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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

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RESEARCH ARTICLE OPEN ACCESS

DESIGNING OF A MODEL FOR HUMAN AMELOGENIN FOR PREDICTING ITS ROLE IN MINERALIZATION DURING SYNTHESIS OF TOOTH ENAMEL, AN IN SILICO APPROACH

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ABSTRACT

The Amelogenin protein found in developing tooth enamel is believed to organize enamel crystals during tooth development by acting as a crystallization centre for mineralization. The motive of the current study was to predict the 3-D structure of human amelogenin X and mechanism of its self-aggregation using computational methods. Homology modeling followed by threading and ab-initio methods were used for structure prediction to obtain a protein model suitable to study the self-aggregation of amelogenins in-silico. The model obtained from HHPred was selected, refined, and optimized using different bioinformatics servers and softwares. On analysis, the model gave acceptable Procheck, Verify-3D, Errat and ProQ results. The predicted model could be validated by studying its intermolecular interaction to form nanospheres which is in agreement with literature reports. This predicted protein model can be used further to study the protein-mineral-protein interactions taking place in the process of amelogenesis for hypothesizing on new restorative materials.

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KEY WORDS

Tooth enamel; In silico; Homology Modeling; ramachandran plot; amelogenin

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[I] INTRODUCTION

Biological materials like enamel exhibit intricate architecture and outstanding physical properties, unobtainable by traditional methods of materials synthesis. As a result, despite enormous effort no ideally biocompatible artificial tooth enamel material has so far been developed [1]. In case the mechanism of the natural process of enamel formation is understood, better materials based on the same principle can be designed which would have properties closer to the natural enamel. Enamel is composed of proteins amelogenin, ameloblastin, enamelin and tuftelin. Of these more than 90% is constituted by amelogenin. Thus, it can be considered as major protein responsible for enamel formation and has been studied under the present work.

The protein has been found to exist in two major isoforms - AMELX (Amelogenin X isoform) and AMELY (Amelogenin Y isoform), encoded by AMGX and AMGY genes present on the short arms of the human X and Y chromosomes [2]. Amelogenin X was chosen for this study because it is reported that mutations in AMELX may cause Amelogenesis imperfecta, a disorder of tooth enamel development in which teeth may become usually small, discolored, pitted or grooved, and prone to rapid wear breakage [3]. Also, AMELX is the structural constituent of tooth enamel and is involved in hydroxyapatite binding and Amelogenin-Amelogenin protein binding [2].

The matrix-mediated enamel biomineralization involves secretion of the enamel specific amelogenin proteins that through self-assembly into nanosphere structures provide the framework within which the initial enamel crystallites are formed [4]. Amelogenin protein is found in the developing tooth enamel and belongs to the family of extracellular matrix (ECM) proteins. Its function is believed to be in organizing enamel crystals during tooth development. It has been found that amelogenin is primarily hydrophobic, rich in proline (25%), glutamine (14%), leucine (9%), and histidine (7%), which altogether account for more than 50% of the amino acids. The amino acid sequence can be divided into 3 domains, based on differences in composition of amino acids [1]:

a). The N-terminal domain with 45-amino-acids is rich in Tyr (TRAP),

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b). The hydrophobic central region, primarily composed of Xxx-Yyy-Pro- repeat motifs (where Xxx and Yyy are primarily Gln) and
c). The 11-Amino-acid-long C-terminal domain, being charged and hydrophilic, contains a number of potential electrostatic binding sites—acidic aspartic acid and glutamic acid and basic lysine and arginine.

The entire sequence shows high levels of homology in short conserved regions of protein (motifs) as determined by the MEME motif discovery method. The list includes the following motifs: DKTKREEVD, SYGYEPMGGW, GYINFS/LYE, LKWYQSMIR, MGTWILFACLL LLGAAF, DLPLEAW, MMPVPGQ/HHSMTPTQHHQPN, LHHQIIPVL/VSQ, S/AHA/ TLQPHHHI/ LPV/MVPAQQPV, QQPFQPQ [5].

A phylogenetic tree was constructed using the amelogenin sequences of human, pig, horse, goat, bovine, guinea pig, hamster, mouse, rat, crocodile, xenopus, snake, dog, platypus, porcupine etc., which showed closest homology between pig and human amelogenin sequences [5]. Due to this fact most of the study related to this protein has been done using pig amelogenin, which is readily available from slaughterhouses.

Amelogenin is a unique biomineralization protein because it self-assembles to form supramolecular structures called "Nanospheres"- spherical aggregates of monomers that are 20−60 nm in diameter, consisting of spherical substructures that are 4-8 nm in diameter [1, 6]. Amelogenin nanosphere assembly proceeds through intermediate structures (perhaps represented in vivo by "stippled material") of some 4-5 nm hydrodynamic radius, and is computed to comprise 4-6 amelogenin monomers [7]. Nanospheres are best described by a dense, predominantly hydrophobic, protein core, surrounded by a loose shell comprised of the negatively charged hydrophilic C-terminus that is exposed to the aqueous environment. It has been suggested that this negatively charged surface prevents the aggregation of nanospheres at room temperature and below. Conservation of above mentioned functional sites may indicate a role for these residues in nanosphere formation [1].

Biomineralization is regulated by an interplay between hydrophobic and hydrophilic molecules, whereby the hydrophobic molecules provide a skeletal or space-filling structure and the hydrophilic (acidic) molecules are involved in the regulation of crystal nucleation and growth. Thus, amelogenin possesses both properties required for biomineralization of enamel [4].

Two key proteinases have been identified within the enamel matrix - MMP20 (Enamelysin) and Kallikrein (KLK4), along with a serine proteinase ESMP1. MMP-20 is expressed during the secretory stage, when the crystallites are growing predominantly in length. As MMP-20 slowly degrades these proteins, the crystallites grow in width and thickness so that there is a net replacement of protein by minerals [8].

KLK4 is a protease with broad target sequence specificity that can degrade enamel proteins. This protein is secreted during the transition and maturation stages of amelogenesis, immediately preceding the point where the quantity of enamel proteins in the matrix drops precipitously. KLK4 functions during the later stages of dental enamel formation to degrade the accumulated enamel matrix, allowing the crystals to grow in width and thickness until adjacent crystals contact. This activity is essential for hardening of the enamel [9,10].

Thus if this natural process of enamel formation can be simulated and additionally synthetic material based on parallel process can be generated, it may have desirable properties of a biocompatible restorative material. For working on this idea, our first objective was to predict the 3-D structure of the protein amelogenin X and study its role in enamel formation. .

[II] MATERIALS AND METHODS

2.1. NCBI sequences

The protein sequence of amelogenin X (Genbank id: AAA51717.1) zz was downloaded from the NCBI database, 191 amino acids are found in total size of the sequence and the source of this protein is Homo sapiens in origin [11].

2.2. NCBI-Blast

The sequence obtained from the NCBI Reference Sequences database was submitted to NCBI-Blast and searched against the Protein Data Bank (PDB) database to extract suitable structural templates [12].

Since, on doing BLAST of query sequence with PDB database, none of the matches obtained had even up to 30% similarity score, it could be concluded that homology modeling cannot be performed as no appropriate template could be found. Therefore, secondary structure predictions (2.3), along with threading and ab-initio predictions (2.4) were used to obtain the suitable 3-D amelogenin X protein model.

2.3. Prediction of the 2D structure of Amelogenin X using the following list of the bioinformatics tools and servers

1. Genamics Expression (http://genamics.com/expression/strucpred.htm) - It is a windows application program for DNA and protein sequence analysis. It offers an interface to a large array of secondary structure prediction algorithms including DPM, DSC, GOR 4, HNN, MLRC, PHD, PREDATOR, SIMPA96, and SOPMA [13].

2. PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/psiform.html) - It is a protein structure prediction server that allows users to input a protein sequence, do a prediction and get the output of the prediction textually via e-mail as well as graphically via the web [14].

3. JPRED (http://www.compbio.dundee.ac.uk/www-jpred/) - It is not an individual routine but assembles various scores for secondary structure. The consensus of structure prediction algorithms is obtained as result [15].

4. GOR4 (http://npsa-pbil.ibcp.fr/cgi-in/npsa_automat.pl? page=npsa_gor4.html) - It is the fourth version of GOR. It is based on information theory and does not have a defined decision constant, i.e., the standard against which the values of pair frequencies have to be compared. GOR

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IV employs all likely pair frequencies inside the window of 17 amino acid residues. A mean accuracy of 64.4% has been obtained for a three state prediction (Q3) after carrying out cross validation for a database having 267 proteins [16].

5. HNN (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl? page=npsa_nn.html) - Hierarchical Neural Network prediction method is constituted by two networks. One of these is a sequence-to-structure network and the other one is a structure-to-structure network. This makes only local information as the basis of prediction [17].

6. PROF pred (http://www.aber.ac.uk/~phiwww/prof/) - It is a service provided by Predict Protein serving sequence analysis, structure prediction and function prediction [18].

7. 3D-Jigsaw (http://bmm.cancerresearchuk.org/~3djigsaw/) – It is an automated, comparative modelling server which may be used for predicting the structure and/or function of a protein sequence [19].

8. PORTER (http://distill.ucd.ie/porter/) – It is a server used for protein secondary structure prediction. A collection of 45 bidirectional recurrent neural networks (BRNNs) forms the basis of its algorithm. It has been tested by a rigidly accurate 5-fold cross validation method and achieves 79% correct classification on the "hard" CASP 3-class assignment [20].

9. PORTER+ (http://distill.ucd.ie/porter+/) – It is a server for the prediction of local structural motifs. The motifs are constructed by using multidimensional scaling (MDS) and clustering for pair-wise angular distances of multiple Φ and Ψ dihedral angle values compiled from high-resolution protein structures. This method allowed visualization of the protein backbone fragments in a scaled down 3D conformational space from the earlier multiple dimensions and led to the recognition of a few conformational clusters which are populated by real or validated backbones. In Porter+ these clusters are mapped into a conformational alphabet of 14 letters which represent structural motifs for tetra-peptides. Porter+'s architecture is similar to Porter's. It classifies nearly 60% of residues as the right structural motif and around 30% to be above a base-line statistical predictor [20].

2.4. Prediction of 3D structure of amelogenin X using following bioinformatics servers and softwares

1. I-Tasser (http://zhang.bioinformatics.ku.edu/I-TASSER/) – It is an online service for predicting the protein structure and function. Based on multiple-threading alignments, models are constructed by LOMETS and I-TASSER simulations [21].

2. LOMETS (http://zhang.bioinformatics.ku.edu/LOMETS/) - Local Meta-Threading-Server is an internet service for protein structure prediction. It returns 3D models by assembling consensus target-to-template alignments from nine locally-installed threading programs which include FUGUE, PROSPECT2, PAINT, SPARKS, PPA-I, SP3, PPA-II, HHsearch, SAM-T02 [22].

3. Genesilico (http://genesilico.pl/) - It is a metaserver which comprises of many constituents including PSIPRED, PROF, HMMPFAM, TMHMM, local PDB-BLAST, several 3-D structure prediction methods (RAPTOR, 3DPSSM, FUGUE, mGENTHREADER, FFAS, SAM-T02 and BIOINBGU). Based on the target-template alignments obtained as FR results and backbone of the template, primary 3D models of query structure are constructed using SCWRL. All FR alignments received from various servers undergo united appraisal by energetic standards implemented in VERIFY3D alongwith the grading criterion proposed by the PCONS server [23].

4. Bioinfobank (http://meta.bioinfo.pl) – The structure prediction Meta Server provides access to various fold recognition, function prediction and local structure prediction method. It takes the amino acid sequence of the query protein. All results of fold recognition servers are translated into uniform formats. The information extracted from the raw output of the servers includes the PDB codes of the hits, the alignments and the similarity (reliability) scores specific for every server. Mapping of the hits to the SCOP and FSSP classifications are made either using known PDB representatives or alignment of the template sequence with the databases of proteins in both classifications. The secondary structure assignments for all hits are taken from the mapped FSSP [24].

5. Loop refiner (http://genesilico.pl/) [23].

6. HHPred (http://toolkit.tuebingen.mpg.de/hhpred) – It is a sensitive protein homology analysis and structure prediction utilizing HMM-HMM-comparison. HHpred constructs a profile HMM using a query sequence and equates it to a database of HMMs which is having footnoted protein families or domains having known structure (PDB, SCOP). Output obtained is a list of nearest homologs and their alignments [25].

7. MODELLER: Selected templates were used in modeling the protein to construct the theoretical three dimensional protein structure using the Modeller software version 9v7 [26].

8. SwissPdbViewer: The theoretical model was subjected to SwissPdbViewer for energy minimization and for correcting the stereochemistry of the model [27].

