash granules can be used for removal of dyes from wastewater as exemplified by the uptake of methylene blue dye. The dye adsorption obeyed Langmuir and Freundlich isotherm models. This study highlights the use of granulated fly ash for environmental pollution control that would aid in easy disposal and/or reuse of the fly ash. In the present studies, fly ash was utilized as a potential low-cost adsorbent for the removal of methylene blue dye from aqueous solutions. Adsorption studies were carried out in a batch experiments with different concentrations of the dye and adsorbent and different exposure times. In order to facilitate the removal of the fine particulate adsorbent after use, the fly ash was granulated in calcium alginate gel. The granules could be used for removal of methylene blue from solutions, albeit with a lower efficiency. The removal of methylene blue varied from 0.0034 to 0.2, mgg^{-1} respectively when the initial dye concentration was raised from 1 to 20 mgL^{-1}. The dye removal by fly ash granules followed Langmuir and Freundlich adsorption isotherms.

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ARTICLE

EFFECT OF SUBLETHAL EXPOSURE OF HERBICIDES ON THE BIOCHEMICAL PARAMETERS OF EARTHWORM *EUTYPHOEUS WALTONI* MICHAELSEN [OLIGOCHAETA: OCTOCHAETIDAE]

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ABSTRACT

The earthworms play an important role in recycling of organic wastes and maintain fertility of agricultural fields. Herbicides are the plant growth hormones and generally used for removal of unwanted weeds. The aim of the present study was to observe the effect of sub-lethal exposure of [40 and 80%] of LC50 24 h and 240 h of herbicides 2,4-D and Butachlor on the biochemical parameters [Proteins, Amino acid, DNA, RNA, Acid/Alkaline phosphatase and AChE activity] of gonads of earthworm of Eutyphoeus waltoni in feed materials of buffalo dung with agro-wastes. Effect of sub lethal 40% and 80% of LC50of feed material of buffalo dung with agro-wastes of different exposure periods on the biochemical estimation on of earthworm Eutyphoeus waltoni. The maximum reduction [in per cent change] in Protein, Amino acid, DNA, RNA and inhibition in alkaline/ Acid and AChE activity was observed in the treatment of 80% of 240h LC50 of butachlor [14.74%], [35.29%], [29.16%], [26.12%], [28.27%], [33.04%], [17.28%] respectively. There was significant [P< 0.05, t- test] difference between control and 40% and 80% of LC50 of butachlor and 2,4-D. After withdrawal of experiment for 7 days there was no recovery was observed.

INTRODUCTION

Abundant uses of chemical fertilizers, pesticides and herbicides have made our soil sick and problematic. The long-term use of chemical fertilizers and herbicides has lead to imbalance of our natural ecosystems. The use of chemical herbicides and fertilizers in the agricultural field found to cause adverse effect on soil organisms [1,2].The need to produce more food for the ever increasing world population especially in the developing economics requires extensive use of agrochemicals such as pesticides and herbicides but they have adverse effects on non-target soil organism like the earthworm [3]. Herbicides are commonly known as weed killers to kill unwanted plants. Selective herbicides kill specific targets, while leaving the desired crop relatively unharmed. Some of these act by interfering with the growth of the weed and are often synthetic mimics of natural plant hormones [4].

The 2,4-D [2,4 dichlorophenoxy acetic acid] is a herbicide and a plant growth regulator [5]. Although research into chemical herbicides began in the early 20th century. The first modern herbicide, 2,4-D, was first discovered and synthesized by W. G. Templeman at imperial chemical industries. The chemical; composition of 2,4-D is 2,4-D Acid technical 59% w/w, dimethyl amine 30% w/w, Seqvesting agent [lignin sulphonate] 1% w/w, Diventacqua sufficient 10% w/w. 2,4-D is an herbicide as well as a plant growth regulator [5]. The chemical name of 2,4-D [2, 4-dichlorophenoxyacetic acid] was given by International Union of Pure and Applied Chemistry [IUPAC] which include esters, acids, and several salts [6]. The herbicide 2,4-D and butachlor have toxic effect against earthworm *Eutyphoeus waltoni* in feed materials of buffalo dung with agro-wastes as well as soil alone [7,8].

Butachlor [2-chloro-2', 6'-diethyl-N-[butoxymethyl] acetanilide] is used for the control of undesirable grasses and broadleaf weeds in transplanted paddy and barley fields. Butachlor was the first rice herbicide to be introduced in India. The application of huge amount of herbicides, affect not only target plants but also other non-targeted ones. The pathway of attack can arise from intentional direct consumption, improper application resulting in the herbicide coming into direct contact with people. Under some conditions, certain herbicides can be transported via leaching or surface runoff to contaminate groundwater or distant surface water sources. The use of herbicides has adverse effect on the survival of earthworms as well as its growth and reproduction [9-13].

Earthworms are considered as one of the most important biotic components of the soil because their role in mineralization and breakdown of organic matter which results formation of soil structure and humus soul for maintains the soil fertility. Earthworms are one of the best bio-indicator of pesticides contaminated soil. Due to high concentration of organo-chemicals at upper surface layer of soil, reduced earthworms activities in their habitat [14, 15]. Earthworms have the high biomass of terrestrial invertebrates which play an important role in structuring and increasing the nutrient content of the soil. Therefore, they can be suitable bioindicators of chemical contamination of the soil in terrestrial ecosystems providing an early warning of deterioration in soil quality [16, 17]. The suitability of earthworms as bioindicators in soil toxicity is high due to the fact that they ingest large quantity of the decomposed litter, manure, and other organic matter deposited on soil [18, 19]. Earthworms are often used as test organism because of their important function as decomposer and their sensitive reaction towards environmental influence. In terrestrial ecosystem, earthworms are so important experimental organism for ecotoxicity because their high growth and reproduction rate.

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The aim of the present study was to observe the effect of sub-lethal exposure of [40% and 80%] of LC$_{50}$ 24 h and 240 h of herbicides 2,4-D and Butachlor on the biochemical parameters [Proteins, Amino acid, DNA, RNA, Acid/Alkaline phosphatase and AChE activity] of gonads of earthworm of *Eutyphoeus wa*ltoni in feed materials of buffalo dung with agro-wastes.

MATERIALS AND METHODS

Collection and culture of earthworm *Eutyphoeus waltoni*

 The earthworm *Eutyphoeus waltoni* was collected from agricultural fields of Gorakhpur District. of Uttar Pradesh, India and was reared in the Vermibiotechnology Laboratory, Department of Zoology, Deen Dayal Upadhyaya, Gorakhpur University, Gorakhpur, UP. INDIA at room temperature. Adult earthworms of same age group were used in the experiments.

Collection of biological wastes

 The feeding materials [buffalo dung , wheat straw and gram bran] for the earthworm *Eutyphoeus waltoni* were collected from different parts of Gorakhpur district in U.P., India.

Herbicides

 Commercially available herbicides butachlor [2-chloro 2,6 diethyl N, butoxymethyl acetanilide] was purchased from Aristo biotech and life science Pvt. Ltd., E-26, G.I.D.C. Manjusar, savli,Distt.Vadodara-391755 , Gujrat [India] and 2,4-D [2'-4' dicholorophynoxy acetic acid] was purchased by Earth Care Pvt. Ltd. Kolkatta [India], used in the experiment at different concentrationsat laboratory conditions.

For biochemical estimation of earthworm *Eutyphoeus waltoni,* the experiment was conducted on cemented earth surface. One kg of different combinations of animal dung, agro wastes in different ratio were kept on 30 × 30× 10 cm in bed form at room temperature in dark. The vermicomposting beds were turned over manually every 24 hours for 10 days in order to eliminated volatile substances. After treatment 40% and 80% of LC$_{50}$ of 2,4-D and butachlor of feed materials of all exposure periods, ten adult earthworms were incubated in each vermibed for the observation of biochemical effects e.g. Proteins, Amino acid, DNA, RNA, Acid/Alkaline phosphatase, and AChE on earthworms by 2,4-D and Butachlor herbicides

Biochemical Estimation

 Protein levels in the gonads of earthworm *Eutyphoeus waltoni* were estimated according to lowery et al. [20]. Animals were dissected, their gonads were removed, weighed and homogenates [1mg/ml, w/v] were prepared in 10% TCA. Values have been expressed as µg protein/mg gonads.

Estimation of total free amino acids in gonads of *Eutyphoeus waltoni* were made according to the method of Spies [21]. The gonads were homogenized in 96% ethanol [10:1 w/v] in an electrical homogenizer for 5 min and centrifuged at 8000 g for 20 min.

Estimation of nucleic acid [DNA and RNA] in gonads of *Eutyphoeus waltoni* were estimated according to Schneider [22] by use of diphenylamine and orcinol reagents, respectively. Homogenates [1mg/ml, w/v] were prepared in 5% TCA at 90˚C, centrifuged at 5000 g for 20 min and supernatants were used for estimations.

Activity of acid and alkaline phosphatase in the gonads was determined according to Andersch and Szcypinski [23] as modified by Bergmeyer [24] and Singh and Agarwal [25] by use of p- nitrophenyl phosphate as substrate. Homogenates of gonads [2% w/v] were prepared in ice cold 0.9% Nacl solution and centrifuges at 5000 g [37'C] for 20 min. The supernatants were used as enzyme source.

Acetylecholinesterase activity was measured according to the method of Ellman et al. [26]. Acetylecholinesterase hydrolysis into the product choline and acetate. The method is based on the principle that free 'SH' groups released from the thioesters of choline. The AChE hydrolyze the dye DTNB to form a yellow product.

The gonads of earthworm *Eutyphoeus waltoni* were removed from the body, and homogenized in 1.0 ml of 0.1 M phosphate buffer cold pH 8.0 [50mg/ml] for 5 min. in ice bath. Therefore, centrifuge the homogenate at 1000g for 30 min. in a refrigerated centrifuge [0-4˚C]. Protein estimated was done by the method of Lowry et al. [20].

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In withdrawal experiments the earthworms were transferred from sub lethal exposure of 24h and 240h LC$_{50}$ of 2,4-D and butachlor in to the fresh vermibed. The fresh vermibed was changed daily up to 7 days, thereafter biochemical parameters were estimated.

Statistical analysis

The data have been expressed as mean ± SE of six replicates. Student's t-test was applied between control and treated groups to locate significant [p < 0.05] variations. Product momentum correlation coefficient was determined between exposure time and different value of LC [27]. Slope values, confidence limits, t-ratio, heterogeneity factors and g-values for different toxicity study were calculated by the method of Robertson et al. [28].

RESULTS

Table 1: Effect of sub lethal exposure (40% and 80% of 24 h LC$_{50}$) of 2,4-D and butachlor on the protein, amino acid, DNA and RNA level (µg/mg) in the gonads of *Eutyphoeus waltoni*.

Parameters	Sub lethal exposure of 24 h LC$_{50}$	Control	2,4-D	Butachlor
Protein	40%	96.18 ± 1.06 (100)	70.16±1.40*(72.94)	53.87±1.46*(56.09)
	80%		45.33±1.52*(47.13)	32.83±1.37*(34.13)
Amino acid	40%	38.33±1.30 (100)	31.66±1.04*(82.59)	24.68±1.81*(64.38)
	80%		25.50±1.12*(66.52)	17.43±1.29*(45.47)
DNA	40%	110.85±2.16 (100)	98.15±1.13*(88.54)	92.66±1.33*(83.59)
	80%		65.50±1.21*(59.08)	54.83±1.57*(49.46)
RNA	40%	58.68±1.15 (100)	47.35±1.19*(80.69)	38.31±1.14*(65.28)
	80%		37.18±1.42*(63.36)	21.33±1.22*(36.34)

Each value is mean ± SE of 6 replicates. Values in parentheses are per cent change with control taken as 100%. * Significant (p<0.05, t- test) difference between control and treated groups

Table 2: Effect of sub lethal exposure (40% and 80% of 240 h LC$_{50}$) of 2,4-D and butachlor on the protein, amino acid, DNA and RNA level (µg/mg) in the gonads of *Eutyphoeus waltoni*

Parameters	Sub lethal exposure of 24 h LC$_{50}$	Control	2,4-D	Butachlor
Protein	40%	96.18 ± 1.06 (100)	31.33±1.76*(32.57)	24.22±1.10*(25.18)
	80%		20.66±1.35*(21.48)	14.48±1.07*(14.74)
Amino acid	40%	38.33±1.30 (100)	19.05±1.03*(49.69)	17.15±1.18*(44.74)
	80%		15.21±1.04*(39.68)	13.53±1.11*(35.29)
DNA	40%	110.85±2.16 (100)	75.23±1.19*(67.86)	69.16±1.79*(62.39)
	80%		36.66±1.02*(33.07)	32.33±1.22*(29.16)
RNA	40%	58.68±1.15 (100)	31.86±1.13*(54.29)	28.18±1.37*(37.79)
	80%		18.73±1.24*(31.91)	15.33±1.08*(26.12)

Each value is mean ± SE of 6 replicates. Values in parentheses are per cent change with control taken as 100%. * Significant (p<0.05, t- test) difference between control and treated groups

Table 3: Effect of sub lethal exposure (40% and 80% of 24 h LC$_{50}$) of 2,4-D and butachlor on the acid, alkaline phosphatase (µm/mg protein/30 min) and AChE activity (µm 'SH' hydrolyzed/min/mg protein) in the gonads of *Eutyphoeus waltoni*

Parameters	Sub lethal exposure of 24 h LC$_{50}$	Control	2,4-D	Butachlor
Alkaline Phosphatase	40%	4.74 ± 0.69 (100)	3.68±0.55*(77.63)	3.36±0.15*(70.88)
	80%		2.86± 0.47*(60.33)	2.51±0.06*(52.95)
Acid phosphatase	40%	3.42±0.50 (100)	2.89±0.20*(84.50)	2.56±0.10*(74.85)
	80%		2.27±0.27*(66.37)	2.11±0.24*(61.69)
AChE	40%	0.081±0.003 (100)	0.056±0.002*(69.13)	0.048±0.002*(59.25)
	80%		0.038±0.005*(46.91)	0.032±0.003*(49.46)

Each value is mean ± SE of 6 replicates. Values in parentheses are per cent change with control taken as 100%. *Significant (p<0.05, t- test) difference between control and treated groups

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Table 4: Effect of sub lethal exposure (40% and 80% of 240 h LC$_{50}$) of 2,4-D and butachlor on the acid/alkaline phosphatase (µm/mg protein/30 min) and AChE activity (µm'SH' hydrolyzed /min/mg protein) in the gonads of *Eutyphoeus waltoni*.