2.5. Evaluation of protein model

A) UCLA server (http://www.doe-mbi.ucla.edu/) - It consists of Procheck [28], Errat [29], Verify-3D [30]. Procheck assures the stereochemical character of a protein structure by examining its residue-by-residue as well as overall structure geometry, Errat examines the statistics of non-bonded interactions within different atom types. It also maps the location of a 9-residue sliding window value versus error function. The location of the 9-residue sliding window is computed by a search for similarity and differences with statistics of highly refined structures, Verify-3D assigns a structural class to the atomic model (3D) based on its environment and location (polar, nonpolar, loop, alpha, beta etc.) and compares the results to good structures. In this way it decides the harmony of the atomic model to its own amino acid sequence (1D). The results of different models obtained were compared using this server.

B) PROQ: it is a neural network-based method to predict the quality of a protein model that extracts structural features, such as frequency of atom–atom contacts, and predicts the quality of a model [31].

2.6. Docking of Amelogenin monomers

ClusPro docking server (http://nrc.bu.edu/cluster/) – It presents as the first fully automated, web-based program for computational docking of protein structures. Coordinate files of the proteins are uploaded. It assesses many acknowledged complexes, holding back a predetermined number of complexes with favorable surface complementarities. A filter is then employed for this set of structures. Structures having correct electrostatic and desolvation free energies are selected for further clustering. The output of the program is a short list of acknowledged complexes, i.e., complexes which have been produced by server after clustering and checking for feasible values of electrostatic and desolvation free energies, which are ranked in accordance with their clustering properties [32].

[III] RESULTS

3.1. Blast output results

The PDB Blast results indicate poor homology of known PDB structures to human Amelogenin X sequence [Figure-1]. The templates were 2CSD/A (Topoisomerase V), 2CSB/A (Topoisomerase V from Methanopyrus Kandleri), 2BLE/A

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(Human GMP Reductase in complex with GMP), 2ESH/A (Consereved potential transcription factor) having identity of 17%, 17%, 29%, 26%, respectively and similarity of 39%, 36%, 43%, 37%, respectively. From these results it is clear that homology modeling will not yield good results. So, alternative methods were undertaken.

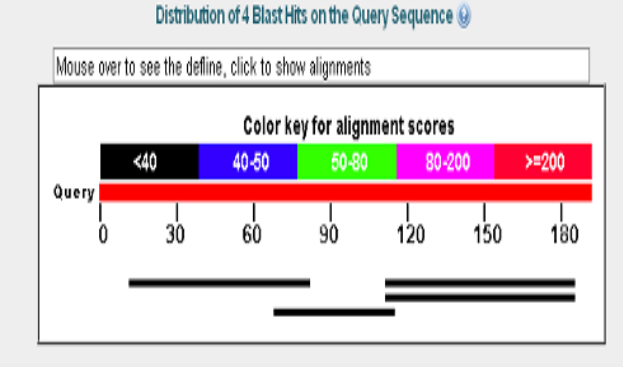

Fig: 1. Blast output result of Amelogenin X showing poor homology between the templates and Amelogenin amino acid sequence.

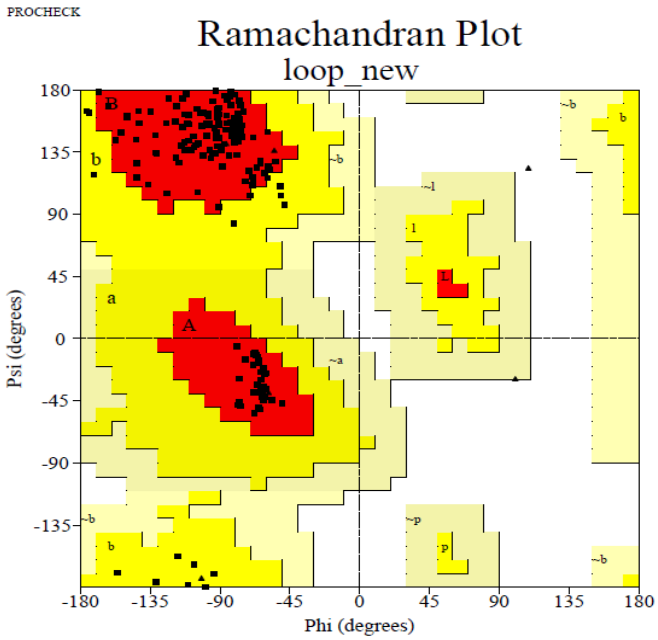

Fig: 2. Ramachandran plot for final model of amelogenin X showing most of the residues falling in the core and allowed regions of the plot.

3.2. Secondary structure prediction of Amelogenin X

Using the above mentioned tools (2.3) for secondary structure prediction, the predicted results were evaluated for the helix, strand and coil content of the protein. These results showed that most of the protein structure consists of coil and from the consensus it is evident that a very small part is constituted by helical elements. A still smaller or negligible part is constituted by strand **[Table-1]**.

3.3. *3D structure prediction of amelogenin X*

Using the various threading and ab-initio modeling servers (2.4) a number of models were obtained and analysed using various in-silico parameters like the Ramachandran scores, Verify 3D, Errat, ProQ scores and the energy values. The I-TASSER and Genesilico prediction servers gave low Ramachandran scores and high energy values. LOMETS though gave very good Ramachandran scores, the Verify-3D and Pro-Q scores were not in an acceptable range. The model predicted by Bioinfobank also gave unacceptable Verify-3D and LG scores. From this analysis it is concluded that HHPred gives the only reliable 3-D protein model on the basis of the Ramachandran scores (Core – 83.6%, Allowed – 12.5%, Generously allowed – 2.3% and Disallowed – 1.6%), energy (-1167.12) and other parameters in an acceptable range. This HHPred model was taken for further loop refinement, energy minimization and analysis **[Table-2]**.

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3.4. Final model analysis

The model predicted by HHPred server was subjected to further refinement and energy minimization. It shows an improvement in Ramachandran scores and decrease in energy at each step, values of other scores also being acceptable, resulting in the 5th model as best approximation **[Table-3]**. Aiming for further refinement rendered the parameters become unfavorable, i.e., the Ramachandran score decreased, energy values increased, verify-3D, Errat and ProQ scores also entered unfavorable ranges. From this the 5th model was taken for further study as the values of all parameters analyzed were acceptable and the Ramachandran plot showed the phi-psi torsion angles for all residues in the structure (except those at the chain termini) in an acceptable area **[Figure-2]**. The secondary and tertiary structure of the predicted model obtained from HHPred **[Figure-3(a)]**, and the final predicted tertiary structure of Amelogenin X after refinement of model obtained from HHPred are shown in **[Figure-3(b)]**.

3.5. Self-aggregation of amelogenins

In order to further check the validity of the model predicted above, ClusPro was used. Its output gave ten configurations of the aggregation of amelogenin X to form clusters, and based on the energy values, the best configuration was selected **[Figure 4]**. Being evident from the results, the configurations match the literature findings that the hydrophobic central region of Amelogenin is forming the core and N- and C-termini are facing the outside in the formation of nanospheres upon interaction of the six monomers [7].

Table: 1. Secondary structure prediction of human Amelogenin X sequence using different servers and analyzing the helix, strand and coil content (in %) as predicted by these tools for reaching a consensus

Sl. NO.	Programs/Tools	Helix	Strand	Coil
1.	PSIPRED	3.60%	-	96.40%
2.	JPRED	10.99%	-	89%
3.	GOR4	-	17.80%	82.20%
4	HNN	6.28%	-	93.72%
5	SOPMA	10.47%	10.99%	71.73%
6	PROFPRED	5.76%	3.14%	91.10%
7	PORTER	6.80%	-	93.20%
8	PORTER+	9.95%	6.80%	83.25%
9	LOOPP	4.71%	1.05%	94.24%
10	I-TASSER	4.19%	-	95.81%
11	PHYRE	4.19%	-	95.81%
12	3D-PSSM	8.90%	-	91.10%
13	3D-Jigsaw	6.80%	-	93.20%
14	Prof prediction	8.38%	6.28%	85.34%
	Genamics Expression			
15	DPM	-	-	100%
16	DSC	8.90%	3.14%	87.96%
17	GOR4	-	17.80%	82.20%
18	HNN	5.76%	8.90%	85.34%
19	MLRC	14.66%	4.71%	80.63%
20	PHD	6.28%	9.95%	83.77%
21	Predator	6.28%	3.66%	90.06%
22	SIPMA96	15.70%	2.60%	81.70%
23	SOPMA	13.09%	10.99%	75.92%

[IV] DISCUSSION

The process of biomineralization of enamel is of great biological interest due to its uniqueness. This process is different from e.g. bone mineralization of bone, as in the latter process the protein collagen is an integral part of the mineralized tissue, whereas in mineralization of enamel the protein amelogenin is itself degraded in the process, ultimately leaving only the mineral content, thus making enamel the hardest tissue in humans. Therefore, for simulating this natural process of amelogenesis, the structure of amelogenin X protein was predicted, and by using a protein-protein docking approach we identified self aggregated amelogenins in form of cluster **[Figure-4]**. Next, binding interaction of amelogenin X to MMP20 and to the mineral Calcium hydroxyapatite needs to be done. These interactions provide an idea about the natural process of enamel formation, which can then be used to propose a material by substituting some components of this system by other components which may lead to a more biocompatible material that can be used for restoration.

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Table: 2. Comparative result of Ramachandran Score (in %), Verify 3-D, Errat, ProQ and Energy of different models predicted by I-Tasser, LOMETS, Genesilico Model server, Hhpred and Bioinfobank server.

MODEL No.	Ramachandran Score (in %)				Verify 3-D	Errat	ProQ		Energy
	Core	Allowed	Gener	Disall		Overall quality factor	LG Score	SF Score	
I-Tasser Prediction									
1	51.50	30.30	14.40	3.80	10.42	0	51.50	30.30	14.40
2	77.30	11.40	9.10	2.30	0.52	0	77.30	11.40	9.10
3	75.80	12.90	0.80	4.50	0.00	12.766	75.80	12.90	0.80
4	44.70	28.00	18.20	9.10	48.96	0	44.70	28.00	18.20
5	54.50	28.30	15.20	1.50	24.48	0.637	54.50	28.30	15.20
LOMETS Prediction									
1	85.60	10.60	2.30	1.50	4.17	9.259	1.191	0.121	-2079.12
2	89.40	7.60	0.80	2.30	11.46	11.765	1.176	0.107	-1740.55
3	92.90	7.10	0.00	0.00	25.52	0	0.603	0.095	41.95
4	77.30	18.90	2.30	1.50	36.46	34.973	1.855	0.18	-1158.61
5	81.80	12.10	0.03	3.00	11.98	8.523	1.047	0.074	-534.403
6	92.90	7.10	0.00	0.00	29.69	0	0.286	0.048	192.27
7	100.00	0.00	0.00	0.00	0.00	0	0.91	0.081	-1745.75
8	85.00	15.00	0.00	0.00	0.00	0	1.073	0.094	-1531.43
9	75.00	20.50	3.00	1.50	18.23	12.429	0.937	0.089	-479.24
10	83.30	14.40	2.30	0.00	0.00	5.369	1.354	0.134	-1490.82
Gensilico Prediction									
Best model	59.80	28.80	6.80	4.50	33.33	2.21	1.714	0.105	16336.2
HHPred prediction									
	83.60%	12.50%	2.30%	1.60%	3.13%	13.376	0.479	0.057	-1167.12
Bioinfobank prediction									
Best model	73.90%	21.70%	0.00%	4.30%	29.17%	1.109	2.16	0.186	-2110.81

Table: 3. Showing comparative results of Ramachandran Score (in %), Verify 3-D, Errat, ProQ and Energy of different models obtained after loop refinement and energy minimization of the optimal protein model at various stages during refinement and energy minimization

Model No.	Ramachandran Score (in %)				Verify	Errat	ProQ		Energy
	Core	Allowed	Gener	Disall		Overall quality factor	LG Score	SF Score	
1	84.40%	12.50%	2.30	0.80%	14.06%	17.033	0.81	0.098	-1651.03
2	85.20	13.30	1.60	0.00	8.85	27.011	1.50	0.17	-3125.13
3	88.30	10.90	0.80	0.00	23.44	39.412	2.44	0.307	-3910.89
4	88.30	11.70%	0.00	0.00	15.63%	49.123	2.62	0.341	-3832.98
5	**88.30**	**11.70**	**0.00**	**0.00%**	**12.50**	**49.123**	**2.42**	**0.336**	**-3929.03**

[IV] CONCLUSION

The From the results obtained for secondary and tertiary structure prediction of human amelogenin X, a model could be constructed. Since this model is giving reliable results on validation and also the docking results obtained to check the proteins self-aggregation show similar results as given in literature [7], this model can be considered as the desired protein model for predicting the exact mechanism of enamel matrix formation, leading to more cost effective and biocompatible synthetic materials.