Parameters	Sublethal exposure of 24 h LC$_{50}$	Control	2,4-D	Butachlor
Alkaline Phosphatase	40%	4.74 ± 0.69 (100)	2.61±0.18*(55.06)	2.42±0.05*(51.05)
	80%		1.84±0.07*(38.81)	1.34±0.06*(28.27)
Acid phosphatase	40%	3.42±0.50 (100)	1.98±0.31*(57.89)	1.25±0.04*(36.54)
	80%		1.39± 0.23*(40.64)	1.13±0.05*(33.04)
AChE	40%	0.081±0.003 (100)	0.035±0.004*(43.20)	0.018±0.003*(22.22)
	80%		0.021±0.002*(25.92)	0.014±0.002*(17.28)

Each value is mean ± SE of 6 replicates. Values in parentheses are per cent change with control taken as 100%. * Significant (p<0.05, t- test) difference between control and treated groups

Table 5: The correlation coefficient (r-values) between different biochemical parameters of data Table 19-20.

Bio chemical parameters	DNA	RNA	Protein	Amino acids	Alkaline phos-phatase	Acid phos-phatase	AChE
DNA	1.00	0.915	0.852	0.768	0.681	0.498	0.633
RNA		1.000	0.888	0.892	0.681	0.607	0.724
Protein			1.000	0.883	0.573	0.527	0.667
Amino acids				1.000	0.595	0.579	0.643
Alkaline phosphatase					1.000	0.641	0.711
Acid phosphatase						1.000	0.711
AChE							1.000

All values are correlation coefficient (r-values) at significant 0.01 level, ns=not significant.

There was significant reduction in the level of biochemical parameters [protein, total free amino acid, nucleic acid and inhibition in acid/ alkaline phosphatase and AChE activity] in the gonads earthworms *Eutyphoeus waltoni* after sub-lethal exposure of [40% and 80%] of LC$_{50}$ 24 h and 240 h of herbicides 2,4-D and Butachlor. Table 1 shows that there was significant dose dependent decrease in protein level in the gonads of *Eutyphoeus waltoni* exposed to 40% and 80% of 24 h LC$_{50}$ of 2,4-D and butachlor. Maximum reduction [43% of control] in protein level of gonads was observed in the earthworms exposed to 80% of 24 h LC$_{50}$ of butachlor [Fig. 1]. [Fig. 2] shows that treatment of 40% and 80% of 240h of LC$_{50}$ of 2,4 D and butachlor caused a significant reduction in protein level in the gonads of *Eutyphoeus waltoni*. Maximum reduction [14.74%] in protein level in the gonads of *Eutyphoeus waltoni* was observed after the treatment of 80% of 240h LC$_{50}$ butachlor [Table 2].
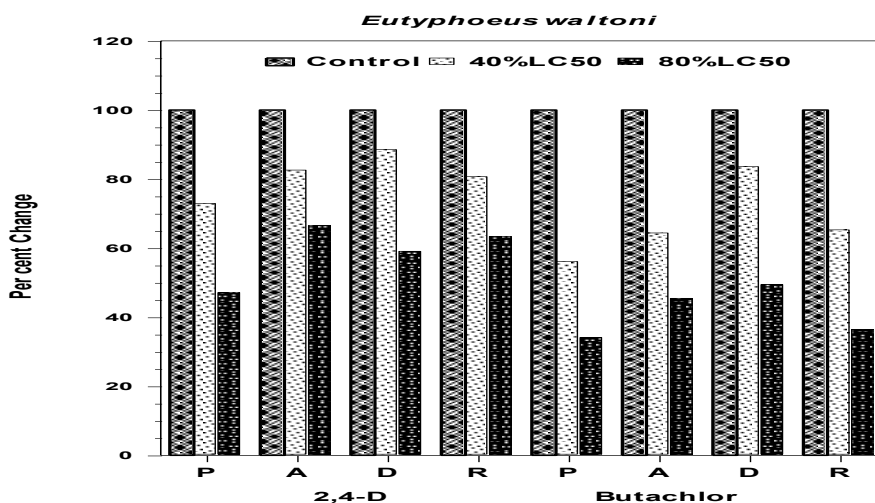

Fig. 1: Effect of sublethal exposure (40% and 80% of 24h LC$_{50}$) of 2,4-D and butachlor on the per cent change in protein, amino acids, DNA, RNA level in the gonad of earthworm *Eutyphoeus waltoni*. P= Protein, A= Amino acid, D= DNA, R= RNA

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[Fig. 1] shows that treatment of 40% and 80% of 24h LC$_{50}$ of 2,4-D and butachlor cause a significant dose dependent reduction. Reduction in total amino acid level [42.42% of control] was observed in the gonads of earthworm exposed to 80% of 24h LC$_{50}$ of butachlor [Table 1]. [Fig. 2] shows that treatment of 40% and 80% of 240h LC$_{50}$ of 2,4-D and butachlor cause significant reduction in the total free amino acid level in gonads of *Eutyphoeus waltoni*. Maximum reduction in total free amino acid level [35% of control] was observed in earthworms exposed to 80% of 240h LC$_{50}$ of butachlor [Table 2].
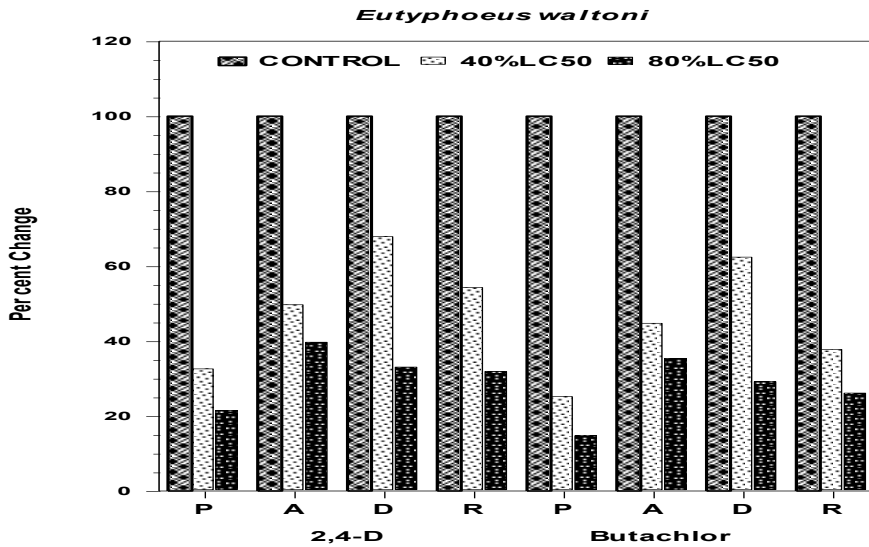

Fig. 2 : Effect of sublethal exposure (40% and 80% of 240h LC$_{50}$) of 2,4-D and butachlor on the per cent change in protein, amino acids, DNA, RNA level in the gonad of earthworm *Eutyphoeus waltoni*. P= Protein, A= Amino acid, D= DNA, R= RNA

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There was a significant dose dependent reduction in the DNA level of gonads in *Eutyphoeus waltoni* exposed to 40% and 80% of 24h and 240h LC$_{50}$ of butachlor and 2,4-D [Fig.1,2]. Treatment of 80% of 24h LC$_{50}$ of 2,4-D caused a significant reduction in DNA level to [59.08%] of control [Table 1]. Maximum reduction to [29.16% of control] was observed in the gonads of earthworms exposed to 80% of 240h LC$_{50}$ of butachlor [Table 2].

[Fig. 1] shows that the exposure of 40% and 80% of 24 h LC$_{50}$ of butachlor, 2,4-D caused reduction in the RNA level of *Eutyphoeus waltoni*. Maximum reduction in the RNA level to [36.34 of control] was observed in the gonads of earthworm exposed to 80% of 24 h LC$_{50}$ butachlor. Treatment of 40% and 80% of LC$_{50}$ of 2,4-D and butachlor caused a significant reduction in RNA level of gonads of *Eutyphoeus waltoni*.[Table 1, 2].

Fig. 3: Effect of sublethal exposure (40% and 80% of 24h LC$_{50}$) of 2,4-D and butachlor on the per cent change in ALP= Alkaline phosphatase, ACP= Acid phosphatase, and AChE= Acetylcholineesterase activity in the gonad of earthworm *Eutyphoeus waltoni*
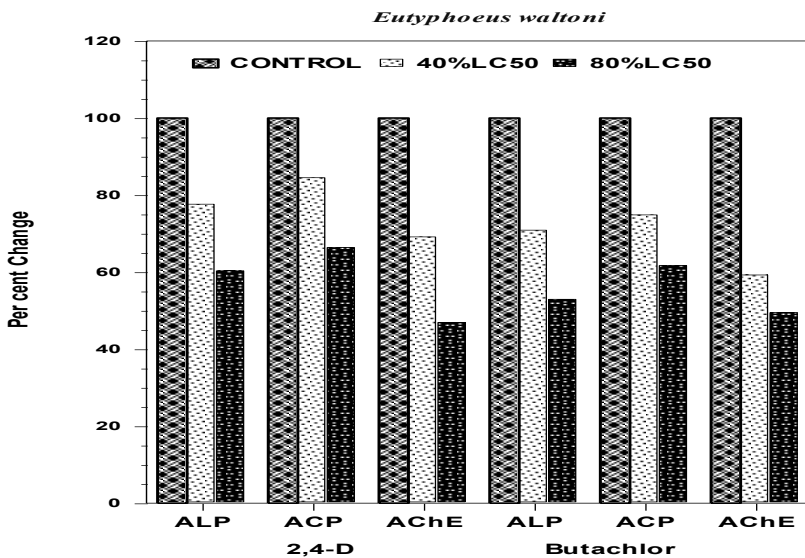
………..

Fig. 4: Effect of sublethal exposure (40% and 80% of 240h LC$_{50}$) of 2,4-D and butachlor on the per cent change in ALP= Alkaline phosphatase, ACP= Acid phosphatase, and AChE= Acetylcholineesterase activity in the gonad of earthworm *Eutyphoeus waltoni*.
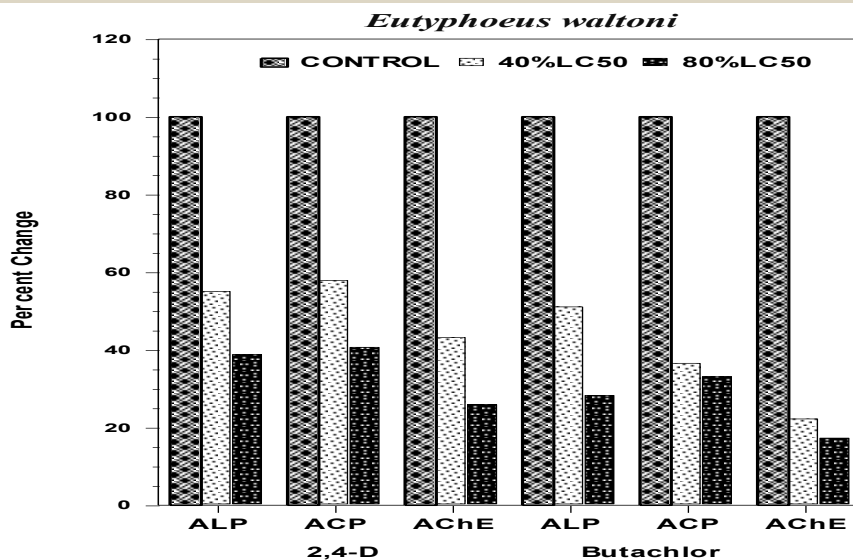
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[Fig. 3 and Fig. 4] shows that there was a significant dose dependent of acid/ alkaline phosphatase and AChE activity in the gonads of earthworms exposed to 2,4-D and butachlor. Treatment of 80% of 24h LC$_{50}$ of butachlor and 2,4-D caused inhibition in alkaline phosphatase to 52.95%of control, respectively [Table-3]. Acid phosphatase activity was inhibit to 66.37%, 61.69% in the gonads of *Eutyphoeus waltoni* exposed to 80% of 24h LC$_{50}$ of 2,4-D and butachlor, respectively. [Fig . 3] shows that the treatment of 40% and 80% of 240h LC$_{50}$ of 2,4-D and butachlor caused significant inhibition in acid and alkaline phosphatase activity in the gonads of *Eutyphoeus waltoni*. Maximum inhibition in alkaline phosphatase activity [28.27% of control] and acid phosphatase [33.04% of control] was observed in the gonads of *Eutyphoeus waltoni* exposed to 80% of 240h LC$_{50}$ of butachlor [Table 4].

The earthworms treated with sub lethal exposure [40% and 80% LC$_{50}$] of 2,4-D and butachlor showed time and dose dependent inhibition of acetylcholinesterase activity in gonads of *Eutyphoeus waltoni*. Treatment of 80% of 24h LC$_{50}$ of butachlor and 2,4-D caused the inhibition in AChE activity was observed [Table 3, 4]. [Fig 4.] Maximum inhibition was observed in 80% of LC$_{50}$ of 240h in butachlor [17.28%] than the sublethal exposure of 2,4-D. Whereas minimum inhibition in AChE activity was observed 40% LC$_{50}$ of 2,4-D [43.20%]. [Table 3, Fig. 3, 4].

In this withdrawal experiment attempts have been made to observed the level of different biochemical parameters [Protein, amino acid, DNA, RNA, acid and alkaline phosphatase and AChE activities] in the gonads of *Eutyphoeus waltoni* after sub lethal exposure of 80% of 24h LC$_{50}$ treatment of butachlor and 2,4-D for 7 days. There was no recovery in the level of protein, amino acid, DNA, RNA and acid/ alkaline phosphatase activity and AChE activity in the gonads of *Eutyphoeus waltoni*.

DISCUSSION

It is evident from results that the 2,4-D and butachlor significantly change the different biochemical parameters i.e protein, amino acid, DNA, RNA, acid, alkaline phosphatase and AChE activity in the gonads of *Eutyphoeus waltoni* within 24h and 240h.The effect of 2,4-D and butachlor in 240h of exposure on different biochemical parameters in gonads of earthworm *Eutyphoeus waltoni* was more pronounced. It seems that increase in exposure periods from 24h to 240h may cause penetration of earthworm body which cause more reduction in protein, amino acid, DNA, RNA, and acid/alkaline phosphatase and AChE activity with respect to *Eutyphoeus waltoni*.

Data obtained from the withdrawal experiment clearly indicates that effect of 2,4-D and butachlor is non reversible as the level of protein, amino acid, DNA, RNA, and acid/alkaline phosphatase and AChE activity within 7 days. Mommensen and Walsh [29] reported that Proteins which are the main source of the nitrogenous metabolism, are mainly involved in the architecture of the cell and during chronic periods of stress they are also a source of energy. Increment in the free amino acids level was the result of breakdown of protein for energy requirement and impaired incorporation of amino acids in protein synthesis. The herbicide acetochlor caused adverse effect on the sperm number and DNA in *Eisenia fetida* [30]. Gobi et al.[31]. Reported that they were glandular cell enlargement and vaccualization in the

intestine of the earthworm *Perionyx sansibaricus* exposed to sub lethal concentration of herbicide butachlor.

AChE inhibition of different animals by many organophosphate insecticides is well established. It reduced the survival rate of earthworms in agricultural soils. The present study assessed the sublethal exposure 40% and 80% LC_{50} of 2,4-D and butachlor to under laboratory conditions with special reference to the biochemical changes of earthworms. Navarro and Obregon [32]. reported that treated *Eisenia fetida* with organophosphate insecticide, Malathion and found that decreased the spermatic viability in spermathecae, altering the cell proliferation and modifying the DNA structure of spermatogonia.

This pesticideis known to retard growth and reproduction in earthworms such as *Eisenia fetida* [33]. and *Perionyx sansibaricus* as well as damage epithelial tissue in *Eisenia fetida* [34]. Butachlor is neurotoxic to land snails and genotoxic to toad and frog tadpoles, flounder, and cat fish where it causes DNA strand break induction in erythrocytes [35-38]. It is also genotoxic to cultured mammalian cells where it causes DNA strand breaks and both micronucleus and chromosomal aberration inductions [39,40].

The earthworm *Eutyphoeus waltoni* treated with sub lethal exposure [40% and 80% LC_{50}] of 2,4-D and butachlor showed time and dose dependent inhibition of acetylcholinesterase activity in gonads of *Eutyphoeus waltoni*. AChE is responsible for the termination of cholinergic impulse by the hydrolysis of acetylcholine esterase [AChE] in the choline and acetic acid [25].

The enzyme AChE units have two active sites, an anionic site, which attracts the cationic part of the substrate or inhibitor by coulombic or hydrophobic force, and anesteric site. AChE has been secreted into the synapse it binds to receptor sites on the next nerve cell, causing the latter to propagate the nerve impulse. Before the transmission of second impulse through the synapse, ACh secreted after the first impulse must be hydrolised by the AChE in the junction. Inhibition of AChE resulted in abnormal accumulation of acetylcholine which cause eventual paralysis of the muscle. Death occurs as a result of asphyxia caused by the paralysis of respiratory muscle [41]. Result obtained from the this section indicate that 2,4-D and butachlor inhibit the acetylcholinesterase of *Eutyphoeus waltoni* in dose dependent manner.

The dose dependence effect on AChE activity could be due to serveral factors like role of penetration in the animal, rate of inactivation, variability or increased competition with the natural substrate at the active sites [42,43]. Singh and Agarwal [44]. also reported that carbamate and organophosphate compounds [e.g. mexacarbate, phorate] also show a dose related response presumably due to conversion to more toxic metabolites in the body of animals. Denoyelle et al. [45]. reported that AChE activity significant decreased in earthworm from treated orchards. Jarden et al. [46]. reported that inhibition of cholinesterase activity after treatment of pesticide azinphos- methyl on juvenile earthworm *[Eisenia andrei]*.

CONCLUSION

Among all treatment of sub-lethal exposure of LC_{50} of herbicides 2,4-D and butachlor against *Eutyphoeus waltoni* in the different feed materials, the tertiary combination of buffalo dung with wheat straw and gram bran have more potency to increase the tolerance power of earthworm *Eutyphoeus waltoni* in agricultural fields. The use of vermicompost of buffalo dung with wheat straw and gram bran is beneficial for better productivity of crops as well as provided better nourishment to enhance the population and tolerance power against herbicides and other chemicals.

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ARTICLE
COST ANALYSIS OF STRABISMUS SURGERY BY ACTIVITY BASED COSTING

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ABSTRACT

The aim of this study was to estimate the cost of strabismus surgery through activity- based costing (ABC) and compare it with the tariffs approved by the Iranian Ministry of Health. Costing study was conducted in Nikookari Ophthalmology Hospital of Tabriz from April 2014 to April 2015 with a retrospective approach by activity-based costing method (ABC). The required data and information were collected interviewing experts, hospital officials, and relevant units, direct observation of activities, and reviewing the documents in the financial department and HIS software system. Then, the cost of strabismus surgery was estimated using Excel 2013. According to the findings of the present study, the amount and the share of the total costs of the activities identified in the main centers are as following: human resources ($2,818,722), depreciation of medical equipment ($277,233), consumable medicals and supplies ($218,484), building depreciation ($138,004), depreciation of non-medical equipment ($43,640), consumable non-medicals and supplies ($ 26,804), utilities cost ($4,724), and costs of indirect units ($ 864,455). Ultimately, the cost of a strabismus surgery was estimated to be $ 464 which is $ 236 higher than its approved tariff. The difference between the approved tariff and the cost of strabismus surgery in the studied hospitals was considerable. This price gap can negatively affect the performance and quality of services provided by hospitals in the long term. Since the costs of personnel, indirect costs, and depreciation of medical equipment account for 90% of total costs of strabismus surgery, hospital managers should pay more attention to these items in order to improve efficiency of their services and reduce costs.

INTRODUCTION

The tendency of policymakers to satisfy people and increase pressure to improve efficiency and effectiveness have forced the managers of public sectors to pay special attention to issues such as determining the exact unit cost of goods and services, improvement of processes, evaluation of outsourcing or privatization options, and aligning activities with the strategic missions and programs. One of the most important service sectors is health sector which plays a decisive role in` the general health of society. Costs in hospital as one of the most vital health sectors has a special place and there are many problems and challenges facing the establishment of balance between limitation of resources and costs and providing satisfactory and desirable services. Technological progress, population growth, and rising public expectations have caused an increasing growth in hospital costs and made managers to manage the costs in order to provide qualitatively acceptable services at the lowest cost and develop their activities [1-3].

Due to the fact that no country has sufficient resources to meet all needs of its population, hospital managers are in need of accurate and exact information to improve efficiency and performance. One of the important information that is needed by managers of hospitals is the cost information that allows the managers to make correct decisions regarding pricing of services. However, hospital administrators still rely on the information of traditional costing method which has been developed when competition was not stiff as today and quality and efficiency were among less important factors of success. Nowadays, due to present economic development as well as stiff competition, search for a new method of costing consistent with the present high competition and the increasing demands of health sector became more prominent. Therefore, the need for developing an appropriate and efficient costing method compatible with the diversities and complexities of activities which works well with evaluation of their effects on the cost and quality of various services were felt more than ever [4-8].

Nowadays, many companies have found that activity-based costing (ABC) is one of the best methods of costing. This method allows organizations to well benefit from their resources for providing products and services to their customers. As a tool for efficiency and cost control, ABC was developed between 1970 and 1980 in order to estimate the costs of different activities. The main motivation for using this method can be attributed to the accurate estimation of costs and full unit cost. The implementation of this method in health sectors supports its suitability for developing countries. In this method, it is assumed that cost targets create activities and activities consume the resources. Comparison between ABC and other methods shows that ABC is both more accurate in the allocation of costs (according to the specific cost driver for each activity)

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and more detailed in the unit cost of supporting and main activities. These features form a fundamental tool for improving the efficiency and quality of care services [9-13]. Given the considerable features of activity-based costing and its suitability for developing countries, the present study aims at estimating the cost of strabismus surgery in Nikookari Ophthalmology Hospital of Tabriz and its comparison with the tariffs approved by the Iranian Ministry of Health.

MATERIALS AND METHODS

Study design

Nikookari Ophthalmology Hospital of Tabriz is a specialized and teaching hospital with 82 beds, 60 approved beds with a bed occupancy rate of 50%.

Strabismus costing method

The present costing study was conducted in the above mentioned Hospital from April 2014 to April 2015 with a retrospective approach by activity-based costing method (ABC). The reason why the current study was carried out in this hospital was that it was possible to randomly select some cases from among several surgical operations performed here daily. The required data and information were collected through face-to-face interview with experts, hospital officials, and relevant units, direct observation of activities, and reviewing the documents in the financial department and HIS software system. The cost of strabismus surgery was estimated using ABC in Excel 2013. The steps of strabismus surgery costing in the present study were as follows:

Identification of all activities associated with providing the desired service

In this step, by studying the documents and instructions on how to provide services and face-to-face interviews with relevant experts, various types of offered services and their sequence from admission to discharge were determined.

Costing in activity centers

The costs identified in this study are as following:
A) **Human resources cost:** First, information on the staff working in each unit was gathered. Then, the cost of permanent employee together with the cost of employees hired on a contractual basis working in each unit was extracted from the payroll system and the financial software. Finally, pension, premium, overtime payment, and remuneration were added to obtain total personnel cost.

B) **Utilities cost:** Total cost of utility services which includes water, electricity, gas, and telephone was estimated by referring to the accounting unit. Cost of water, electricity, and gas was determined on the basis of the area and telephone and internet costs were divided by the number of lines available.)Due to the unavailability of information about the equipment and according to references this cost driver was used.

C) **Consumable medications and supplies, consumable non-medicals and supplies:** a list of all items delivered to various wards of the hospital was prepared. This list included medical supplies and medicines, technical equipment and goods, devices, and general goods. Considering the price and the number of items, total cost of each activity center was estimated.

D) **Medical and non-medical equipment depreciation cost:** A list of equipment available in each activity center and detailed information on their purchase time, price, and maintenance were prepared. Equipment was divided into two groups; equipment that their useful life had not finished and those that their useful life had finished. With the help of experts, equipment was re-evaluated and their useful life was determined. To determine the depreciation of equipment, the method of Sum-of-years' digits is a depreciation method was used.

E) **Building depreciation:** Firstly, according to the hospitals' map and with the help of technical experts, the area of each activity center was determined. Since the technical management experts of Tabriz University of Medical Sciences declared that the useful life of the building is finished, approximate monthly rental cost of the hospital was estimated. Then, the cost of each hospital ward was determined based on its area.) Considering that the purchase price of the equipment was not available, to calculate the cost, first the inflation was applied and the price was updated)

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Identification of appropriate cost driver

Appropriate cost drivers for each of the main and overhead activity center have been identified.

Allocation of costs to activity centers

Cost Allocation was done in two steps. First, the cost of each overhead activity center was allocated to strabismus surgery centers. Then, the cost of main strabismus surgery centers was allocated to activities and services.

Calculation of the cost of strabismus surgery

The following formula was used: [Total cost of operation room × (Number of strabismus surgeries × Relative value of strabismus surgery)]/ sun of (Relative value of each surgery conducted in the operation room × Number of that surgery)

RESULTS

Camel First, after reviewing the hospital processes, interviewing experts and officials, and observation of activities, hospital units were divided into two main (direct) and support units (overhead and indirect). Then cost drivers related to main strabismus surgery unit and supporting unit were identified. They have been shown in [Table 1] and [Table 2].