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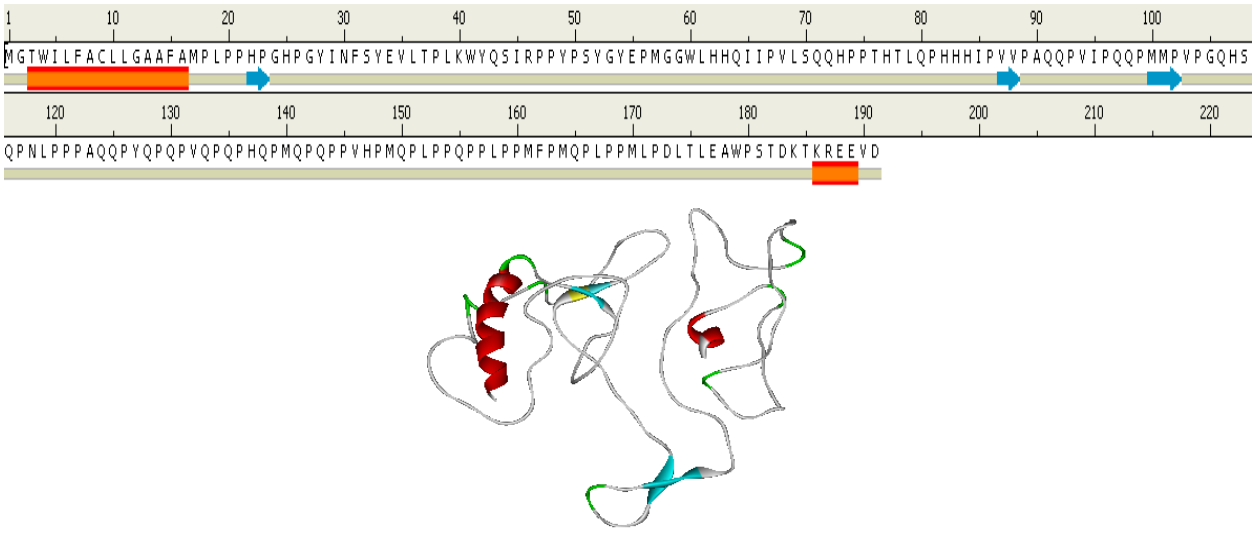

Fig: 3(a) Secondary and tertiary structure of the predicted model obtained from HHPred

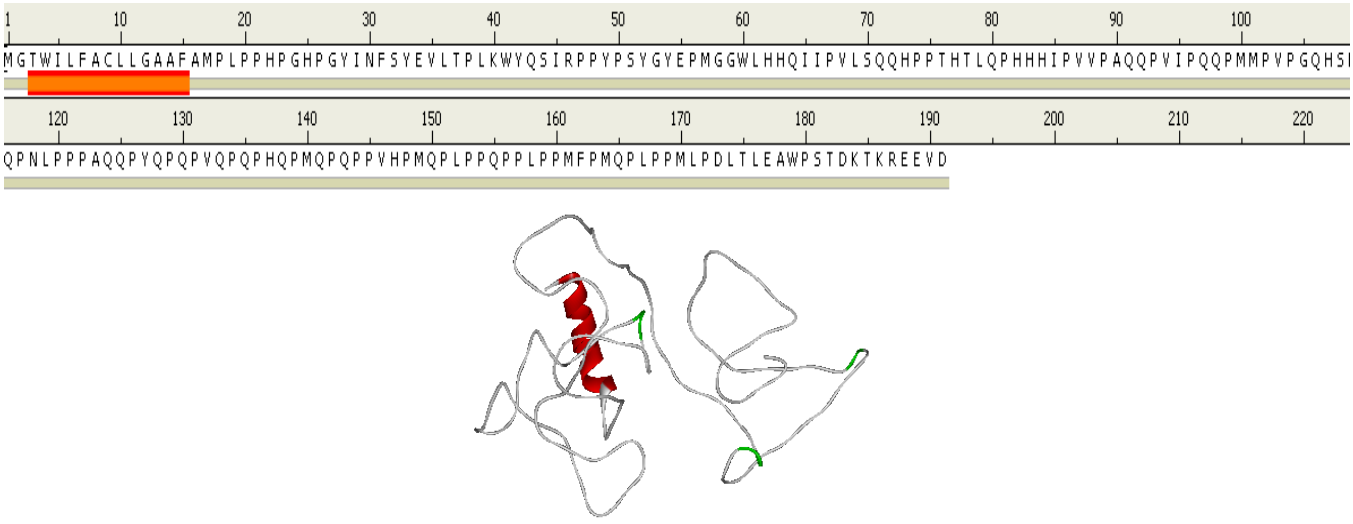

Fig: 3(b) Predicted final secondary and tertiary structure of amelogenin X after model refinement obtained from HHPred.

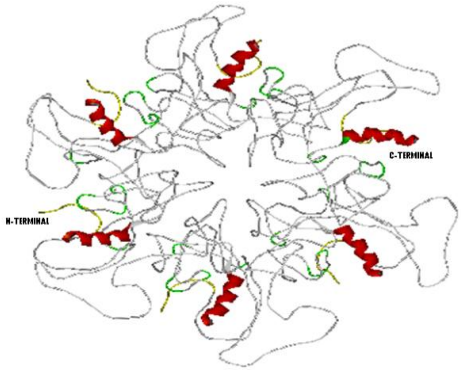

Fig: 4. Docking result of amelogenin with amelogenin to form aggregates; six amelogenin monomers interact with each other with the hydrophobic central region of Amelogenin forming the core and N- and C-termini facing outside.

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REVIEW ARTICLE OPEN ACCESS

MANAGEMENT PRACTICES TO MITIGATE THE IMPACT OF HIGH TEMPERATURE ON WHEAT: A REVIEW

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ABSTRACT

Climate change is a reality and agriculture is highly vulnerable, particularly in the semi-arid and arid regions of India. The climate is changing rapidly through the processes such as CO2 and changed pattern of precipitation resulting in heat and drought stresses, respectively. This changing climate could strongly affect the wheat production worldwide. The effect of increasing temperature during grain filling stage of wheat causes substantial reduction in grain yield. In this review paper are discussed the factors responsible to reduce the grain yield of wheat under the climate change and agronomic management practices as time of sowing, alternative method of planting, mulching, seed priming, foliar spray of salts, use of potassium fertilizer with municipal waste water, use of extra irrigation water and foliar spray of micronutrients to mitigate the high temperature effect on the productivity of wheat. Priming with moringa water extract and ascorbate substantially improved the tissue water status, membrane stability, gas exchange, water productivity of the plant. Timely sowing of wheat crop generally gives higher yield as compared to late sown crop. Late sown wheat crop faces high temperature stress during ripening phase. Late planting reduces the tillering period and hot weather during critical period of grain filling lead to forced maturity thereby reduces the grain yield. Planting of wheat with zero tillage, bed planting and conventional tillage with mulching produced higher grain yield than conventional tillage. Organic mulches provided better soil water status and improved plant canopy in terms of biomass, root growth, leaf area index and grain yield, which subsequently resulted in higher water and nitrogen uptake and their use efficiencies. The foliar spray of KNO3 (0.5%) at 50 percent flowering stage, 1.0 per cent KNO3 during anthesis stage, 2.5 mM of arginine, spray of zinc, extra irrigation water during grain filling stage, use of potassium fertilizers with municipal waste water increased the productivity of wheat under high temperature conditions.

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Climate change; mitigate; Mulching; seed priming; foliar spray; extra irrigation water

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[I] INTRODUCTION

Crop performance and yield depend largely on environmental interaction. So, the knowledge as how to environment influences crop growth, development and yield of great importance. Non-stress environment is ideal for successful cop production. A biological stress can be defined as any environmental factor capable of inducing a potentially injury strains in living organisms [1]. Growth and development of wheat is adversely affected by environmental stresses like high temperature, soil moisture deficit, low light intensity, etc. Among these, temperature plays an important role in growth, development and yield of wheat. Wheat growing in India is a gamble of temperature [2]. High temperature at the both ends of wheat growing season in India is a limiting factor. In case of November sown crop, high temperature at sowing, helps wheat plant to accelerate its growth and thus shortens the tillering phase by inducing the crop to enter the elongation stage more quickly and this result in poor tillering of the crop. In case of delayed sown crop, stages like flowering and grain filling

Table: 1. Effect of dates of sowing on growth and yield contributing characters of wheat

Date of sowing	Plant height (cm)	Dry matter accumulation at maturity (q/ha)	Number of tillers (m^{-1}row length)
25 November	82.4	65.9	84.1
10 December	80.1	43.9	54.1
CD (p=0.05)	1.3	5.9	7.1

Source [27]

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coincide with rise in temperature and atmospheric drought during March and April, which causes poor growth and low grain yield.

Continual heat stress affects approximately 7 million hectare of wheat in developing countries, whereas terminal heat stress is problem in 40 per cent of the temperate environments that encompass 36 million hectare. Spring wheat is grown in these areas experience severe heat stress in certain phases of crop growth. In India also almost the entire wheat growing area falls under the tropical and sub-tropical environment. A sudden rise in temperature during grain filling prior to maturity is a widely prevalent phenomenon in almost all parts of India, causing significant reduction in yield [3]. Wheat grown under late sown conditions is exposed to very low temperature up to booting stage, but the later stages face higher temperature that inhibits grain development, resulting into poor grain yield.

Upon exposure to heat stress, development of various growth stages is accelerated to such a degree that the environment cannot supply necessary inputs (radiation, water and nutrient) fast enough [4]. In wheat, period from onset of spike ignition to flowering is very sensitive to temperature acceleration in this phase seems to be the main reason for reduction in sink size under high temperature conditions. Heat stress affects the production of wheat by causing reduction in duration of grain filling phase, kernel size, biomass, tiller number, etc. heat stress adversely affected days to appearance of first node, tiller per plant and spikelet's per plant, thereby resulting in reduction of sink capacity and future sources capability of the plant [5]. An increase in 10C can cause decrease of 4 mg in grain weight [6].

Many investigators have established the effects of temperature at the later stage of the development of wheat crop particularly after the heading stage. Average temperature of 150C during grain filling is optimum for maximum grain weight [7, 8]. High temperature during grain filling period of wheat affects the kernel weight and grain yield through reduction in grain filling [9]. However, an increase of 0.50C temperature resulted in decrease in the duration of wheat crop by seven days, which reduced the yield by 0.5 t ha-1 in north India [10]. In another studies, the effect of high temperature on grain development in wheat and rice studied [11]. It was found that wheat yield decreased by 5 per cent for each 10C rise in post anthesis daily mean temperature in the range between 17.70C and 32.70C. In another investigation was observed that a 20C increase in temperature in wheat and rice resulted in 15-17 per cent decrease in grain yield of both crops but beyond that the decrease was very high in wheat [12]. High temperature has many detrimental effects on wheat. High temperatures after anthesis hastens the leaf senescence shorten the period of grain growth and decrease the grain yield [13]. Under high temperature conditions, tillering and root growth are reduced but heading and maturity are accelerated. High temperature and drought reduced the duration of grain development period, thus reducing the grain size [14]. In this review paper are discussed the factors responsible to reduce the grain yield of wheat under climate change particularly the effect of high temperature during reproductive stage.

[II] FACTORS AFFECTING ON WHEAT UNDER CLIMATE CHANGE

2.1. Effect seed priming

The climate is changing rapidly through the processes such as CO_2 and changed pattern of precipitation resulting in heat and drought stresses, respectively. This changing climate could strongly affect the wheat production worldwide. In study evaluated the role of seed priming with inorganic salts ($CaCl_2$, KNO_3, KCl, plant water extracts (sorghum, moringa) and organic molecules (ascorbate, salicylicate, proline) in improving the wheat performance under heat and drought stresses [15]. For stress treatments, there were four sets in the experiment viz., optimal conditions, drought stress (at 50 % of the field capacity), heat stress (4^0C) higher than the ambient temperature and both drought and heat stress. For priming wheat seeds were soaked in aerated solutions of respective osmotic/plant water extract while water soaked and dry seeds were taken as control. Each of the stresses substantially reduced the wheat performance and the effects were more severe when both the plants were exposed simultaneously to both the stresses. Priming treatments substantially improved the tissue water status, membrane stability, gas exchange, water productivity and plant was better than the other inorganic salts, however, priming with moringa water extract and ascorbate was better than other treatments in the respective group. Seed priming techniques may therefore be employed to improve the wheat performance in changing climate.

2.2. Effect of dates of planting

Time of planting is one of the most important non-monetary inputs for optimizing the growth according to prevailing agro-climatic conditions and genotypes. The performance of wheat varies with different dates of planting.

2.2.1. Growth parameters

Conducted an experiment at Punjab Agricultural University, Ludhiana on loamy sand soil [16] and reported that plant height was maximum of the crop sown on 25 October (81cm), which was at par with crop sown on 4 November (76 cm) and 14 November. It was significantly superior to the crop sown on 24 November (72 cm). Another experiment was conducted at Palampur on silty clay loam soil [17] and noted that wheat crop sown in the 1st week of November had significantly higher plant height (98.1 cm) as compared to crop sown on 1st week of December (82.8 cm) and 1st week of January (75.5 cm). In another studies, the similar findings were reported from Gurdaspur, Punjab [18]. However, other experiments were conducted [19, 20] **[Table–2]** at Punjab Agricultural

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University, Ludhiana on loamy sand soil and reported that plant height was significantly higher of crop sown at November 15 (78.5 cm) as compared to December 20 (71.6 cm). Similar results were reported from Chiplima [21], and from Gurdaspur [22]. However, it was reported that plant height was not significantly affected by the date of sowing [23, 24]. From HAU, Hisar, reported that crop sown on 20 November produced significantly more leaf area index (4.4) than that of 20 December (3.9) sown crop [25]. However, at Ranchi, Bihar conducted an experiment on sandy loam soil [26] and reported that timely sown crop (24 November) produced significantly more leaf area index (4.31) as compared to late sown crop (3.01). Dry-matter accumulation was also significantly higher with timely sown crop (879.9 gm-2) than late sown crop (662.6 gm-2). However, plant height, dry matter accumulation and tillers per meter row length were significantly higher in early sown crop of 25 November than late sown crop of 10 December. So that early sown wheat crop was superior to late sown crop in various growth parameters [27] **[Table-1]**. Another investigation was conducted at Haryana Agricultural University, Hisar on sandy loam soil and noted that planting dates did not affect the dry-matter accumulation significantly. Plant height was significantly higher of 30 November (81.9 cm) sown crop than 1 November (76.5 cm) and 15 November sown crop [28]. However, conducted an another experiment on sandy loam soil and observed that plant height was maximum of crop sown on 1 December as compared to crop sown in December and January at Umerkote [69] and Bathinda [29], respectively.

Conducted an experiment at Pantnagar on silty loam soil and found that a significant reduction in plant height was recorded in late sowing wheat crop [30]. However, from Jharkhand noted that the plant height of timely sown wheat was 11.47, 29.25 and 54.06 per cent higher in the first year [31], while 15.08, 33.44 and 44.06 per cent higher in second year compared to moderately late, late and very late sown wheat, respectively. Dry matter accumulation was decreased with delay in sowing from timely (21 November) to very late (7 January).

In conclusion, delayed sowing caused marked reduction in growth parameters like plant height, dry matter accumulation and leaf area index through the reduction in duration of maturity. There are many factors responsible for reduction in growth factors but high temperature is one of the major factors which play vital role in the reduction of growth parameter.

2.2.2. Grain yield

Conducted an experiment at Gurdaspur (Punjab) on loamy sand soil and found that crop sown on 15 October was produced higher grain yield as compared to crop sown on 25 October, 4 and 14 November. The increase in yield was due to increase in effective tillers and 1000-grain weight of early sown crop [18]. However, from Ludhiana (Punjab), reported that grain yield was significantly higher of crop sown on 25 October as compared to crop sown in November and 5 December on loamy sand soil [19,20] **[Table–2]** . Another studies conducted on silty clay loam [17], loamy sand [35], sandy loam [28, 36, 37, 38] **[Table–3]** and loamy [39] soils found that grain yield was significantly higher of crop sown in the 1st week of November as compared to crop sown in December and January, respectively.

Table: 2. Effect of sowing dates on the grain yield and yield attributes of wheat

Treatments	Grain yield (qha^{-1})	Straw Yield (qha^{-1})	Tiller per meter row length	1000-grain weight (g)	Plant height (cm)
Dates of sowing					
November 15	45.3	69.9	89.4	38.3	78.5
December 20	31.6	49.2	75.1	37.6	71.6
CD (p=0.05)	2.2	3.0	3.2	0.5	0.8

Source: [20]

Table: 3. Effect of sowing dates on the grain yield and yellow berry incidence in wheat

Treatments	Grain yield (qha^{-1})	Yellow berry (%)*
Dates of sowing		
November 3	43.2	33.1 (31.0)
November 15	41.0	30.7 (26.5)
December 15	28.7	18.7 (10.8)
CV (%)	9.4	15.4
CD (p=0.05)	2.0	7.3

Source: [37]

- arc-sine transformed values
- figures in parenthesis are the original values

However, maximum grain yield was recorded from the crop sown on 15 November and significantly differed from crop sown in December and January on silty loam, sandy loam and loamy sand soils of Pantnagar, Kanpur and Ludhiana, respectively [20, 32, 33] **[Table–2]**. Similar results were reported from Chiplima [21]. From Jamalpur, Bangladesh [34] reported that higher grain yield was obtained from the crop sown on 20 November (1795.4 kg ha-1) than 20 October (653.8 kg ha-1), 5 November (895.5 kg ha-1), 5 December (1358.9 kg ha-1) and 20 October (771.2 kg ha-1). However, from Jharkhand [31] noted that grain and straw yield of wheat were significantly affected by the time of sowing. Delay in sowing beyond timely sowing (21 November) reduced the grain yield by 16.2, 37.4 and 59.9 per cent under moderately

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late (7 December), late (21 December) and very late (7 January) sown condition, respectively. The similar findings were reported from Ludhiana, Punjab [20] **[Table–2]**. Timely seeded crop produced maximum spike-bearing tillers m-2, grains per spike and 1000-grain weight. Similar results were reported from Hisar (Haryana and Ludhiana (Punjab) [20,25] **[Table–2]**. The wheat crop sown early consumed more photo-thermal units and helio-thermal units in comparison with late sown crop at physiological maturity. This could be explained by the fact that delayed sowing resulted in forced maturity of wheat because of high temperature prevailed during reproductive phase of the late sown crop. Due to that maximum grain yield was recorded in early sown wheat crop in comparison with late sown crop.

Conducted an experiment at Pantnagar on silty loam soil [30] and found that crop sown on 27 November produced significantly higher yield (36.6 q ha-1) as compared to crop sown on 27 December (33.7 q ha-1). The grain yield was higher due to significantly higher number of seedlings and effective tillers per unit area and more grain weight per spike and 1000- grain weight. Similar findings were reported from Ranchi, Bihar [26], Niphad, Maharashtra [40] and Ludhiana, Punjab [27] **[Figure–1]**, respectively. The grain yield of wheat under early sown crop could be attributed to better basic infrastructural frame work of plants in early sowing as supported by higher taller plants, dry matter accumulation, tillers per meter row length, number of effective tillers per unit area, number of grains/ear, 1000-grain weight, ear length, straw yield and also utilized higher growing degree days and photo-thermal units. Similarly, straw yield was significantly higher in early crop sown might be due to higher growth and development contributing characters such as plant height, dry matter accumulation, tillers per meter row length and number of effective tillers per unit area. However, conducted an experiment on sandy-loam soil and observed that highest grain yield was obtained from the crop sown on 1 December than November, December and January sown crop [29, 41]. Sowing time is one of the most important management factors involved in obtaining higher yield [44]. Timely sowing of wheat crop generally gives higher yield as compared to late sown crop. Late-sown wheat crop faces high temperature stress during ripening phase. Late planting reduces the tillering period and hot weather during critical period of grain filling lead to forced maturity thereby reduces the grain yield. The rate of dry-matter accumulation remains faster at higher temperature than at lower temperature. Spike dry weight increases due to the deposition of grain protein and carbohydrates, which are partially on the expense of, assimilate transfer from stem and root. Whereas, from Udaipur noted that grain yield was significantly higher of normal sown crop (37.7 q ha-1) as compared to early (33.6 q ha-1) and late sown crop (28.2 q ha-1). The grain yield was higher due to increase in effective tillers per meter row and grain weight/ear in normal sown crop [42].

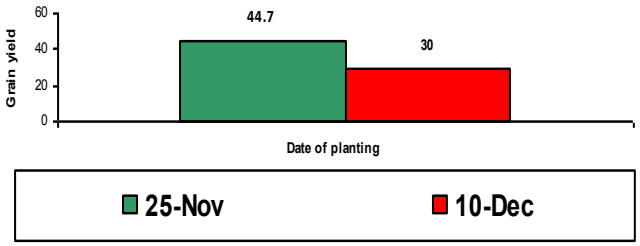

Fig: 1. Effect of dates of planting on grain yield of wheat. Source: [26]

In the end, it can be concluded that late sowing caused substantial reduction in biological and economic yield of wheat through the reduction in number of seedlings and effective tillers per unit area, more grain weight per spike, number of grains per ear head and 1000- grain weight irrespective of location and variety. It may be occurred due to the adverse climate change particularly high temperature during the reproductive stage and low temperature effect on germination.

2.2.3. Quality parameters

The late sown crop had maximum protein, ß-Carotene, gluten content, sedimentation value as compared to normal and early sown crop [28, 36, 38, 42, 43]. The lowest incidence of yellow berry was reported in wheat grains of 30 November sown crop [28, 43]. However, another an experiment conducted at Gurdaspur (Punjab) on sandy loam soil and reported that the incidence of yellow berry decreased significantly with successive delay in sowing from 3 November to 15 December [37] **[Table–3]**. Whereas, other experiment conducted at Jobner (Rajasthan) on loamy soil [39] and reported that sowing dates had no significant effect on grain appearance, test weight and sedimentation value during both the years of study. However ß-Carotene was higher in early sown crop (4.91 ppm) as compared to late sown crop (4.65 ppm). The yellow berry incidence was higher in late sown crop (6.49 %) as compared to early sown crop (5.74 %).

In general, it has been observed that the quality parameters such as protein, ß-Carotene content, gluten content,

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sedimentation value were higher in late sown crop; however, the incidence of yellow berry was lowest.

2.3. Effect of methods of planting

The selection of suitable method of planting plays an important role in the placement of seed at proper depth, which ensures better emergence and subsequent crop growth. Wheat is planted using different planting methods depending upon the available soil water, time of planting, amount of residue in the field and availability of planting machine.

2.3.1. Growth parameters

Conducted an experiment at Punjab Agricultural University, Ludhiana on loamy sand soil and observed that plant height and dry matter accumulation was significantly higher with bed planting method as compared to conventional method [45]. The similar findings were reported from Modipuram, U.P. [46] and Hisar [47, 48]. However, the leaf area index and dry matter accumulation was significantly more in wheat sown with bed planted method with 3 rows as compared to flat planting method [47]. The crop growth rate in bed planting was significantly maximum as compared to flat planting throughout the crop season. In another study from Hisar [49] reported that plant height was significantly higher in bed planted wheat (92.11 cm) in comparison to conventionally sown crop (83.23 cm). However, in another investigation from Hisar, it was observed that furrow irrigated raised bed sowing (FIRBS) resulted in less LAI as compared to conventional flat sowing during both the years [25]. Whereas, from Ludhiana reported that numbers of tillers, effective tillers per square meter and ear length were found to be highest in conventional tillage with mulching which was at par in crop sown with conventional tillage without mulching but significantly higher than zero tillage in standing stubbles after removal of loose straw [27].

It can be concluded that growth parameters such as plant height, dry matter accumulation and leaf area index were found higher in wheat sown with bed planting than conventional method. It shows modification in planting method could alleviate the adverse impact of high temperature during the reproduction stage of wheat.

2.3.2. Grain yield

Conducted field experiment on sandy-loam soil at New Delhi and noted that grain yield was maximum of wheat sown with bed planted method with 3 rows (53.06 q ha-1), which was at par with conventional planting method (50.85 q ha-1) and significantly superior to bed planted method with 2 rows (42.23 q ha-1) [54] **[Table–4]**. Yield of no-tilled sown wheat was poorer than conventional method of sowing, mainly due to failure of herbicide to control perennial and moisture stress [55]. The mean yield of winter wheat sown with direct drilling or shallow cultivation was about 10 and 7 per cent less, respectively, than after ploughing [56]. While from Bukua (Nigeria) reported that wheat grain yield was lower in zero tillage (1.5 t ha-1) than conventional tillage (1.83 t ha-1) sown crop on a sandy soil [57] probably due to low fertility and low water holding capacity of soil. Similarly, two year study conducted at Birsa Agricultural University, Ranchi during 1982-83 and 1983-84 on silty loam soil showed that wheat sown with conventional tillage after puddle transplanted rice gave 7 percent more grain yield than no-tillage [58].