Table 1: Main center of strabismus surgery

row	Cost center	
1	Patient Admission	
2	Diagnostic activities	Laboratory
3		Electrocardiography
4	preoperational procedures (in ward) and patient care	
5	surgeries	
6	Patient Discharged	

Table 2: Secondary center of strabismus surgery, Cost driver, Cost

Supporting Units	Cost drivers	Costs($)
Presidency and management, accounting, administrative services, telephone center, security	The number personnel	312597
Kitchen	The number of staff, days of care	82929
Warehouse equipment, facilities and food	The number of delivered items in terms of activity center	17480
Pharmacies and drug warehouse	The number of delivered items	39584
Security station, information and facilities	The physical size of each unit	164012
Laundry	The number of staff, days of care	14025
Informatics	The number of computers in each department	11119
Medical Equipment	The number of existing equipment in departments	12111
Nursing Office	Number of employees covered by the nursing office	35870
Oxygen stock	The number of cylinders in each section	10542

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For the allocation of costs in the first stage, the cost of supporting activity centers was allocated to the patient's direct activity centers. After allocating overhead costs to the activity centers mentioned in this study, total cost of patient's main activity centers was obtained [Table-3].

Table 3: The costs of the main center of strabismus surgery

Row	Activity Center		Cost of human resources	Depreciation			Non-medical Consumable Materials	Medical consumable materials	utility facilities	Indirect costs	total
				Depreciation of building	Depreciation of medical equipment	Depreciation of non-medical equipment					
1		Admission	134116	26162	0	2,052	7472	0	1,757	114140	285699
2	Diagnostic activities	Laboratory	51579	9811	2,609	1,734	965	3188	237	28533	98656
		Electrocardiography	9442	981	195	457	51	350	24	5,676	17175
3		Preoperative and postoperative care	307920	71945	7821	16836	9,362	20218	1,915	504642	940660
4		surgery	2283947	26162	266608	21846	8667	194728	720	187558	2990237
5		Discharge	31718	2,943	0	714	285	0	71	23905	59636

In the second stage of allocation, the cost of main activity centers was allocated using appropriate cost drivers to the activities.

Calculation of the costs of these centers and their allocation to patients were done as follows:

Admission
Annual cost per patient admission was 285,699$. Of which 85% goes for inpatient admission and 15% for outpatient admission. Therefore, total cost for admission of outpatients was estimated to be $ 242,844. The number of patients admitted to the studied hospital was 11243. As a result, the cost for hospitalization of each patient was 22$.

Diagnostic activities cost

Laboratory cost
Laboratory cost amounts to $ 98,656 per year. Since the same tests are performed for all patients at this hospital, laboratory cost was considered to be the same for all patients. In total, 13824 patients had referred to the laboratory and the cost obtained for each patient was $ 7.

Electrocardiography service cost
Total cost of electrocardiography unit per year was estimated to be $ 17,175. In 2013, 6130 cases of electrocardiography have been recorded and the cost for each patient was estimated to be $ 3.

Preoperative and postoperative care
The costs related to preoperative and postoperative care was estimated to be $ 940,660. Allocation of the costs related to this part was based on the number of occupied days for a bed. Occupied bed days in the studied hospital was 16036 and the cost for each day of hospitalization was calculated to be $ 59. Given that the stay duration of strabismus surgery is 2 days, the cost per patient was obtained 117$.

Surgery cost
These costs include the cost of the recovery room, sterilization and operating room. To estimate the cost of strabismus surgery in the operation room, the following formula was used:
[Total cost of operation room × (Number of strabismus surgeries × Relative value of strabismus surgery)]/ sum of (Relative value of each surgery conducted in the operation room × Number of that surgery)
The total cost of a strabismus surgery is $ 162,777. Total number of strabismus surgeries conduced in the studied hospital was 529. Thus, the cost of each strabismus surgery was found to be $ 308.

Patient discharge cost
A total of 8046 patients had been discharged from the studied hospital in 2013 and the cost for discharge of any patient was $ 7.

Finally, the cost of each strabismus surgery was obtained to be $ 464. This is $ 235 more than the tariff for this surgery approved by the Iranian Ministry of Health. [Table 4]

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Table 4: Comparison of the tariffs approved by the Ministry of Health and Activity Based Costing

Surgery Name	Global Surgery Tariff in 2013	Activity Based Costing	The difference
Strabismus surgery (one or both eyes)	229	464	-235

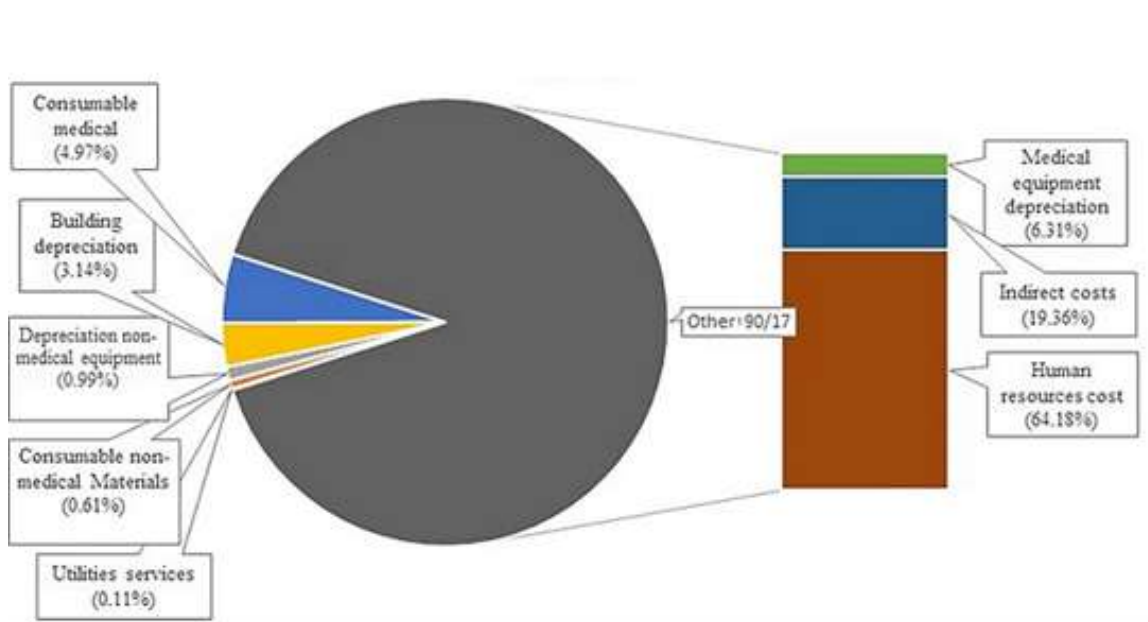The share of costs related to the main and secondary activity centers has been shown in [Fig. 1].

Fig. 1: The share of costs in the main surgery centers of Nikookari Ophthalmology Hospital of Tabriz
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DISCUSSION

Camel The present study aimed to estimate the cost of strabismus surgery in Nikookari Ophthalmology Hospital of Tabriz and compare it with the tariff approved by the Iranian Ministry of Health. Based on the study results, the cost of strabismus surgery in the studied hospital was obtained $ 464.

According to the findings, personnel cost (64.18%) comprised the highest share of allocated costs. In the studies conducted by Negrini et al. [14] and Lievens et al.[15], human resources cost has been reported to be 49% and 43.3% of total costs. In a study carried out in the intensive care unit of a hospital in Germany, personnel cost (42%) accounted for the highest share of the total costsn [16]. These findings confirm the results obtained in the present study. Although the share of human resources cost relative to total costs have varied in these studies, personnel cost in all of them accounted for the highest share of total costs. Improving performance of human resources as critical part of provision of services can play critical role in reduction of unit cost.

The second major part of costs belonged to indirect costs (19.68%). Analyzing the components of these costs can greatly help the hospital management in providing required information for budgeting the activity center and the whole hospital system. Given the high share of indirect costs in the cost of services, efficient use of resources to increase productivity and provide further services reduces the shared average cost of services and leads to lower total cost of services. For instance, by sharing activity centers' cost (presidency and management, accounting, administration, telephone center, and security) in the current study to the laboratory activity center, based on the sharing of the number of employees, the figure of $ 28,533 was obtained. By dividing this figure to the number of surgeries done in the same period, the average cost of these centers for providing each service will be $ 2 on average. Increasing the number of tests to 20000 services, the average cost per test would be only $ 1.5. These calculations can also be done regarding other overhead parts. In fact, by determining the parts that have the highest impact on the cost of services, the costs can be controlled and reduced.

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Medical equipment depreciation cost (6.31%) ranks third after personnel costs and indirect costs. This is one of the significant findings of the present study which can be attributed to excessive obsolescence of equipment, installation of new equipment (the highest depreciation of equipment occurs in the first years and the last years of their useful life), and existence of specialized equipment in this hospital and high cost of their maintenance.

Other shares of costs belonged to medical supplies and medicine (4.97%), building depreciation (3.14%), depreciation of non-medical equipment (0.99%), consumable non-medical (0.61%), and utilities cost (0.11%), respectively. Lower ratio of energy costs in comparison with other hospital costs may be the result of governmental subsidies paid to the health sector. Since the studied hospital is a teaching one and teaching tariff should be taken into consideration. Energy cost should also studied without considering the subsidies.

According to the results of the present study, the cost of strabismus surgery is considerably higher than the tariff approved by the Ministry of Health. In a study conducted by Suthummanon et al. [17] in the medical sector of Thailand, it was shown that there is a considerable difference between the cost of services and the costs estimated by Activity Based Costing (ABC). Also, the results of a study carried out by Antikainen [18] showed that the costs estimated on the basis of ABC are higher than the approved tariff which was attributed to not considering the cost of unused capacities which agrees with the findings of the present study. The price difference in the present study could be due to the weakness in tariffing system in Iran which not follows an accurate and scientific method for determining the rate of services. Investment cost is another reason which includes the depreciation of property, building, and equipment which is one of the major costs in determining the cost of services. However, since most of the facilities existing in hospitals are state-funded and public hospitals provide medical services to patients as nonprofit institutes, the costs related to depreciation of property, building, and equipment are not estimated and taken into account, which can lead to a considerable difference in the cost of services.

CONCLUSION

The difference between the approved tariff and the cost of strabismus surgery in the studied hospital was considerable. This suggests non-recognition of the actual costs of strabismus surgery and lack of proper financing by the Ministry of Health. This price gap can negatively affect the performance and quality of services provided by hospitals in the long term. Since the costs of personnel, indirect costs, and depreciation of equipment account for 90% of the total costs of strabismus surgery, to improve efficiency of the services and reduce costs hospital managers should pay more attention to these items.

One of the proposals in relation to the calculation of the cost of hospital services is concerned with the development and application of a comprehensive system in hospitals which can improve the system of costing which in turn brings about more accuracy to estimating costs. Using this system, operations related to ordering, follow-ups, and control of consumables in different wards of a hospital can lead to improvement in management process. In addition, by determining the consumables in each ward during a certain period, costs can be exactly calculated and the reasons for deviations and weaknesses can be identified by juxtaposition and comparison of the obtained figures with standards. Also, by developing work standards in different wards of a hospital, efficiency and performance of human resources can be improved, unused potentials can be identified, and solutions necessary for improving the status quo can be proposed.

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ARTICLE

INVESTIGATION ANXIOLYTIC EFFECTS OF HERBAL TEA OF VALERIANA SISYMBRIFOLIA VAHL. COMPARED TO LAVANDULA ANGUSTIFOLIA ON THE FEMALE RATS

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ABSTRACT

Introduction: *Anxiety is a natural human reaction that involves both mind and body. Anxiety is defined by a diffuse, unpleasant, vague sense of apprehension. It is often concomitant with autonomic symptoms, such as perspiration, palpitations, headache, and tightness in the chest.* ***Method:*** *This study was carried out to compare the anxiolytic effects of the herbal tea of Lavandula angustifolia versus Valeriana sisymbrifolia and these effects were assessed and compared to the control group. In order to do this, 21 female rats weighing 100 to 150 grams were applied. The rats were divided into three groups including control, VS (treated group by Herbal tea of Valeriana sisymbrifolia) and LA (treated group by Herbal tea of Lavandula angustifolia). Treatment groups (n = 7/group) had ad libitum access to the tea from Valeriana sisymbrifolia 0.3% (w/v) for VS Group and tea from Lavandula angustifolia 0.3% (w/v) for LA Group , during a period of 24 hours before the test. Then, the behavior of rats was tested in order to sedative (locomotor activity) and anxiolytic (elevated plus maze) activity. All the data were given as Means±S.E.M. Data were analysed by one-way ANOVA following by Tukey test.* ***Finding****: The study indicated that the anxiolytic effect of the herbal tea of Lavandula angustifolia is stronger than herbal tea of Valeriana sisymbrifolia on the female rats.*

INTRODUCTION

Anxiety is a natural human reaction that involves both mind and body [1]. Anxiety is defined by a diffuse, unpleasant, vague sense of apprehension. It is often concomitant by autonomic symptoms, such as perspiration, palpitations, headache, and tightness in the chest [2]. Pharmacological treatment plays an important role in the therapeutic concept Benzodiazepines have been the most widely used anxiolytics in general practice for many years [3] and are relatively safe drugs for a short term treatment of anxiety despite their drug dependence potential and side effects [4, 5]. However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many research to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects [6, 7]. There are so many herbal teas to have anxiolytic effects. *Lavandula angustifolia* (LA) is part of the Labiatae family and belongs to the Lavender genus which is a natural growth in the Mediterranean region [8]. Lavender is reported to be an effective medical plant treating inflammation, depression, stress, seizure and of migraine headache [9-11]. Lavender is also reported to be an effective medical plant in treatment of restlessness in case of anxious mood. Intake administration of LA has been shown to the anxiolytic effect in clinical studies [12-14]. *Valeriana sisymbrifolia* (VS) (Valerianaceae family) is a medicinal plant used in complementary and alternative medicine for its sedative and anxiolytic properties [15, 16]. Valerian's effects on the central nervous system have been well documented and attributed to many of it active compounds: valepotriates, baldrinals, valerenic acid, valerenal, valeranone, and other constituents in the essential oils [15, 17-22]. Albeit, the anxiolytic properties of valerian have been demonstrated in animals [23, 24]. This study evaluated the effectiveness *Valeriana sisymbrifolia* versus *Lavandula angustifolia* and which is more effective for anxiolytic effects.

MATERIALS AND METHODS

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This was an experimental study in which 21 female Wistar rats weighing 100 to 150 grams were randomly selected and tested. All animals were housed under standard environmental conditions of temperature, relative humidity and light (at 23±2 °C, 40—60% humidity, 12 h light: 12 h dark cycle (lights on at 08:00 h). Animals are divided into three groups including control, VS (treated group by Herbal tea of VS) and LA (treated group by Herbal tea of LA). The Used dosage was considered based on leaflet in the pocket of the herbal tea (it was equivalent to 3g/lit/24h) and The VS rhizome and LA flowers were used for this study. VS and LA Groups rats (n = 7/group) had ad libitum access to the tea from VS 0.3% (3gram per 1000 ml w/v) in drinking water and tea from LA 0.3% (w/v), respectively, for a period of 24 hours before the test. Then, the behavior of rats was tested in order to sedative (locomotor activity) and anxiolytic (elevated plus maze) activity. Elevated plus maze (EPM) is made up of wood and includes two open arms (each 50×10 cm) and two closed arms (each 50× 10 × 40 cm) and a central plate (10 ×10 cm). Open arms are across from each other and so are the closed arms and are located 50 cm above the floor of the room. This is an experimental non-conditional anxiety testing model and does not require any animal training and learning [25, 26]. In the day of the test, the animals were transferred to the laboratory in the afternoon between

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17:00 p.m. and 20:00 p.m., and then in order to test the anxiety level , the animal was located in an elevated plus-maze (in the plate and across from the open arm) and the important anxiety testing indices, including the number of entrances to open and closed arms and the time of staying in open and closed arms were tested and recorded for 5 minutes [1, 25-29].The total number of entrances into two arms are considered as a locomotor activity [30]. The statistical analysis of data was performed by one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis. In all cases, differences were considered significant (p< 0.05).

RESULTS

The ANOVA showed that there was a significant difference in rat behavior on time spent in the open arms of EPM between VS and LA groups compared to control group. Tukey test analysis showed a significant increase in time spent on open arms in treatment 2 group compared to the control group (p<0.05) but the time spent on open arms in the treatment1 group compared to control group was not significant [Fig. 1]. The number of entries into the open arms in treatment 2 increased significantly, [Fig. 2]. Time spent on closed arms for the treated group by Herbal tea of LA decreased significantly but this decrease was not significantly in the treated group by Herbal tea VS [Fig. 3]. Number of closed arms entries and total number of open and closed arms increased but not significantly [Fig. 4 and 5].
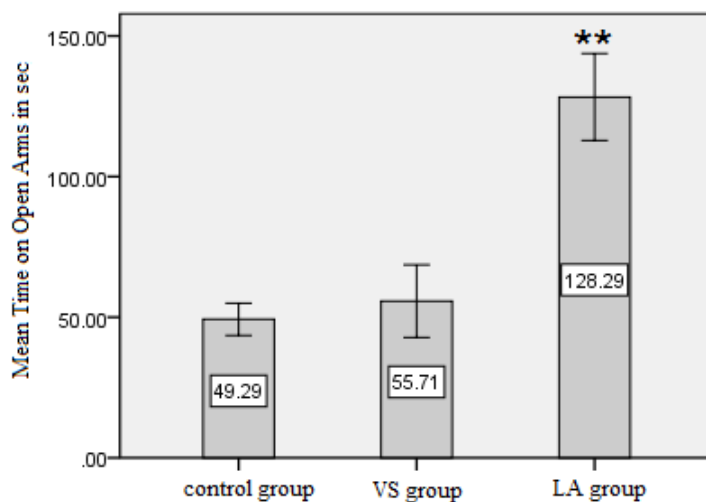

Fig. 1: The period of staying in open arms for LA group is significantly more than the control group using ANOVA following by Tukey test. **shows the significant difference (P<0.001)
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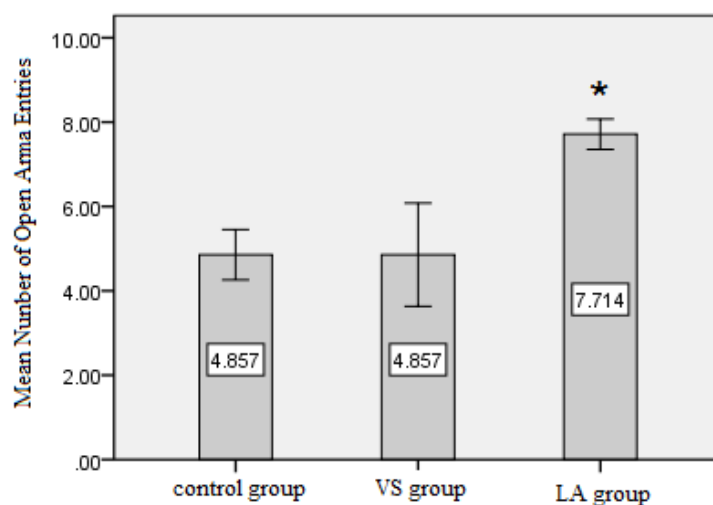

Fig. 2: The number of entrances into the open arm arms for LA group is significantly more than the control group using ANOVA following by Tukey test. *: shows the significant difference (P<0.05)
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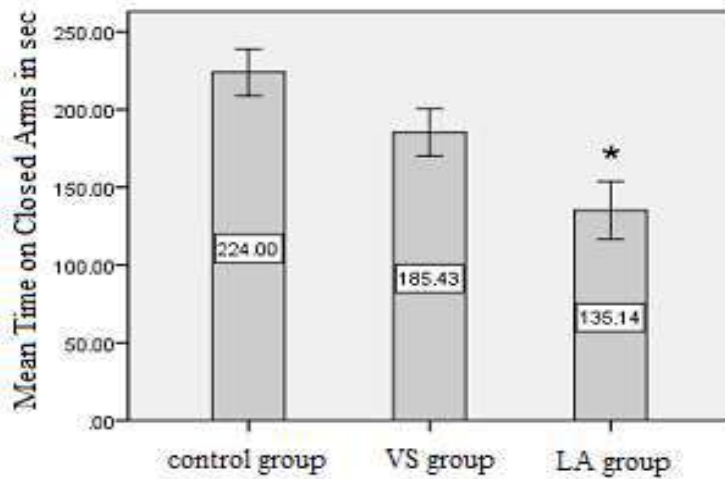
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Fig. 3: The period of staying in closed arms for LA group is significantly less than the control group using ANOVA following by Tukey test. *: shows the significant difference (P<0.05)
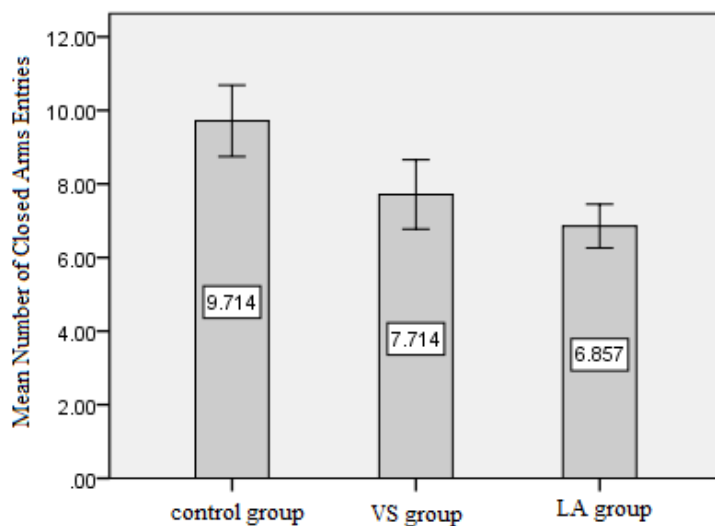
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Fig. 4: The number of entrances into closed arms in the treatment groups

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DISCUSSION

An increase in the time and the proportion of the entrances into the open arms without a changed locomotor activity are regarded as a powerful marker for an anxiolytic substance effect [29]. The close arm entries are selectively correlated with the locomotor activity [31]. The drugs that cause stimulation and increase the locomotor activity were reported to increase the number of close arm entries [32]. In the elevated plus maze, an anxiolytic or anxiogenic like-effect is evaluated by the relation of entries into the open arm and the time spent on the open arms of the plus maze in comparison to the same parameters of the control group. An increase in the time spent and number of entries into the open arm without changing locomotor activity was regarded as a powerful marker for the anxiolytic effect [29]. The enhancement of total arm entries might suggest a nonspecific locomotor stimulant effect which is the co-load on "locomotor activity" and "anxiety", whereas closed arms entries load highly and selectively on locomotor activity [31, 33]. Increase time spent in open arms, percent entries in open arms, total entries and closed arms entries indicated anxiolytic effect. The present study showed the treated groups by of herbal tea of VS and LA induced anxiolytic behavior but did not increase locomotor activity and this indicates herbal tea of LA has anxiolytic effects stronger than herbal tea of VS. The active components of LA are thought to be linalool, linalyl acetate, cineole, terpinen-4-ol and camphor [34-37]. The presence of linalool, linalyl acetate in the plant extract supports the claim that the extract has a sedative effect [38]. Some studies reported the parable mechanisms. Chronic Injection of Lavender oil altered dopamine D3 receptor subtype homeostasis in the olfactory bulb and induced behavioral change [39]. Also, Lavender oil potent anxiolytic properties via modulating voltage dependent calcium channels [35]. Linalool, a monoterpene compound prevalent in essential oil of Lavender, interferes with glutamatergic transmission [36]. Lavender oil is also suggested to modulate GABAergic neurotransmission, especially on GABAA receptors, and enhance

inhibitory tone of the nervous system [40-43]. Cholinergic system is suggested to play a role in lavender analgesic, antianxiety, antidepressant, and anticonvulsant effects of lavender [43, 44].
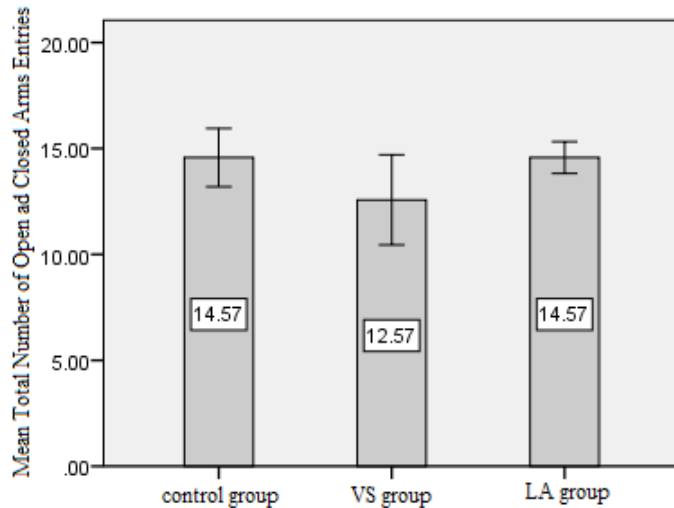

Fig. 5: The total number of entrances into open and closed arms in the treatment groups

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Previous studies showed the binding of valerian extract to GABAA receptors in rat cortical membrane preparation [18]. It has been shown that valerian extract, aqueous or hydro-alcoholic, contained GABA and other amino acids that could displace labeled muscimol [18], suggesting that specific constituents of valerian extract can directly bind to GABAA receptors. The GABA content of valerian extract could also be responsible for the stimulated release and reuptake of GABA. This could be an indirect mechanism of GABA agonistic activity of valerian extract [45, 46]. Additionally, derivatives of valerenic acid inhibit the local catabolism of GABA by inhibition of the enzyme GABAse, which could also increase GABA concentration [47]. These mechanisms might have been operational in our *in vitro* brainstem model, but in *in vivo* models, the role of exogenous GABA in producing central nervous system (CNS) sedative effects, is questionable because of the very low permeability of GABA across the blood-brain barrier [18]. The significance of the inhibition of GABA catabolism by valerenic acid derivatives in *in vivo* models is not yet known. But in this study valerian had not anxiolytic effects. The previous studies have shown anxiolytic effects of valerian in female rats aged six to ten months [24] and in the male mice [48] while in this study female rats aged two to three months were used. Perhaps this contradiction is because of the age testing, used dosage, method of extraction, route of administration and/or sex-dependent. However, the relationship between sex hormones and anxiety behaviors should be discussed. For example the relationship between estrogen and anxiety behavior in different findings may reflect several experiences. Frick et.al. [49] observed in their laboratories that female rats spent less time in elevated plus-maze in open arms. This indicates that exists more anxiety more in female rats. But they had not considered the sexual cycle and it also was not regarded in this study. However, Galeeva et.al. [50] indicated that explore in open arms was reduced in female rats in Diestrus phase, i.e. when the level of estrogen is in its minimum state, there is more anxiety; therefore, the reduction of estrogen results in more anxiety and this estrogen includes the anxiolytic effect. Again, in another experiment, the opposite was shown so that the ovariectomized female mice receiving estrogens in comparison with ovariectomized mice that did not receive any estrogen, showed an increased anxiety behavior [51]. However the effects of estrogen are exerted through two receptors consist of alpha and beta. but an anxiolytic effect of estrogen is more focused on beta receptors [52]. Also, some of the results show that the beta hormone receptors of estrogen are a potential facilitator for serotonin and dopamine neuro-transmitters. Hence, according to different reports by other scholars estrogen increases anxiety behavior. There is a possibility that estrogens on beta receptors are active in a time phase more than other times. However, it seems that the effects some of the herbal medications are sex-dependent [53]. Hence, more research is needed to investigate the anxiolytic effects of *Valeriana sisymbrifolia* in the female rats.