Table: 4. Effect of planting system on grain yield of wheat

Planting system	Grain yield (tha^{-1})
Conventional	5.085
3 wheat rows per bed	5.306
2 wheat rows per bed	4.223
CD (p=0.05)	0.269

Source: [88]

The lower yield of wheat following rice under no-tillage was observed as compared to conventional tillage [15]. In another study found that no-tillage technique gives more grain yield (12.93%) and straw yield (15.84%) than conventionally tilled wheat [107]. This is because no-till planting allows the timely planting of wheat and so, reduce the estimated 1 per cent loss day-1 ha-1 of wheat yield due to late planting. However, 1 per cent increase in wheat yield with reduced tillage was recorded compared to conventional method [3]. Whereas, when early sowing was made more than 30 days before conventional sowing it increased yield by an average of 11 per cent. So, the objective behind introduction of wheat sowing without seedbed preparation was to improve wheat productivity by sowing wheat in time. This technology improves the grain yield by about 20 per cent over farmers practice entirely under farmers' conditions [2].

Zero, reduced and conventional tillage recorded statistically similar grain yield [92, 57, 20, 103]. In rice-wheat system, no-tillage increased wheat yield by 10 per cent over conventional tillage because of better number of tillers, better establishment and lesser weeds [4]. Similarly, recorded significantly higher grain yield under zero tillage [22, 67, 89, 104, 111] as compared with the yield obtained under conventional tillage. This increase was due to significantly higher effective ear head m-2 , grains spike-1 and spikes m-2 in zero tilled wheat crop. The higher yield and its attributes under zero tillage were due to advancement in sowing time offered by zero tillage [95}. In another study planting of wheat with zero tillage in standing stubbles after removal of loose straw, conventional tillage with mulching and conventional tillage without mulching produced the statistically similar grain yield. It might be due to the similar straw as well as biological yield under different planting methods [26] **[Figure-2]**.

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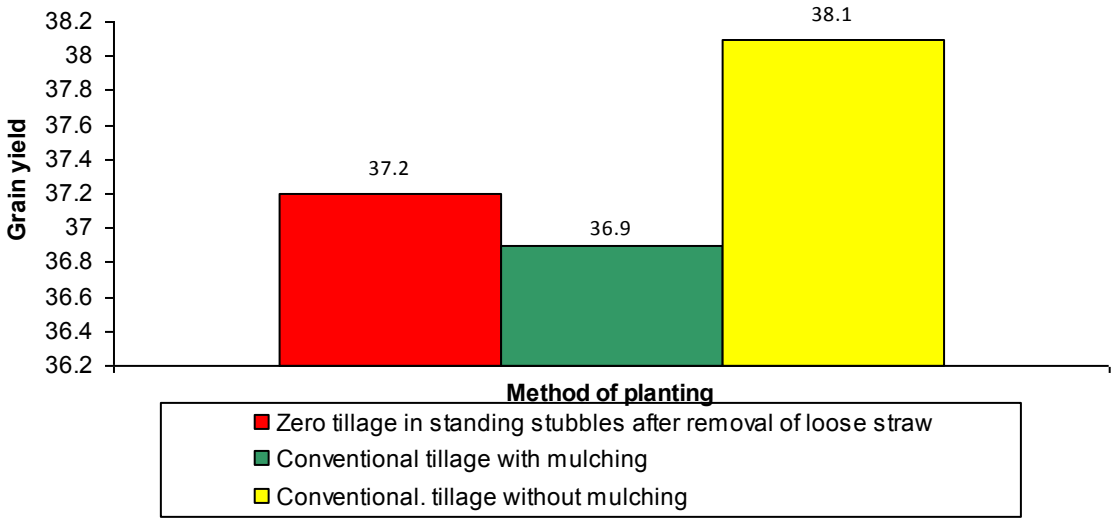

Source: [27]

Fig 2: Effect of methods of planting on grain yield of wheat

In the end it can be concluded that planting of wheat with zero tillage, bed planting and conventional tillage with mulching produced higher grain yield by proving these alternative methods of planting proved to mitigate the effect of high temperature during the reproductive stage of wheat.

2.3.3. Quality parameters

Conducted an experiment at Haryana Agricultural University, Hisar on sandy loam soil and reported significantly higher protein yield in bed planted wheat in comparison to conventionally sown crop [49]. However, the protein content was not influenced significantly due to planting methods. In another study from Hisar, noted that planting system did not affect significantly on protein content of wheat. Also the nitrogen uptake was not significantly affected by the planting methods [48].

From these studies it could be concluded that method of sowing of wheat could reduce the impact of climate change and improve the quality parameters of wheat.

2.4. Mulching

Mulching has been proved to be useful in conserving moisture and increasing productivity in wheat [70, 71, 72, 73, 74] **[Table–5, 6]**. Organic mulches provided better soil water status and improved plant canopy in terms of biomass, root growth, leaf area index and grain yield, which subsequently resulted in higher water and nitrogen uptake and their use efficiencies [75]. Mulching, when catch crop residues are spread and left on the soil surface between successive crops, is well-known and recommended practice for conserving soil and water [76, 77]. The main advantages of mulching are organic matter and nutrient supply.The slow release of nitrogen (N) from decomposing mulch residues is better synchronized with plant uptake than sources of inorganic N, increase N uptake efficiency and crop yield while reducing N leaching losses [78, 79, 80]. Mulched catch crops approach also long-term increases of soil organic matter and microbial biomass [81, 82, 83], further improving nutrient retention and N uptake efficiency. These favorable changes are the main reasons for increase in plant yields [84, 85, 86]. The other benefits are favorable changes in micro-climate within the crop fields and reduction in soil temperatures [87]. Plant residue protects the soil surface against the splash effect of raindrops, crusting and increases aggregate stability measured by wet-sieving. It was confirmed by observing the increase in the soil organic matter content, increased its stability and decreased soil surface sealing [88]. Organic matter addition increased macro porosity and water infiltration rates [89]. Much research has shown that use of surface mulch can result in storing more precipitation water in soil by reducing runoff, increasing infiltration and decreasing evaporation [71, 90, 91] **[Table–6]**. Mulched cover crops also may provide favorable microhabitats for useful insects [92]. Application of plant mulch combined with minimum tillage is known to be effective in reducing soil erosion, maintaining soil structure and conserving soil water in temperate as well as tropical regions.

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Table: 5. Effect of different treatments on biomass yield and grain yield of wheat

Year	Yield (qha^{-1})	Treatments				
		No straw mulch and no irrigation	Straw mulch and no irrigation	No straw mulch and irrigation 15 mm	No straw mulch and irrigation 30 mm	No straw mulch and irrigation 45mm
1997	Biomass	3356±390	4584 ± 350**	4500 ± 364**	4903 ± 510***	5556 ± 493***
	Grain	818 ± 125	1240 + 84**	1250 ± 104**	1384 ± 156***	1650 ± 131***
1998	Biomass	7120 ± 310	8563 ± 439**	9546 ± 480***	10970 ± 570***	12580 ± 520***
	Grain	2061 ± 199	2601 ± 203**	2520 ± 195**	2883 ± 228***	3230 ± 213***

Source: [71]
** statistical significance at p< 0.01 level, when compared to the t1 treatment within the same row and same year
*** statistical significance at p< 0.001 level, when compared to the t1 treatment within the same row and same year

Table: 6. Effect of straw mulch and irrigation on evapotranspiration and soil water depletion

Year	Factor	Treatments				
		No straw mulch and no irrigation	Straw mulch and no irrigation	No straw mulch and irrigation 15 mm	No straw mulch and irrigation 30 mm	No straw mulch and irrigation 45mm
1997	Evapotranspiration	239.2	219.5**	250.6 NS[a]	260.8**	271.1***
	soil water depletion	58.1	38.4***	54.5 NS[a]	49.7***	45.0***
1998	Evapotranspiration	369.6	355.3*	349.9**	338.8***	364.4 NS[a]
	soil water depletion	91.2	76.9***	71.5***	60.4***	85.7 NS[a]

Source: [71]
a statistical significance at p> 0.05 level, when compared to the t1 treatment within the same row and same year
* statistical significance at p< 0.05 level, when compared to the t1 treatment within the same row and same year
** statistical significance at p< 0.01 level, when compared to the t1 treatment within the same row and same year
*** statistical significance at p< 0.001 level, when compared to the t1 treatment within the same row and same year

In conclusion, use of organic mulches provided better soil water status and improved plant canopy in terms of biomass, root growth, leaf area index and grain yield, which subsequently resulted in higher water and nitrogen uptake and their use efficiencies and may reduce expected reduction of economic yield under adverse climate during reproductive stage of wheat.

2.4.1. Foliar spray of chemical

In recent past some encouraging results were obtained with post flowering foliar application of various nutrients on yield of wheat. The higher grain and straw yield of wheat by spraying 0.5 per cent KNO_3 at 50 per cent flowering stage of the crop was reported [93], a number of studies have been conducted [94, 95] confirming the results . The beneficial effect of NO_3- in delaying synthesis of abscisic acid and promoting cytokinin activity [96] and of K+ on photosynthesis, carbohydrate redistribution and starch synthesis in storage organs [97, 98] were presumed to be responsible for higher grain yield. It has been revealed that NO_3- and its counter ions, both K+ and Ca_2+ gave beneficial effect on grain filling and yield of wheat when applied as foliar spray at 50 per cent flowering stage of the crop [99]. Crop sown on 25 November with zero tillage in standing stubbles after removal of loose straw and one foliar spray of KNO_3 (1%) during anthesis was at par in grain yield than those obtained with conventional tillage without mulching + two foliar spray of KNO_3 (1%) during anthesis produced the statistically similar grain yield [27, 100]. However, one foliar spray of KNO_3 (1%) during anthesis gave the highest grain yield followed by two foliar spray of KNO_3 (1%) during anthesis as compared to one extra irrigation during post anthesis and recommended irrigation **[Figure–3]**. A study was designed to explore the role of arginine (0.0, 2.5 and 5.0 mM) in increasing the tolerance of wheat cultivar (Sids-93) to two late sowing (23/12 and 23/1) besides the normal sowing date (23/11) in Egypt [101]. Foliar application of arginine with 2.5 and 5.0 mM on normal or delayed sowing wheat exhibited significant increment in yield and its components in comparison to untreated plants. The magnitude of increments was much more pronounced in response to 2.5 mM of arginine which induce 19.23, 20.53 and 25.51 per cent increases in economic yield per feddan at normally, 30 and 60 days delay, respectively. As well as results show that 2.5 mM arginine treatment induce 8.0 per cent increase in grain yield over the plants sowing late at 23/12 and could reduce the reduction per cent in grain yield from 41.22 to 26.22 per cent at 23/1 sowing

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date.

2.4.2. Foliar spray of micronutrient

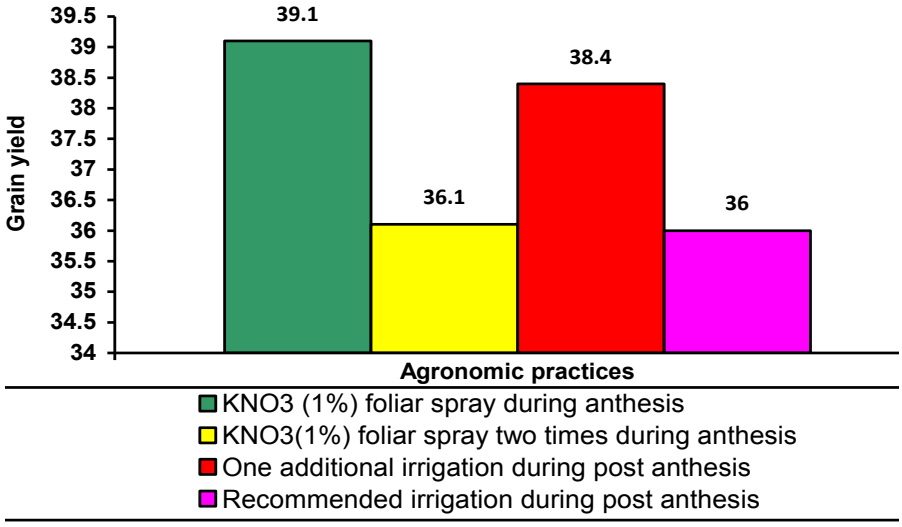
One of the reasons of reduction of crop yield is insufficient supply of micronutrients. Zinc is one of those micronutrients which have an important role in metabolic activities of the most plants. On the other hand, its mobility is low under drought stress conditions, so this element can be sprayed to increase its intake in the plant. It showed that the highest grain and biological yield during the conditions of normal irrigation and spraying with the zinc is 62.40 and 143.00 qha-1, respectively and the lowest of 38.00 and 122.00 qha-in the conditions of discontinuation of irrigation at flowering stage and lack of spraying zinc [102]. On the other hand, application of zinc spraying in drought conditions causes the increase in number of grains per spike, grain weight and harvest index, comparing with the lack of spraying. The spraying the zinc in normal irrigation conditions, especially in drought stress conditions have significant positive effects on yield and yield components of wheat.