CONCLUSION

The results of this study showed that herbal tea of LA in female rats has anxiolytic effects compared to herbal tea of valerian and this effect was significantly than control and valerian groups. But valerian had not anxiolytic effects.

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FINANCIAL DISCLOSURE
None

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ARTICLE

RECONFIGURABLE PLATFORM BASED DESIGN IN FPGA FOR UNDERWATER IMAGE COLOR CORRECTION

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ABSTRACT

Color correction is a serious constraint that comes into play in underwater imagery. Optical properties of light cause issues that can severely degrade the quality of underwater photography. As light with different energies travel through water, it is absorbed at higher rates, it contributes to severe bluishness of the underwater captured images. Thus it creates challenges for imaging in underwater. Attenuation of longer wavelengths occurs more rapidly when compared to shorter wavelengths, resulting overall blue color to the captured image. Scattering, independent of wavelength of visible light is one of the reasons for image loss and it does not influence color. In marine biology studies related to species identification and ecosystem monitoring and surveillance, examination of color is required. Color filters and manual post-processing are used for flooding objects with white light from a very close distance in underwater imaging techniques. However, these techniques are inefficient since it doesn't render correct colors, and it works only for fixed setups, as loss of color depends upon distance. Using post processing methods for underwater images, underwater monitoring of natural and human-engineered environments can be sufficiently enhanced to a great extent. In this paper, hardware implementation of underwater image color correction is performed in Field Programmable Gate Array (FPGA) to be used in Automated Underwater Vehicles (AUV). FPGA platform is preferred as its ability to perform parallel algorithm such as image processing algorithms due to its inherent parallelism.

INTRODUCTION

Recovering process of underwater images involving retrieval of correct colors is a challenging task. This is due to the fact that illumination done for image capture is extremely altered in water since water is refractive and turbid medium. Relevant Image modifications are caused mainly due to following reasons: different attenuation of different wavelength, scattering and absorption of radiation by the underwater environment. For this task, many qualitative techniques based on imaging have been proposed in the literature. They are based on image restoration techniques or subjective techniques such as image enhancement methods. The information regarding medium physical parameters is essential in first case and image adjustments based on certain criterions are performed in second case. Gray world hypothesis is one of the most popular criteria in which average of the captured image is supposed to be gray. Implementing automatic methods based on this assumption yield good results. The color space consists of two opponent color components which encode α and β (yellow-blue and red-green chromaticities), and a luminance component (achromatic).This is applied for color discrimination in human color visual mechanism. With the help of lαβ color space, the chromatic components are relatively altered by displacing their distributions closer to the white point (white balancing).Histogram cut off and stretching of the luminance component is done to advance the contrast of the image. So, by performing a color correction similar to that in human visual system is the way to tackle the difficulties of underwater images based on color constancy. The average information in each image channel tends to gray that is captured color image averages to gray (achromatic). The main drawback of gray-world systems is that they will not reproduce correct colors if extremely huge number of colors is absent in the scene.

The *laβ* color space described by Ruderman et al, [1] examines the human eye insight of natural images. In human color visual mechanism, color space consists of a luminance component I and two adversary color constituents α and β. In applications like 3D imaging, documentation, navigation etc, color correction of underwater images or videos is a significant task. G. Biancoa et. al. [2] present a new method which deals with uniform illumination and gray-world assumptions used in the lαβ space for color correction. White balancing of chromatic components (αandβ) and l,the luminance element is processed to improve the contrast of the image. This procedure is appropriate in underwater imaging to eliminate unwanted color casts and in the gray shades, greenish-blue components are removed.

Underwater monitoring can be improved by automatically capturing underwater color images using robots. In [3] adaptive illumination, a precise-color imaging method is used. A multi-color controllable light source is used to mix the illumination in a distance-dependent way. The color mix pays accurately for loss of color and produces an image and its composition of color which is alike to represent the object in air. Objects closer or far away from the camera will not be rendered appropriately because of the distance-dependent behavior of adaptive illumination. This work helps to capture color-accurate underwater images based on color which facilitates surveillance operations and automating underwater monitoring.

Erik Reinhard and Tania [4] explain a universal technique for correction of color and it takes characteristics of color of one image from other. It describes that a color space with de-correlated axes is a helpful means for manipulating color images. Calculation of standard deviation and mean of the data points generates convincing output results providing suitable input images. The important approach is to select an appropriate color space to perform simple operations .The representation of an image (three channel) in any color spaces results in correlation between different channel values, which is not needed.

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Erik [5] introduced a color space, called lαβ, which reduces relationship between channels for large number of natural scenes. This is based on the hypothesis that for processing natural scenes a human visual system is perfectly suited.

Arjan Gijseni et al, [6] used computational color constancy, an important requirement for computer vision applications. In image processing or computer vision related topics like human–computer interaction, extraction of color features, and color appearance models, color is a vital cue. The colors in images depend on the inherent features of surfaces, objects and the light source's color. For vital color systems, the effect of light source needs to be eliminated. The purpose of color constancy procedures is verification of the (first three) target images (under three different colored light sources) in such a way that they emerge alike to the (fourth) canonical image.

Prabhakaret al,[7] futured a preprocessing tool for images to boost the excellence of the underwater images. The work combines homomorphic filtering, bilateral filtering, wavelet de-noising, and contrast equalization. On degraded underwater images, these filters are used consecutively. The anisotropic filters are used for smoothing the image in preprocessing algorithms. To improve the perception of underwater images, KashifIqbal et al,[8] proposed a method based on slide stretching. Firstly, RGB algorithm for contrast stretching   is performed to balance the contrast of colors in images and after that the saturation and intensity stretching of HSI is used to increase the true color and to rectify the issues of lighting. Interactive software has been developed for the enhancement of underwater image. Alex et.al [9] have implemented homomorphic filtering to enhance underwater images in FPGA.

Modern hardware based solutions for image processing algorithms increasingly utilize Field Programmable Gate Arrays (FPGA) [10-11]. Through parallelism, FPGAs are able to accelerate the execution of image processing algorithms and improve their performance. Considering the cost for making application specific integrated circuits (ASICs), FPGAs are well suited to be used as a prototype for limited number of end users.

In this paper FPGA implementation of underwater image color correction in lαβ space is explained. Xilinx System generator is being used for implementing the design in FPGA. Hardware co-simulation is carried out using Xilinx Virtex 4 development board.

MATERIALS AND METHODS

The algorithm relies on gray-world and unvarying illumination suppositions are performed in the lαβ space model. Color correction is achieved by the white balancing of α and β, while the luminance component is utilized to improve the contrast of the underwater image. The underwater RGB image has to be converted to lαβ space for further analysis.
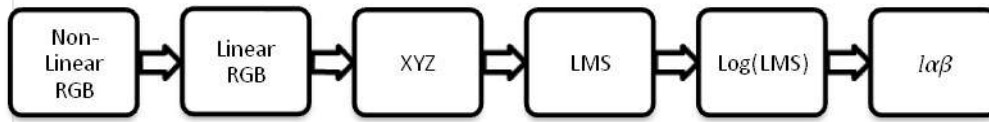

Fig. 1: Conversion of non linear RGB model to *lαβ*

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As shown in [Fig. 1], non-linear RGB image is rectified by the non-linearity correction (gamma correction) so as to get RGB coordinates [5][12]. Then conversion into XYZ tri stimulus values is obtained by multiplying the RGB coordinates $f_i(m,n)$ with $T_{xyz,ij}$ matrix as in equation 1 and 2.

$$x_j(m,n) = T_{xyz,ij} f_i(m,n) \tag{1}$$

$$f_i(m,n) = \begin{bmatrix} 0.5141 & 0.3239 & 0.1604 \\ 0.2651 & 0.6702 & 0.0641 \\ 0.0241 & 0.1228 & 0.8444 \end{bmatrix} \tag{2}$$

From this device-independent XYZ space, image is converted to LMS space using the $T_{lms,ij}$ matrix as in equation 3 and 4.

$$l_j(m,n) = T_{lms,ij} x_i(m,n) \tag{3}$$

$$T_{lms,ij} x_i(m,n) = \begin{bmatrix} 0.3897 & 0.6890 & 0.0787 \\ -0.2298 & 1.1834 & 0.0464 \\ 0.0 & 0.0 & 1.0 \end{bmatrix} \tag{4}$$

Now, the data is transformed into logarithmic space, and finally these axes were de-correlated by utilizing Principal Components Analysis (PCA). It can be achieved by multiplying $l_{log,i}(m,n)$ vector by the decorrelation matrix $T_{pca,ij}$ as in equation 5 and 6.Thus, the vector coordinates of image in lαß space is obtained. The three consequential principal axes are orthogonal, where the axis l represents an achromatic channel, while the other two (α andβ) channels are chromatic yellow-blue and red-green opponent channels.

$$l_{l\alpha\beta,j}(m,n) = T_{pca,ij}l_{log,i}(m,n) \tag{5}$$

$$T_{pca,ij} = \frac{1}{\sqrt{6}}\begin{bmatrix} \sqrt{2} & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & \sqrt{3} \end{bmatrix}\begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & -2 \\ 1 & -1 & 0 \end{bmatrix} \tag{6}$$

HARDWARE IMPLEMENTATION

The proposed method is executed in field-programmable gate array (FPGA) which is a device that can be reprogrammed by a designer or customer even after manufacturing it. Similar to application-specific integrated circuit (ASIC), FPGA configuration is described using hardware description language (HDL).Any logical function performed by ASIC, is easily implemented using FPGAs. Relative to an ASIC design, FPGAs offer advantages for many applications such as the ability for updating the functionality, partial reconfiguration of any part of the design and much low non-recurring engineering expenses.

The main advantage in performing image processing using FPGA [8] implementation is due to it stability to exploit both spatial and temporal parallelism. The FPGA implementation can be designed to partition the image into sections and later on, distributing each section into corresponding pipelines. Thus data processing can be done concurrently. So, constraint of parallelization in FPGA is due to the mode of processing the data and hardware limitations of the system. Xilinx System generator is used to implement the proposed design in FPGA.

The following are the various Xilinx system generator model blocks that are being used for implementing the design in FPGA using Xilinx system generator. [Fig. 2] shows the overall implementation model. [Fig. 3-6] shows various subsystem models for implementing the design.
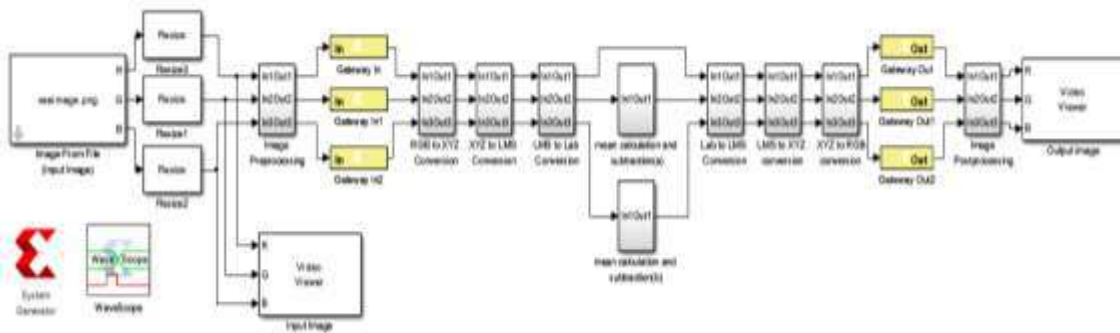
Fig. 2: Implementation of the color correction algorithm using system generator blocks

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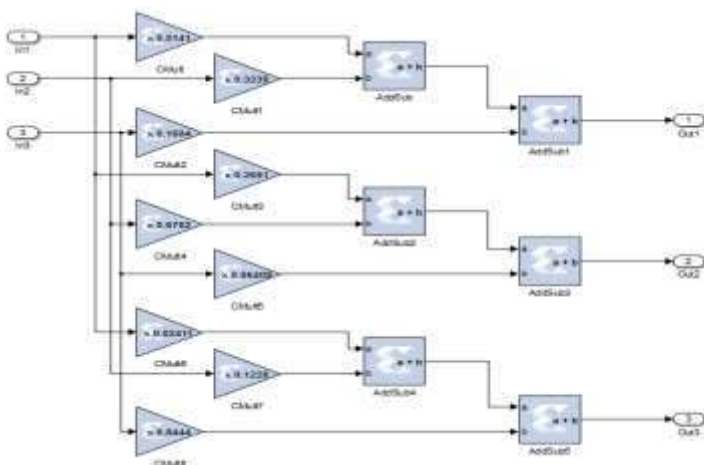
Fig. 3: RGB to XYZ conversion block