2.4.3. Use of extra irrigation water

Additional irrigation over recommended frequencies resulted in increased yields in Sudan and Mexico. In Sudan, increasing irrigation frequency from 14 days to 10 days were increased yields 10 percent, while frequencies of 7 days resulted in an increase of 29 per cent. In Mexico, application of 600 mm of water gave control yields of 3.1 t ha-1, while an extra 150 mm during grain filling gave a 13 per cent yield increase (3.5 t ha-1). Although an extra irrigation did not have any main effect in Bangladesh, extra irrigation with a deep tillage treatment increased yield by 11 per cent. Extra irrigation had no significant effect on plant traits or yield in Bangladesh. In Sudan, increased grain yield in response to extra irrigation was associated with higher harvest index and a tendency for higher 1000-grain weight relative to the control. With extra irrigation in Mexico, yield increases of 13 per cent were associated with similar increases in grains per unit area and biomass at maturity, as well as 2 days delay in maturity [103]. The effects of irrigation on crop production are usually quantified using crop water production functions which relate crop yield to amounts of water applied [104, 105]. The rational irrigation can considerably increase the grain yield [71, 106, 107]. It was also asserted that excessive irrigation delays the maturity, harvesting and decreased grain yield [107]. However, it was also reported that excessive irrigation led to a decrease of crop WUE and that effective deficit irrigation may result in higher production and WUE [108]. On the contrary, it showed that the effect of irrigation on wheat yield was almost solely due to increased transpiration, while WUE and harvest index remained unaffected [109]. It is also indicated that the responses of grain yield and WUE to irrigation varied considerably due to differences in soil water contents and irrigation schedules [110]. It concluded that the impact of limited irrigation and soil water deficit on crop yield or WUE depend on the particular growth stage of the crop [111]. However, the crop sown on 25 November with conventional tillage without mulching with one additional irrigation during post anthesis, conventional tillage with mulching + one additional irrigation during post anthesis and conventional tillage with mulching + recommended irrigation gave the similar grain yield of wheat [27,100] **[Figure–3]**.

2.4.4. Use of potassium fertilizers with municipal waste water

A cadmium level is significant in most urban and industrial waste waters. Hence its accumulation in crops which are irrigated by the urban waste waters is investigable. On the



Source: [27]

Fig 3: Effect of agronomic practices on grain yield of wheat

application of potassium fertilizer reduce cadmium uptake in plants. Conducted an experiment with three levels of

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potassium fertilizer (0, 200 and 400 kgha-1 potassium sulfate) [112]. The results showed that application of potassium fertilizer on wheat grains has decreased cadmium uptake of 0.57 to 0.18 mgkg-1 and also after using potassium fertilizer on wheat straw, the amount of cadmium was decreased from 0.91 to 0.54 mgkg-1. On the other hand after potassium consumption, the percentage of grain protein was also significantly increased from 10.45 to 12.63 per cent. As consumption a large amounts of potassium causes a significant reduction in grain cadmium concentrations, so although potassium intake may not be economically justified, but its application is justified due to the reduced absorption of cadmium and health of crops.

In the end, it could be concluded that the foliar spray of KNO_3 (0.5%) at 50 per cent flowering stage, 1.0 per cent KNO_3 during anthesis stage, 2.5 mM of arginine, spray of zinc, extra irrigation water during grain filling stage, use of potassium fertilizers with municipal waste water could alleviate the adverse impact of high temperature on wheat.

[III] CONCLUSION

From the investigations of various workers, it can be concluded that the adoption of agronomic practices like Seed priming, timely planting , zero tillage, conventional tillage with mulching and bed planting, one additional irrigation in conventional with and without mulching during post anthesis, Foliar spray of 0.5 per cent KNO_3 at 50 per cent flowering stage or 1.0 per cent KNO_3 during anthesis in zero tillage and conventional tillage, use of organic mulch, foliar spray of zinc in normal irrigation conditions and drought conditions, use of potassium fertilizer with waste water can alleviate the adverse impact of high temperature on wheat.

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RESEARCH ARTICLE OPEN ACCESS

THE EFFECTS OF A COMPREHENSIVE HEALTH EDUCATION PROGRAM IN CHINESE PATIENTS AFTER PERCUTANEOUS CORONARY INTERVENTION

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ABSTRACT

OBJECTIVES: *To determine how the comprehensive education program influences CHD risk factors in Chinese patients after PCI, and evaluate the effect of the comprehensive program.* ***METHODS:*** *A consecutive series of patients after PCI were recruited. They were randomly divided into the comprehensive education group and the ordinary education group. Patients were followed up by telephone or hospital visit. At 6 months and 12 months after PCI, the management of CHD risk factors in two groups was evaluated.* ***RESULTS:*** *Finally 64 patients in comprehensive group and 63 patients in ordinary group finished the follow-up study. Twelve months after PCI, patients in comprehensive group had much higher control ratio of all risk factors, compared to ordinary group. At 12-months cutoff date, the reductions of CHD risk factors in comprehensive group were significantly more than ordinary group.* ***CONCLUSIONS:*** *The comprehensive education program had more improved outcomes in CHD risk factors than ordinary one.*

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[I] INTRODUCTION

With the steady improvement in medical technology, Percutaneous Coronary Intervention (PCI) acting as a safe, effective, non-surgical treatment for coronary heart disease (CHD) has become more and more perfect [1]. The demand for PCI has increased exponentially since the 1980s because it can re-canalize vasculature, and relieve symptoms quickly and effectively. However, the procedure does not stop the underlying process of atherosclerosis [2]. Even though the effect of PCI has been strengthened by the technological and pharmacological innovation, potential risk factors of CHD still remain a serious problem [3-5]. In 2007, the American College of Cardiology/American Heart Association (ACC/AHA) Task Force on Practice Guidelines created new "Focused Update" Guidelines for secondary prevention and long term management for patients with coronary and other vascular disease after PCI [6]. Health education has been integrated into the category of secondary prevention, encouraging people to adopt healthier lifestyles and further reducing the risk of CHD [7-9]. If post-PCI patients cannot pay attention to adopt healthier lifestyle because of an absence of health education, irregular follow-up, or other reasons, this might lead to restenosis in endovascular stent, increasing the rate of re-hospitalize and rising mortality. Several studies have shown that the intervention programs resulted in significant reduction of body weight, waist circumference, serum lipids, serum glucose, blood pressure, and resting heart rate [10-13]. However, very few studies have been done on the effectiveness of the intervention programs in Chinese post-PCI patients. In developing countries like China, comprehensive health education programs are not widely spread.

Therefore, we established a comprehensive health education programs including discharge education on lifestyle modification, behavior change, and so on, central training after discharge, and frequent follow-up. The purpose of this study was to determine how this program influences CHD risk factors in Chinese patients after PCI, and evaluate the effect of the comprehensive program compared with an ordinary education program

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[II] MATERIALS AND METHODS

2.1. Multiple Subjects

A consecutive series of patients who accepted the PCI were recruited from a metropolitan hospital in Taiyuan from July 2008 to December 2008. Ethical approval for the study was obtained from the Human Research Ethics Committees of the hospital. The study complied with the provisions of the Declaration of Helsinki. All patients provided written informed consent.

Inclusion criteria: 1. patients who accepted the PCI for the first time; 2.age ≤ 75 years old; 3. the residents of Taiyuan city.

Exclusion criteria: 1. Patients who suffered from an unsuccessful PCI, and needed surgical operation; 2. Patients who suffered from psychotic diseases; 3. Patients who suffered from hearing disabilities and could not receive information in the study; 4. Patients who suffered from serious important organs dysfunctions and complications, such as malignant tumors, etc.

2.2. Study design

Subjects were stratified into 2 sub-strata, according to their sex and age (< 60 years and ≥ 60 years). Then Subjects in each sub-stratum were randomly divided into the intervention group and the control group. In the intervention group, comprehensive health education intervention was implemented. In the control group, ordinary health education was given. Single-blind method was carried out in this study. In order not to influence the results of intervention effects, subjects were informed not to communicate with each other. Patients were followed up by telephone or hospital visit. At 6 months and 12 months after PCI, the management of CHD risk factors in two groups was evaluated, and then conclusion was drawn after statistical analysis.

2.3. Intervention and Control

Comprehensive health education program including centralized training and telephone follow-up was carried out in the intervention groups. Ordinary health education program includes discharge guidance and distribution of health education materials **[Table –1].**

2.4. Contents of comprehensive health education:

Basic medical knowledge about CHD including secondary prevention of post-PCI and correct administration was delivered to patients by cardiac physicians in the form of centralized training.

Healthy diet including the significance of health diet to rehabilitation in CHD, dietary principle of CHD, graphic presentation about healthy diet and correct cookery was instructed by dietitians in the means of centralized training.

Correct post-operation rehabilitation exercise including the effect of exercise to post-PCI rehabilitation, correct concepts of exercise, proper intensity of exercise, optimal exercise forms, appropriate exercise time was directed by both physicians and physical therapists.

Doctors and nurses tutored patients on how to quit smoking and refrain from drinking. The contents of lecture were composed of smoking hazard towards CHD, the benefits of withdrawal of smoking, the harms of great amount of drinking about CHD, and benefits of controlled alcohol intake.

2.5. Index Measurement

Smoking conditions (including smoking, refraining from smoking and non-smoking), exercise, conditions of medicine treatment about controlling risk factors of CHD (β-blockers and/or Angiotensin converting enzyme inhibitors, ACEI to control hypertension, and administrating statins to control hyperlipermia, and hypoglycemic agents to control hyperglycemia recommended in the PCI therapeutic manual) were thoroughly measured and analysed.

Physical check-up was carried out uniformly. The contents of physical check-up consist of CHD risk factors level data, such as height (HT), weight (WH), waistline (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), low density lipoprotein (LDL), fasting blood-glucose (FBG), etc.

2.6. Effects Evaluation Criteria

Goals of controlling risk factors of CHD in 2007 PCI Therapeutic Secondary Prevention Guidelines were selected as the criterion of evaluation. The standards were composed of BP<140/90mmHg, or <130/80mmHg (if patients were accompanied by diabetes mellitus); LDL <2.6mmol/L; FBG <6.1mmol/L; giving up smoking completely or avoiding non-smoking; the time of taking part in moderate aerobic exercise≥150minute/week, except for daily working and life. The predictive value of the 2 risk factors of CHD (BMI and WC) has ethnic difference, therefore, we chosen Asian standards proposed by WHO. The standards consisted of BMI:18.5 to 23.9 kg/m2 , WC: male <90cm, female <80cm [6,14,15].

Table: 1. The comprehensive health education programme

	Content
Object	The education about relevant medical common knowledge for the patients should be a part of training and follow-up visit, every patient received individualized and tailored educational plan;
Requirement	Making sure the patients understand the relation between lifestyle and health; Helping the patients acknowledge the CHD risk factors, and help them to change their lifestyles; Making plans with the patients and discuss more than once; Educating the patients don't rush to adjust many lifestyles at a time; Encouraging family members to join in the course of controlling risk factors so that the patients would be more likely to complete the plans. Monitoring the progress of patient's condition during the follow-up visit, Consulting with other medical personnel when necessary.
Health education practitioner	Clinical cardiologist：health education leaders，determine the health education project Heart disease nurses：telephone follow-up at fixed period，help the patients to change bad habits Register nutritionist：health diet education and consultation Community health service personnel：monitoring the risk factors of coronary disease，answering relevant health questions
Health education form	central training, telephone follow-up visit, community service

Abbreviations: CHD, coronary heart disease.

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2.7. Statistical Analysis

Unless otherwise noted, all study data are described as mean ± standard deviation (SD). Testing for baseline homogeneity across categories of BMI in each medication group was performance with ANOVA for continuous parameters and $\chi 2$ test for categorical parameters. The reductions of CHD risk factors between two groups were compared using a general linear model. $\chi 2$ test was used to assess the goal of control in coronary risk factors at 6 months and 12 months after PCI. A 2-tailed p value of 0.05 or less was considered significant. The statistical package used was SPSS version 17.0 (SPSS Inc., Chicago, Illinois).

[III] RESULTS

3.1. Patients

According to the inclusion and exclusion criteria, 163 patients were eligible for the study. However, 14 patients refused to join the study, and 13 patients could not be contacted. Finally, 136 patients were recruited in the study after endorsing the informed consent. They were randomly divided into intervention group (68 patients) and the control group (68 patients). A total of 4 patients in the comprehensive group and 5 in the control group were lost to follow-up or withdrew consent before the 12-month cutoff date, leaving 64 patients in education group and 63 patients in the control group.

Baseline characteristics were shown in **Table–2**, no significant differences of any characteristics were detected between the two groups. The mean age was 60 years, and 80% of patients were male. About 69% of patients were taking a β-blocker, 57% taking an ACEI, 68% taking a stain, and about 42% of patients are under anti-diabetes therapy. About 17% of patients had a habit of smoking, and did not refrain from smoking before enrollment. The levels of cardiovascular risk factors of CHD were similar in the two groups.

Table: 2. Baseline characteristics

	Comprehensive health education (n=64)	Ordinary health education (n=63)	p value
Mean±SD			
Age	59.4±10.1	60.6±10.7	0.520
SBP	136.1±13.3	135.9±12.9	0.939
DBP	84.9±11.1	84.0±10.2	0.663
LDL	107.1±28.3	107.8±26.4	0.891
FBG	103.7±25.8	104.0±18.4	0.937
BMI	23.7±1.4	23.9±1.5	0.673
WC	91.2±5.9	91.4±5.8	0.862
N(%)			
Male	51(79.7)	51(81.0)	0.858
MI	22(34.4)	21(33.3)	0.821
Hypertension	34(53.1)	32(50.8)	0.702
Diabetes	26(40.6)	28(44.4)	0.663
β-blockers	45(70.3)	43(68.3)	0.801
ACEIs	37(57.8)	35(55.6)	0.797
Anti-Stain	44(68.8)	42(66.7)	0.802
Anti-Diabetes	26(40.6)	28(44.4)	0.663
Smoke	10(15.6)	11(17.5)	0.781

Abbreviations: ACEIs, angiotensin converting enzyme inhibitors; Anti-Diabetes, administrating hypoglycemic agents to control hyperglycemia; Anti-Stain, administrating statins to control hyper-lipermia; BMI, body mass index; DBP, diastolic blood pressure; FGB, fasting blood-glucose; LDL, low density lipoprotein; SBP, systolic blood pressure; WC, waistline; MI, myocardial infarction

3.2. Control ratio of cardiovascular risk factors

Effective control ratio of risk factors of CHD at 6 months and 12 months between two groups were respectively compared **[Table–3, Figure–1]**. At 6 months after their discharge from the hospital, post-PCI patients in the two groups had increased control ratio of cardiovascular risk factors. Furthermore, patients in the comprehensive education group, as compared with the ordinary education group, had significant higher control ratio of certain risk factors (SBP: 89.1% vs 69.8%, p=0.007; WC 42.2% vs 15.9%, p=0.001; respectively).

Twelve months after PCI, patients in the comprehensive education group had much higher control ratio of all risk factors, compared with patients in the ordinary education group (SBP: 100% vs 69.8%, p<0.001; DBP: 98.4% vs 61.9%, p<0.001; LDL: 85.9% vs 41.3%, p<0.001; BMI: 90.6% vs 74.6%, p=0.017; WC: 68.8% vs 22.2%, p<0.001; Smoke: 92.2% vs 71.4%, p=0.002; Exercise: 21.9% vs 6.3%, p=0.012; respectively). However, the control ratio of risk factors in the ordinary education group at 12-month cutoff date became similar to those at the date of discharge.

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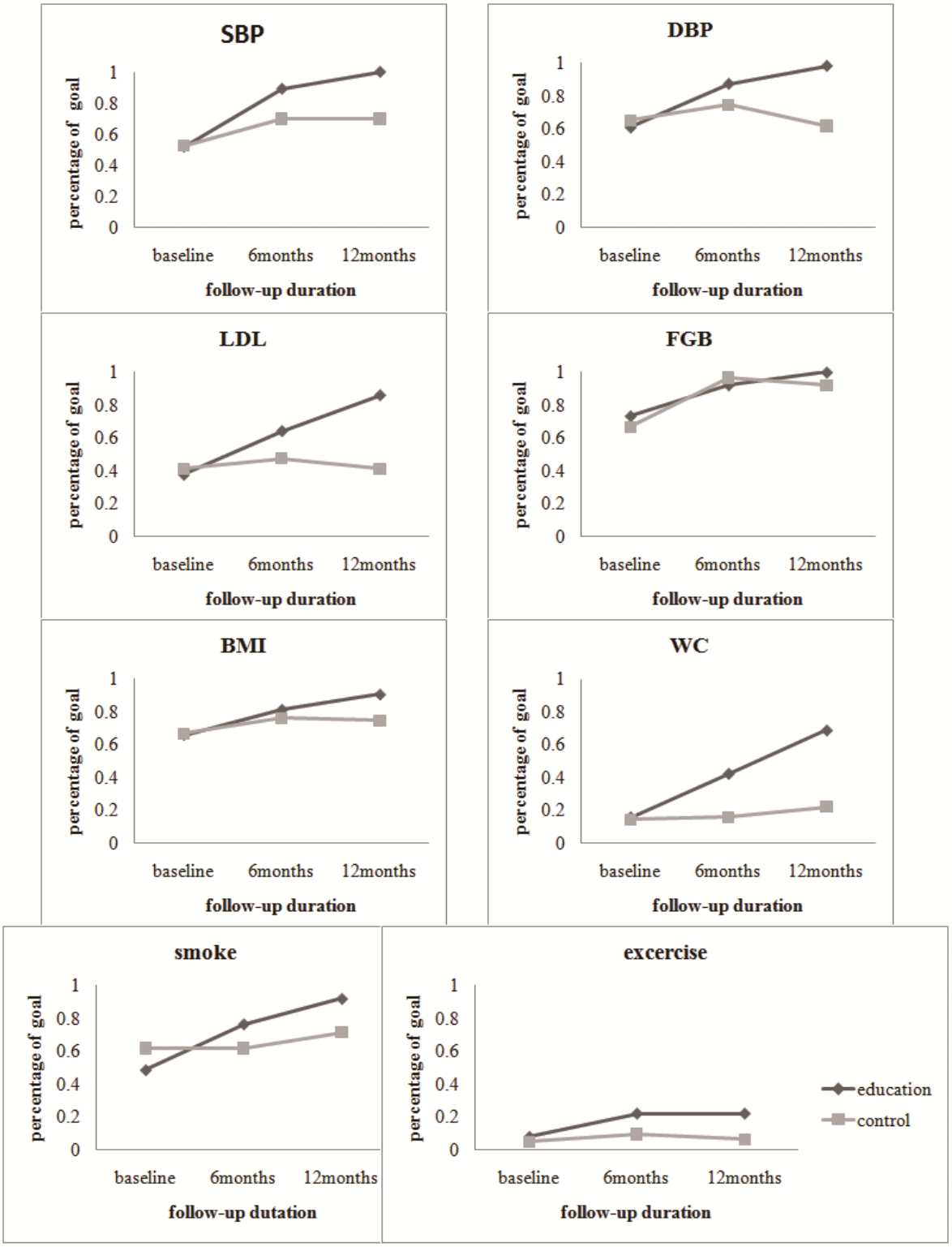

Fig: 1. Comparisons of goals of CHD risk factors between comprehensive and ordinary health education groups at 6 months or 12 months after PCI.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FGB, fasting blood-glucose; LDL, low density lipoprotein; SBP, systolic blood pressure; WC, waistline

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3.3. Reduction of cardiovascular risk factors

After discharge from hospital, the cardiovascular risk factors were reduced in both groups **[Table–3, Figure– 2]**. At 6-month cutoff date, except blood pressure and blood glucose, the reductions of LDL, BMI and WC among patients in the comprehensive education group were significantly more than ordinary education group (LDL: 13.81±1.64 vs 8.45±1.17, p=0.009; BMI: 0.81±0.01 vs 0.28±0.02, p<0.001; WC: 3.17±0.16 vs 0.50±0.05, p<0.001; respectively). At 12-months cutoff date, the reductions of blood pressure and blood glucose in comprehensive education group were also significantly more than ordinary education group (SBP: 11.94±0.99 vs 3.14±0.75, p<0.001; DBP: 7.39±1.02 vs 0.98±0.50, p<0.001; LDL: 23.07±2.10 vs 5.05±0.95, p<0.001; FBG: 13.84±2.37 vs 5.61±1.21, p=0.003; BMI: 1.57±0.01 vs 0.39±0.02, p<0.001; WC: 5.02±0.22 vs 0.93±0.11, p<0.001; respectively).

Table: 3. Comparisons of reductions and goals of CHD risk factors between comprehensive and ordinary health education groups at 6 months or 12 months after PCI

	6 months						12 months					
	Reduction Mean (SE)			Goal N (%)			Reduction Mean (SE)			Goal N (%)		
	Comp	Ordinary	p	Comp	Ordinary	p	Comp	Ordinary	p	Comp	Ordinary	p
SBP	-7.28(0.97)	-5.57(0.80)	0.178	57(89.1)	44(69.8)	0.007	-11.94(0.99)	-3.14(0.75)	<0.001	64(100)	44(69.8)	<0.001
DBP	-4.22(0.99)	-2.78(0.62)	0.221	56(87.5)	47(74.6)	0.019	-7.39(1.02)	-0.98(0.50)	<0.001	63(98.4)	39(61.9)	<0.001
LDL	-13.81(1.64)	-8.45(1.17)	0.009	41(64.1)	30(47.6)	0.062	-23.07(2.10)	-5.05(0.95)	<0.001	55(85.9)	26(41.3)	<0.001
FBG	-9.74(2.14)	-7.06(1.33)	0.291	59(92.2)	61(96.8)	0.252	-13.84(2.37)	-5.61(1.21)	0.003	64(100)	58(92.1)	0.021
BMI	-0.81(0.01)	-0.28(0.02)	<0.001	52(81.2)	48(76.2)	0.486	-1.57(0.01)	-0.39(0.02)	<0.001	58(90.6)	47(74.6)	0.017
WC	-3.17(0.16)	-0.50(0.05)	<0.001	27(42.2)	10(15.9)	0.001	-5.02(0.22)	-0.93(0.11)	<0.001	44(68.8)	14(22.2)	<0.001
Smoke	--	--	--	49(76.6)	39(61.9)	0.073	--	--	--	59(92.2)	45(71.4)	0.002
Exercise	--	--	--	14(21.9)	6(9.5)	0.056	--	--	--	14(21.9)	4(6.3)	0.012

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FGB, fasting blood-glucose; LDL, low density lipoprotein; SBP, systolic blood pressure; WC, waistline; comp, comprehensive.

[IV] DISCUSSION

Presently, the intervention of health education has focused on not only providing health information but also monitoring implementation of lifestyle change. And it is obviously beneficial for convertible risk factors management [16]. In developed countries, the post-PCI patients mostly voluntarily choose to be involved in cardiac rehabilitation programs. As a result, the CHD risk factors are minimized; meanwhile morbidity and mortality are reduced [2]. Current strategies for achieving recommended target risk factor levels include frequent follow-up, intensive diet changes, individualized and group exercise, coaching, group meetings, education on lifestyle modification and behavior change, and formal cardiac rehabilitation programs [16]. In China, such strategies are not widely spread. Furthermore, many post-PCI patients experience immediate symptomatic relief and express the belief that the procedure has cured them [17-21]. They are less likely than other cardiac patients to initiate and maintain behavior changes. As a result, the ordinary health education, including only nominated cardiac rehabilitation advised by cardiologist, specialist nurses or general practitioner, may have less improved outcomes in health behaviors, quality of life or psychosocial wellbeing.

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The present study demonstrated that the comprehensive health education program had more improved outcomes in CHD risk factors, health behaviors and quality of life than ordinary health program. And the superiority was much more significant in the long term (12 months after PCI). Nevertheless, among patients with ordinary health education program the implement of lifestyle change aimed at risk factors was unchanged or even reversed at 12 months after PCI compared with 6 months. It was evident that without comprehensive health program, ordinary heart disease information or education offered by cardiologist or specialist nurse cannot guarantee the implementation of therapeutic lifestyle change. Baseline data showed patients after PCI have limited cardiac related knowledge and poor control status of CHD risk factors. If post-PCI patients can attend the comprehensive health education program, they will obtain enough heart disease information and behavioral skills to adopt healthier lifestyles and as a result, contribute to a major reduction of the re-hospitalize and mortality rates. The services of the comprehensive health program include centralized training and regular telephone follow-ups aiming to develop and implement an education, counseling and behavioral skill-building program.

4.1. The effect of intervention on CHD risk factors

At 6 months cut-off day after PCI, no significant difference was found in blood pressure control between comprehensive and ordinary health education group. It was attributed to widespread knowledge of BP controlling. In the ordinary health education group, educators (especially the nurse) emphasized the importance of BP control. The patients had strong willingness to control blood pressure and good adherence. Hence BP was controlled in appropriate levels. But at one year after PCI, the effect of intervention on BP in ordinary education group was inferior to comprehensive group. It demonstrated that the management of hypertension under comprehensive health education was steady and not rebounded in the long run.

Controlling of blood glucose is necessary to Coronary heart disease [6]. Although no difference on the effective control ratio of blood glucose between two groups was detected, the reduction of blood glucose was lower in comprehensive education group than ordinary group. That reflected either comprehensive health education or routine health education has a good effect on blood glucose control.