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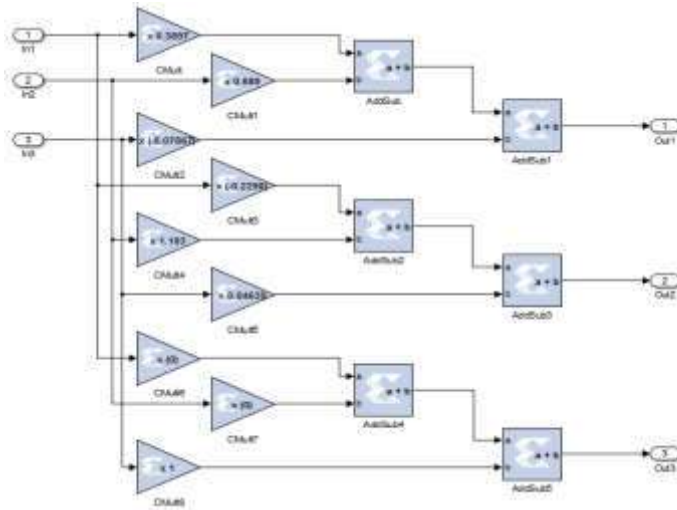
Fig. 4: XYZ to LMS conversion block

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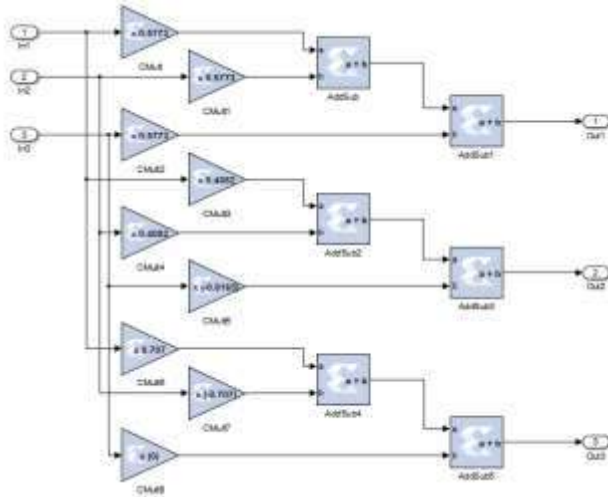
Fig. 5: LMS to Lab conversion block

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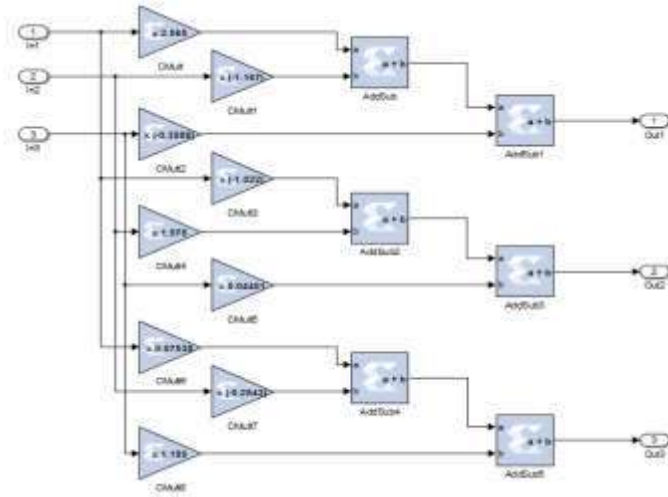
Fig. 6: Implementation of the color correction algorithm using system generator blocks

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RESULTS

Hardware co-simulation is done using Xilinx system generator blocks. Various underwater color images are given as input and output images are shown in [Fig. 7].It is observed that the output image shown is free from any color dominancy. The dominant colors that are present in the input color images have been corrected by the proposed method. The output images which are free from any color dominance can be used for various automatic underwater image applications.

Fig. 7: Input images (left) and corresponding output images (right)

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CONCLUSION

This project investigates an effective tool in underwater images for color correction. The algorithm relies on gray-world and uniform illumination assumptions used in the Iαβ space and it is developed, implemented and tested for color correction of underwater images. Color correction is achieved by the white balancing of chromatic components (α and β) and the luminance component is utilized to improve the contrast of the underwater image. The developed algorithm is implemented in Xilinx FPGA board (ML403) using Xilinx System Generator. Hardware co-simulation was carried out and the developed model is successfully used in conjunction with the ML403 board. From the outcomes of the experiment, it has demonstrated that this method can be used to remove unwanted greenish-blue components in underwater imaging.

CONFLICT OF INTEREST
There is no conflict of interest.

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None

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ARTICLE

SCREENING AND PREVALANCE OF SHV/CTX-M/TEM B-LACTAMASE RESISTANCE GENES IN *KLEBSIELLA* STRAINS ISOLATED BACTERIA FROM URINARY TRACT INFECTION IN PRE-SCHOOL-AGE CHILDREN IN JAHROM, IRAN

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ABSTRACT

Back ground and objective: Today, the existence of extended-spectrum beta-lactamases (ESBLs) in the bacteria isolated from children with urinary tract infection is considered a reality and a major health problem in most countries. Since the agent of these infections mostly has a multi-drug resistant, their treatment is sometimes long and difficult. In recent years, beta-lactamases have become one of the biggest factors for failure of antibiotic treatment. Hence, the present research aimed to phenotypically and genotypically study the antibiotic resistance in Klebsiella strains isolated from urine samples of children in Jahrom, Fars Province. **Materials and methods**: *This descriptive-analytic study was carried out in a period of 9 months. Among the 526 isolates from children under school age with urinary tract infection, 216 Klebsiella strains were identified and examined. Their drug susceptibility pattern to 8 antibiotics was studied using antibiogram test by disk diffusion method and then the phenotype confirmatory test was done using combined disks in order to detect ESBL-producing isolates for resistant isolates to cefotaxime and ceftazidime. Phenotypic identification of beta-lactamase genes (CTX-M, SHV, and TEM) was also done using Multiplex PCR.* **Results**: *In this study, among the 526 isolates examined, 256 isolates were related to Klebsiella . In antibiogram testing by disk diffusion method, the highest resistance and the highest susceptibility were related to amoxicillin (89.35%) and nitrofurantoin (10.18%), respectively. In the phenotypic confirmatory testing, among the 216 isolates, 109 samples (50.4%) were found to be producers of beta-lactamases. According to the genotypic testing (Multiplex PCR), the frequency of TEM, CTX-M, and SHV genes was 73%, 62%, and 36%, respectively.* **Conclusion:** *The findings of the present study suggest the high frequency of Klebsiella stains producing ESBLs in patients under the school age. Rational prescription of beta-lactamases, appropriate infection control policies, and health conditions can reduce the frequency of ESBL-producing bacteria in the urinary tract infections among children.*

INTRODUCTION

Klebsiella pneumoniae is one of the most important cases of infection acquired from community or hospital. This bacterium is one of the most common nosocomial pathogens which causes a variety of infections, especially in infants, such as pneumonia, septicemia, diarrhea, abscesses in the liver, endophthalmitis, meningitis, urinary tract infections, and bacteremia [1]. Increased emergence of multi-drug resistance among nosocomial isolates of *Klebsiella pneumoniae* has limited the treatment options for infections caused by this bacterium. Most of *Klebsiella pneumoniae* isolates are resistant to multiple drugs (MDR) [2]. Among the mechanisms of antibiotic resistance, beta-lactamases are considered the main defense of Gram-negative bacteria, particularly *Klebsiella pneumoniae*, against antibiotics of beta-lactam group. According to Ambler, Bush-Jacoby, beta-lactamases are divided into four groups. Bacteria with extended-spectrum beta-lactamase (ESBLs) are resistant to many antibiotics including penicillins, cephalosporin of the first, second, and third generations, and aztreonam (except cephamycins and carbapenems). These enzymes are inhibited by clavulanate. According to the classification of Bush-Jacoby, some of these enzymes are in 2be group (Class A) and others are in 2d group (Class D). The genes encoding these enzymes are placed on mobile genetic elements such as plasmids or chromosomes [1, 2]. Other enzymes found in *Klebsiella pneumoniae* are *Klebsiella pneumonia* carbapenemases (KPC) which hydrolyze beta-lactam drugs including monobactams, carbapenems, and third-generation cephalosporins. The gene producing this enzyme is placed on the plasmid and is able to transfer many bacteria. The mortality rate in infections caused by the bacteria containing KPC enzymes is about 50% [1, 3]. The most common ESBLs reported from Western and Asian countries are those derived from TEM, CTX-M, and SHV genes which are located on large plasmids and create drug-resistant strains [4, 5]. Recent studies indicate the expansion and dispersion of beta-lactamase genes in different parts of the world. In studies conducted in Spain and Taiwan, the frequency of ESBLs was reported to be 52.1% and 50%, respectively [6, 7]. Studies carried out in some cities of Iran such as Tehran, Tabriz, Shiraz, Kashan, and Amol showed that the frequency of ESBL-producing strains ranges between 23% and 97.87%. [8-12].The polymerase chain reaction (PCR) is regarded an accurate and highly sensitive method. The present research aims to determine antibiotic resistance pattern and frequency of beta-lactamase genes (TEM, CTX-M, and SHV) in *Klebsiella* strains producing ESBLs.

Sampling and isolation of bacteria

Using the cross-sectional, descriptive method, 585 cases of urinary tract infection in children (girls and boys) were collected from laboratories of hospitals in Jahrom, Fars Province in a nine-month period from

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October 2014 to June 2015 (two seasons). In all cases, demographic and clinical data were recorded in a questionnaire.

The bacteria isolated from patients were inoculated in Skim milk medium containing in 1.5 mL microtubes and then kept at a temperature -20°C. After transferring to the laboratory, bacterial samples were cultured on the selective-discriminative medium of EosineMethylen Blue for purification and then incubated for 24 hours at a temperature of 37°C.

Differential biochemical tests and identification of organisms

The colonies were identified by using the differential biochemical tests including fermentation of sugars in the TSI medium, indole production and motility in the SIM medium, and inhibition in Simon Citrate and Urea Agar media and then comparing the results with standard tables.

Determining the susceptibility and resistance of samples to antibiotics

In order to obtain the antibiotic susceptibility pattern of positive samples, disk diffusion susceptibility test (Kirby-Bauer) on Mueller-Hinton agar medium was used. For this purpose, 8 types of antibiotic disks were purchased from MAST Company (England). These 8 antibiotic disks included gentamicin (10 mg), nitrofurantoin (10 micrograms), nalidixic acid (30 mg), cephalexin (30 mg), ciprofloxacin (5 micrograms), cefotaxime (15 mg), ceftazidime (2 mg), and amoxicillin (10 mg). The obtained results were recorded based on CLSI Standard Table as susceptible (S), intermediate (I), and Resistant (R).

Isolation of possible strains producing ESBLs

Like the method for determining antibiotic susceptibility by disk diffusion, the medium was prepared and the bacterial suspension was inoculated to the medium. ESBL production was studied by measuring the increase in the diameter of growth inhibition zone around the ceftazidime-clavulanic acid disk or cefotaxime-clavulanic acid disk by 5 mm or more.

Determining the minimum inhibitory concentration (MIC) using E-test

After determining the antibiotic susceptibility of bacteria using disk diffusion method and identification of methicillin-resistant staphylococcus aureus (MRSA). Susceptibility of these strains to vancomycin, teicoplanin, linezolid, and cybrid was measured by the E-test method (Liofilechem, Italy) in order to determine the minimum inhibitory concentration (MIC). For this purpose, a suspension of the desired bacterium at a concentration of 0.5 McFarland (1.5×10^8) was prepared and cultured on the Mueller-Hinton medium. Then, the E-test strips were put on the inoculated media. The results were controlled after 18-24 hours and interpreted based on CLSI standard. In all phases of the study, standard strains were used as the quality control (QC) of tests. The standard strain of *Klebsiella* : ATCC: 13883 was used.

Determining the beta-lactamase genes using Multiplex PCR

DNA of isolated *Klebsiella* strains was extracted using the Cinanagen kit (Iran) based on the protocol specified by the manufacturer. PCR test for amplification beta-lactamase genes (TEM, CTX-M, and SHV) was done using the primers obtained from Cinnagen Company [Table 1].

Table 1: Sequence of primers used in Multiplex PCR

Primer name	Amplicon size(bp)	Nucleotide sequence(5'-3')
bla- TEM(F)	445	TCGCCGCATACACTATTCTCAGAATGA
bla- TEM(R)	445	ACGCTCACCGGCTCCAGATTTAT
bla- CTX-M(F)	593	ATGTGCAGYACCAGTAARGTKATGGC
bla-CTX-M(R)	593	TGGGTRAARTARGTSACCAGAAYCAGCGG
bla- SHV(F)	973	TCTCCCTGTTAGCCACCCTG
bla -SHV(R)	973	CCACTGCAGCAGCTGC(A/C)GTT

Thermal cycling for PCR included denaturation for 30 seconds at a temperature of 94°C, annealing for 30 seconds at a temperature of 60°C, and extension for 2 minutes at a temperature of 72°C. In this study, the standard strain of *Klebsiella* : ATCC: 13883 was used as the positive control. Finally, PCR products were electrophoresed 1.2% agarose gel medium and then examined under UV light in a transilluminator device [9].

Statistical analysis

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After data collection, they were inserted into SPSS-15.5 software and then the frequency tables were plotted. For statistical analysis, Fisher's exact test, ANOVA, chi-square, and correlation test were used.

Findings

Among the 585 cases of urinary tract infection, 216 isolates of *Klebsiella* were identified and isolated. Among the 216 samples of *Klebsiella*, 117 cases (54%) were related to girls and 99 cases (46%) were related to boys. The highest frequency among boys (22.22%) and girls (29.16%) was found in age groups of 2-4 and 4-6, respectively.

The highest drug resistance and susceptibility of isolated *Klebsiella* strains were against amoxicillin (89.35%) and nitrofurantoin(86.57%), respectively [Table 2].

Table 2: Frequency distribution of *Klebsiella* isolates by the disk diffusion method based on antibiogram pattern

Antibiotic	Resistant	Intermediate	Sensitive	Total
Amoxicillin	193(89.35%)	8(3.70%)	15(6.94%)	216(100%)
Nitrofurantoin	22(10.180%)	7(3.24%)	187(86.57%)	216(100%)
Ciprofloxacin	133(61.57%)	5(2.31%)	78(36.11%)	216(100%)
Gentamicin	42(19.44%)	10(4.62%)	164(75.92%)	216(100%)
Nalidixic Acid	181(83.79%)	7(3.24%)	28(12.96%)	216(100%)
Ceftazidim	137(63.42%)	8(3.70%)	71(32.87%)	216(100%)
Cefotaxime Sodium	146(67.59%)	10(4.62%)	60(27.77%)	216(100%)
cephalexin	84(38.88%)	3(1.38%)	129(59.72%)	216(100%)

Among the 216 sample of *Klebsiella*, 109 samples were evaluated positive in terms of owning the phenotype of ESBLs. Frequency of ESBL$^+$ in females and males was 29.16% and 21.29%, respectively. Using ANOVA analysis, it was observed that there is a significant relationship between gender and the results of combined disk diffusion based on gender in *Klebsiella* isolates producing beta-lactamases (P=0.028). The highest and the lowest frequency of *Klebsiella* isolates producing ESBLs were found in the age groups of 2-4 (17.59%) and under 2 (16.20%), respectively. Using the correlation coefficient test, no significant relationship was found between age group and combined disk test in *Klebsiella* isolates. The highest drug resistance and the highest drug susceptibility among ESBL$^+$ *Klebsiella* isolates were related to amoxicillin (44.90%) and nitrofurantoin (37.96%), respectively. MIC test was performed for 109 ESBL$^+$ *Klebsiella* isolates. Isolates showed a resistance of 100% to both ceftazidime and cefotaxime. The frequency of each of the beta-lactamase genes in different antibiotic classes in *Klebsiella* isolates. In all antibiotic classes has been shown [Table 3].

Table 3: Multiple resistance of Klebsiella isolates to different antibiotic classes

Nitrofurans (Nitro)		Penicillin (Amox)		Aminoglycosid (Genta)		Fluroquinolones (Cipro)		Quinolones (Nali)		Cephalosporins (Cefa,Cefo,Cefta)	
26		102		35		86		101		109	
0	CTX-M	17	CTX-M	0	CTX-M	12	CTX-M	17	CTX-M	18	CTX-M
0	TEM	18	TEM	2	TEM	15	TEM	20	TEM	21	TEM
0	SHV	8	SHV	0	SHV	6	SHV	8	SHV	8	SHV
1	CTX-M/TEM	24	CTX-M/TEM	5	CTX-M/TEM	18	CTX-M/TEM	20	CTX-M/TEM	24	CTX-M/TEM
0	CTX-M/SHV	5	CTX-M/SHV	0	CTX-M/SHV	5	CTX-M/SHV	5	CTX-M/SHV	5	CTX-M/SHV
0	TEM/SHV	7	TEM/SHV	2	TEM/SHV	6	TEM/SHV	6	TEM/SHV	7	TEM/SHV
25	CTX-M/SHV/TEM	26	CTX-M/SHV/TEM	26	CTX-M/SHV/TEM	24	CTX-M/SHV/TEM	25	CTX-M/SHV/TEM	26	CTX-M/SHV/TEM

CONCLUSION

Antimicrobial resistance has always been considered a serious problem for human health. Infection control is highly beneficial for patients and causes a reduction in mortality rate around the world. The family Enterobacteriaceae, particularly Escherichia coli and Klebsiella, creates a variety of infections in people and especially babies, as 4 million babies die of bacterial infections every year [13]. The highest rate of deaths has been reported to be related to pneumonia, septicemia, meningitis, and diarrhea. It seems that babies are more vulnerable due to incompleteness of their immune system at this age [14]. In this study,

585 cases of urinary tract infection were studied and 216 Klebsiella strains were isolated using the confirmatory tests. Among these 216 strains, 54% (117) were isolated from females, while 46% of them (99) were isolated from males. In studies conducted by Zhanel et al. (2008) in Canada and Kang et al. (2004) in Korea, frequency of isolates was higher in males (59% and 63%, respectively) [15, 16]. In terms of age distribution, patients ranged from 1 month to 6 years. The highest and the lowest isolates were related to the age groups of 2-4 and under 2, respectively. In the study of antibiotic resistance in isolates of Klebsiella , the highest drug resistance and susceptibility were against amoxicillin (89.35%) and nitrofurantoin (86.57%), respectively. In a study conducted by Arkin et al. (2013) on Klebsiella isolates, the highest resistance and the highest susceptibility were found to be related to amoxicillin (100%) and imipenem (1.66%), respectively [10]. Since imipenem and nitrofurantoin are considered nosocomial antibiotics in Iran and are not taken without a prescription, resistance to these drugs is fortunately low in Iran [17]. On the other hand, because of the small molecular size, these two antibiotics can overcome the poor permeability of beta-lactamase in bacteria and especially Pseudomonas species by penetration through purines [18]. Studying the pattern of susceptibility to antibiotics showed that amoxicillin has the lowest antibacterial effect (6.94%). This is also corroborated by other studies [19]. According to the present study, nitrofurantoin and gentamicin are among the drugs which can be used for treatment of patients, if antobiogram is done accurately.

Among the 216 Klebsiella isolates in the urine samples of children, 189 isolates (87.50%) showed multiple drug resistance. Among the 526 samples of Escherichia coli and Klebsiella , 245 cases (47%) were resistant to ceftazidime and cefotaxime or both of them. After performing the combined disk susceptibility test, it was revealed that 136 isolates of Escherichia coli (44%) and 109 isolates of Klebsiella (49%) owns the phenotype producing ESBLs. Using the MIC and agar dilution method, the highest production of ESBLs in Escherichia coli in Iran has been calculated to be 96.70% [19]. In a study conducted by Lavigen et al. (2007) in France, ESBL enzyme production in Escherichia coli strains has been reported to be less than 3% [20]. The results of a study conducted by Mohammadi et al. (20006) in Isfahan showed that ESBLs production in organisms causing urinary tract infection is 80%[21].In India, Lebanon, and Russia, ESBLs production has been reported to be relatively high (42%, 77.77%, and 15.5%) [22-24]. Comparison of these results shows that the frequency of ESBLs may be different in strains isolated from different countries and event different geographical areas of a country. This depends on sanitary conditions, infection control systems, and treatment methods. The main risk factor for the increase in bacteria producing ESBLs is the overuse of antibiotics (including the third-generation cephalosporins). In studies conducted by Nakhai and Moghadam (2011) in Mashhad, the frequency of positive ESBLs was reported 19% [25]. These figures are less than those obtained in the present study. In studies carried out by Sultan (2011) and Mirsalehian (2010) in Tehran, the frequency of ESBLs isolates was reported to be nearly similar to the results of the present study (56.69% and 59.36%) [26, 27]. In studies conducted in Austria by Eisner et al., ESBL producing isolates showed a growth rate of 58% in the period 1988-2004 [28]. In addition, in a study conducted in 2011 in India and a study by Sasaki in Thailand, the results were close to the findings of the present study [29]. Considering the comparison of different percentages with each other, it should be noted that regional differences in different parts of the world or even within a country create different therapeutic responses to antimicrobial drugs. The origin of these differences in different locations includes genetic differences between individuals, genetic differences between strains, and differences in other fields. Accordingly, treatment models used in different parts may be different based on the specific features of each region. Therefore, regular and ongoing investigations should to be conducted around the world [30]. For organisms that showed an intermediate resistance pattern to cefotaxime and ceftazidime at antibiogram test and were phenotypically positive in the confirmatory test, MIC (minimum inhibitory concentration) was calculated using E-test. The results showed that 109 ESBL isolates of Klebsiella have a 100% resistance to ceftazidime and cefotaxime. The highest frequency of MIC in Klebsiella isolates for cefotaxime and ceftazidime belonged to 25 micrograms per ml and 12.5 micrograms per ml. In this study, genotype off positive ESBLs was evaluated by Multiplex PCR. According to this method, frequency of TEM, CTX-M, and SHV was 73%, 63%, and 36%, respectively. The most common ESBL found in this study and similar studies [27, 31-32] is TEM. However, many other studies have reported that CTX-M is the most common ESBL [33-38]. There are few studies in which SHV has been introduced as the most common ESBL enzyme [31, 39, 40]. For instance, Paterson et al. reported a wide variety of ESBLs from 7 different countries in 20 years and showed that SHV is the most abundant enzyme among ESBLs (67%) [41]. In this study, frequency of TEM and SHV genes together in positive ESBLs was (8.97%) and less than TEM alone (20%). This is inconsistent with the findings of LAL et al. (2007) in India who reported that the frequency of TEM and SHV genes together and TEM alone was 67.3% and 20%, respectively [42].

In the present study, the highest frequency of TEM in beta-lactamase-producing strains of Klebsiella was related to the age group of 4-6. Among the isolates resistant to cefotaxime and ceftazidime, Multiplex PCR showed that all of them did not contain CTX-M. This is due to the extension of ESBLs scope of action. This means that a bacterium can hydrolyze cefotaxime and ceftazidime by producing ESBL enzymes other than CTX-M. In addition, although the effect of CTX-M is somewhat specific to cefotaxime, it has the ability to hydrolyze other antibiotics of cephalosporin family [43]. In the present study, the extent of resistance of isolates producing ESBLs to nalidixic acid, ciprofloxacin, and gentamicin was relatively high. Some strains were also resistant to nitrofurantoin. However, place of effect and mechanism of nitrofurantoin and gentamicin are different from beta-lactam drugs. The results showed that the presence of beta-lactamase genes (SHV, TEM, and CTX-M) is significantly related with the resistance of strains producing beta-lactamase to these antibiotics. Genes encoding resistance to these antibiotics are possibly transferred

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with ESBL genes. In a study conducted in the US, among the 20 bacteria resistant to antibiotics isolated from patients in hospitals or nursing homes, 17 bacteria contained 54-kb plasmid which codes resistance to ceftazidime through TEM, ESBL. This plasmid was also the mediator of resistance to gentamicin [44]. In the present study, among the ESBL-producing isolates, those encoding blaCTX-M / TEM / SHV gene showed higher resistance to gentamicin and nitrofurantoin than the isolates encoding other genes. The isolates that simultaneously have all these three genes are more likely to transfer the gene of resistance to gentamicin and nitrofurantoin in plasmid. Confirmation of this require further studies. According to what mentioned above, it is recommended that development of resistance in bacteria to be prevented by monitoring the consumption of antimicrobial agents in the treatment of infections eligible for multiple drug resistance. ESBLs are mostly of plasmid type and since these plasmids are easily transferred between different types of bacteria of Enterobacteriaceae family, accumulation of resistant genes leads to creation of strains with multi-drug resistance. In fact, plasmids, because of their replication that is independent of chromosome, are replicated in large numbers within the cell and cause antibiotic resistance in the case of transfer to other strains [45-48]. Previous studies show the high prevalence of beta-lactamase genes and especially TEM in urinary tract infection. Therefore, in order to identify this type of resistance, it is necessary to apply molecular methods along with phenotypic ones. Patients afflicted with infectious agents producing ESBLs, in addition to lack of treatment by ESBLs, often show resistance to other antibiotics. ESBLs have been of particular importance in treatment strategy, because this is due to treatment failures resulting from prescription of antibiotics without a susceptibility test which itself increases the mortality rate, prolongs the hospitalization period, and increases treatment costs. Therefore, rapid identification of ESBL-producing strains in microbiology laboratories is very important and essential [49, 50]. Production of ESBLs is considered a great threat to the consumption of cephalosporins with extended spectrum. Thus, to treat infections that are suspected to ESBL-producing organisms, the most appropriate antibiotic should be selected very carefully. In addition, strains that their susceptibility to ceftazidime and cefotaxime has reduced should be studied in terms of ESBL genes. Due to the high rate of prevalence of ESBL-producing bacteria in the urinary tract infections, it is recommended to identify the ESBL-producing strains among *E. coli* and *Klebsiella* . It is obvious that beta-lactam antibiotics are not recommended for treatment of infection in these patients. In addition, many of these bacteria may be probably resistant non-beta-lactam antibiotics such as nalidixic acid, ciprofloxacin, and gentamicin. In this case, antibiotics such as nitrofurantoin and imipenem are recommended to be applied for treatment.

CONFLICT OF INTEREST
The authors report no conflict of interest

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