To keep LDL-C level in a normal range, patients in comprehensive education group were advised to take health diet, do proper exercise, control bodyweight, and take stains. To control bodyweight and modify abdominal obesity, BMI and WC were evaluated at each time when followed up. And patients were encouraged to take physical exercise (low to moderate intensity exercise) to keep or lose weight.

Quitting smoking or drinking is a gradual process which is easily repeated. When necessary, drug substitution therapy can be induced into cessation process. Furthermore, smoking and drinking is psycho-social problems. In addition to nicotine and alcohol addiction, patients aged less than 60 years should be pay special attention to. They have to face social interaction, entertainment, heavy social responsibility, and psychological stress. So they are difficult to refrain from smoking and drinking.

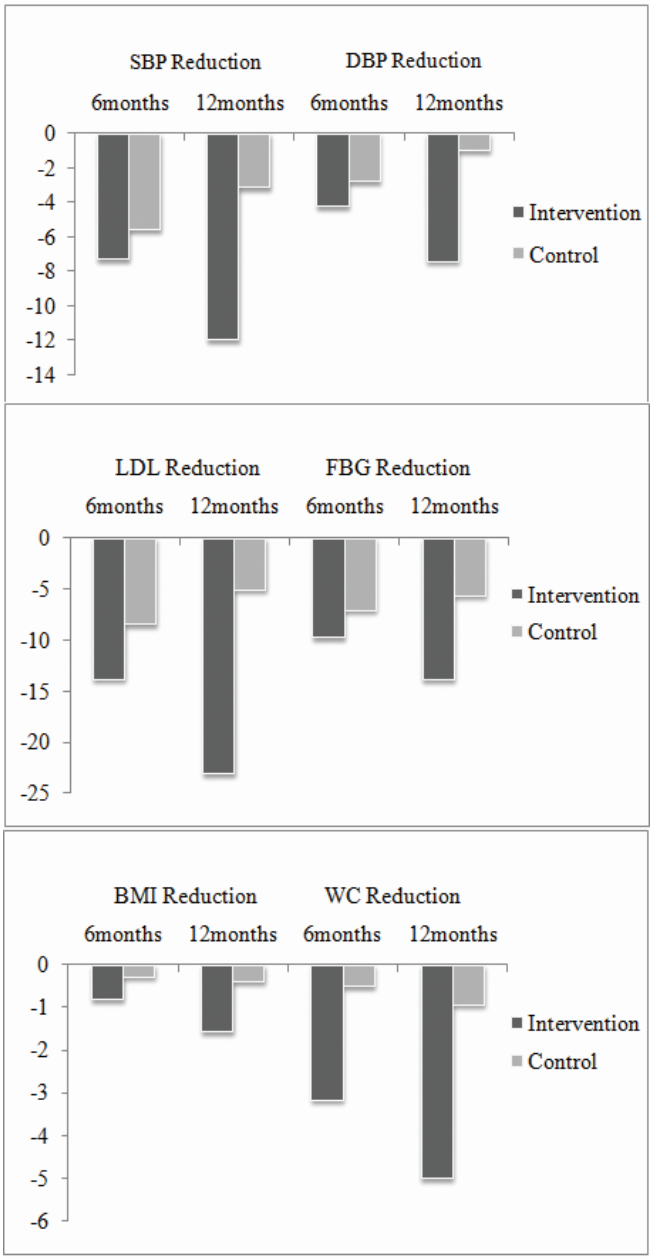

Fig: 2. Comparisons of reductions of CHD risk factors between comprehensive and ordinary health education groups at 6 months or 12 months after PCI.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FGB, fasting blood-glucose; LDL, low density lipoprotein; SBP, systolic blood pressure; WC, waistline.

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4.2. The effect of centralized training

The centralized training organized by nurses, cardiologists, nutritionists and physical therapists can help the patients gain the comprehensive knowledge of rehabilitation: medicine, nutrition, and physiotherapy. Through this study, we found the lack of necessary and accurate information of heart disease would influence the effective control of the coronary heart disease risk factors. For example, the reason why post-PCI patients with diabetes mellitus have poor control of blood pressure may be they don't know the blood pressure should be controlled under 130/80mmHg; normal range of LDL-C level is 0~3.12mmol/L, but most patients don't know the goal level of LDL for post-PCI patients is less than 2.6 mmol/L. And post-PCI patients cannot understand the significance of exercise after PCI. Because of busy lifestyles, patients who are younger than 60 think daily physical activities can take the place of exercises, which is required after PCI. On the other hand, the patients who are older than 60 find that it is difficult to choose appropriate exercise intensity. The post-PCI patients need a certain exercise to promote the recovery of oxygen-poor organs. However, low intensity exercise only plays a consolation; high intensity exercise increases the load on cardiovascular system. Therefore, health information is the premise of promoting lifestyle improvement. The centralized training can provide professional and accurate health information for the patients

4.3. The effect of telephone follow-up

Comprehensive health education programs can make rehabilitation plans understandable to every patient .The plan includes medication, a healthy diet, exercise, quitting smoking, and controlled drinking The plan is mainly achieved by lifestyle improvement. However, due to long-time bad habits, it will be difficult for patients to change their lifestyles in the short term, or continue in the long term. Lifestyle change is a long process, and a short-term health education will not be effective correcting bad lifestyles. Although the centralized training can help the patient begin a new lifestyle, it doesn't promise the new style will last for a long time [22, 23]. Long-term monitoring and guidance is required to achieve the lifestyle improvement. Thus, Regular follow-ups by telephone play an important part in long-term monitoring and guidance. It allows one to educate the patient not to adjust many styles at once, but to make specific stages in the plan, then achieving it step by step. And it can help us to find the factors which hinder the improvement of lifestyle during the various stages in rehabilitation process. Then we discuss with patients, revise the rehabilitation plan, and supervise implementation of plan. Finally, the regular fellow-ups by telephone can reduce the possibility of giving up rehabilitation program or lowering the performance standards for various reasons or excuses of patients. With regards to some patients who can't persist for a long time, we should encourage their family members joining in the rehabilitation plan and help the patient accomplish plan better.

[V] CONCLUSION

The study demonstrated that the comprehensive health education program have more improved outcomes in CHD risk factors, health behaviors and quality of life than ordinary health program. And the superiority was much more significant in the long term. Central training can provide professional and accurate health information and telephone follow-up can monitoring the implement of lifestyle change. The comprehensive education program is obviously beneficial for convertible risk factors management.

AUTHOR CONTRIBUTION

† Drs Gao and Li have equally contributed to this work.

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RESEARCH ARTICLE OPEN ACCESS

STIMULATED RAMAN SCATTERING INSTABILITY OF LASER BEAM IN A PLASMA CHANNEL INCLUDING THE EFFECT OF THERMAL CONDUCTION

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ABSTRACT

This paper presents an analysis of the Stimulated Raman scattering instability of a laser beam in a plasma channel. Considering the nonlinearity to arise from collisional and thermal – conduction phenomena and following the approach of Sodha et al. [1] and Short et al. [17] the phenomenon of Stimulated Raman scattering instability including the effect of thermal conduction is studied. Thermal conduction plays an important role in temperature equilibrium when the electron mean free path λ_m is greater than the beam. Inside a filament, the laser undergoes stimulated Raman backscattering (B-SRS). A nonlocal theory of stimulated Raman scattering (SRS) reveals that growth rate and the threshold power for B-SRS is significantly reduced due to the geometrical factor and enhanced temperature inside the filament. It remains an important process in laser produced plasma. It is also a limiting process in a plasma- loaded free electron laser (FEL).

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KEY WORDS

Laser beam, stimulated Raman scattering, self-focused filament, Langmuir wave, thermal conduction

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[I] INTRODUCTION

It has been shown in recent years [1-3] that a high amplitude electromagnetic beam propagating in plasma is unstable to small-amplitude perturbations. This instability causes the breaking of the beam into filaments and is known as filamentation instability [1]. On the time scale $t>\tau_h$ (which is more relevant to laser – plasma interactions), where τ_h is the heating time of electrons, the nonlinearity arises through nonuniform heating and redistribution of electrons [4]. The understanding of filamentation of laser light may be important to the success of laser fusion. In the long scale length plasmas envisioned for reactor targets, local intensity hot spots caused by self-focusing or laser light filamentation can drive the plasma above parametric instability thresholds. These instabilities tend to be saturated by the creation of super thermal electrons [5]. The hot electrons can penetrate deeply into the pellet, heating the interior, making high compressions difficult. Directly driven targets require very uniform driving pressures. Filamentation could spoil this uniformity, making large compressions difficult. The laser light absorption, penetration, and conversion to X-rays could also be affected by self-focusing [1] and filamentation. The earlier investigations of filamentation of laser beams on a long time scale are restricted to large-scale perturbations where the thermal conduction effects may be neglected [6]. But in the cases of real interest one is much more concerned about the growth of small-scale perturbations where thermal conduction could play a dominant role in determining the energy dissipation of electrons. The relative size of perturbations depends on the ratio m_i/m, since beam radius r_o is generally of the same order as electron mean free path λ_m. In this paper we have studied the filamentation of laser beams in plasmas where both collisional and thermal-conduction losses are present simultaneously. At short wavelengths collisional effects considerably influence laser plasma interaction. The nonlinear process of stimulated Raman scattering is seen to require laser power greater than a threshold value, determined by collisions. In several experiments the observed values of threshold power are far below the values predicated theoretically [7-19]. Liu and Tripathi [14] have developed self-consistent theoretical model to obtain B-SRS growth rate in a cylindrical filament in collisionless plasma. They take the size of the filament to correspond to the maximum linear spatial growth rate for the filamentation instability, with the result that for typical laser intensities the SRS growth rate is only marginally changed from its value in the unfilamented incident beam. Afshar-rad et al. [15] have studied the evidence of stimulated Raman scattering occurring in laser filaments in long scale length plasmas. Drampyan [16] has studied the evidence of self-focusing and stimulated Raman scattering beam break-up into several filaments and filament

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development in a self-focused beam, as a result of azimuthal-angle instability. Sajal et al [19] have studied the relativistic forward stimulated channel in a plasma Raman scattering of laser in a plasma channel. Recently Yin et al. [20] have studied Onset and saturation of backward stimulated Raman scattering of laser in trapping regime in three spatial dimensions. Ghanshyam et al. [21] have studied model of stimulated Raman scattering from underdense collisional plasma without considering thermal conduction in which the laser intensity profile and plasma density have been modified by the filamentation instability. A circularly polarized Gaussian laser beam propagating through a low density plasma creates a partially electron depleted channel. The laser generates stimulated forward Raman scattering, producing a plasma wave and two radially localized electromagnetic sideband waves. The laser and the sideband waves exert an axial ponderomotive force on electrons driving the plasma wave. The latter couples with the pump to drive the sidebands. The radial width of the electromagnetic sideband is of the order of the spot size of the pump, r_0, whereas the radial width of the plasma wave is determined by the growth rate of the Raman process. The localization effect reduces the region of interaction and the growth rate. There is a significant motivation to operate free electron laser (FEL) in a plasma medium. The plasma aids electron beam guiding via charge and current neutralization and allows beam current higher than the vacuum limit. It may also help radiation guiding [17] via optical duct formation. Plasma, however, is a nonlinear medium. When FEL interaction takes place in a plasma medium and laser radiation acquires large intensity, it may bring about the onset of parametric instability, e.g., stimulated Raman scattering and Brillouin scattering that may stabilize the FEL instability and increase radiation band width. If one limits the plasma density to a lower level, then the Langmuir wave generated in the Raman process possesses smaller phase velocity and undergoes Landau damping. SRS is considered to be an important process in laser produced plasma also, here severely limits the deposition of the light energy. In this process, intense laser light interacts with a Langmuir wave and is scattered backward. The scattered wave and the laser pump exert a Ponderomotive force on the electron driving the Langmuir wave.

In this paper, we examine stimulated Raman backscattering of laser radiation in a performed plasma channel considering the effect of thermal conduction and follow the approach adopted by Short et al. [17] and Tripathi et al. [14]. The channel provides radial localization of the pump and decay waves. However, since the mode structure of three interacting waves is different, the nonlinear coupling coefficients are significantly diminished. In section 2, we examine fluid equations and Maxwell's equations to the coupled mode equations inside the filaments. These equations are solved using first-order perturbation theory neglecting pump depletion effects. The result is a nonlinear dispersion relation which is solved to obtain growth rate. In section 3, we discuss the results.

[II] METERIAL AND METHODS

2.1. Instability analysis

Let us consider the propagation of a plane uniform laser beam in collisional plasma along the z-axis,

$$\vec{E} = \vec{A}_0 (r, z) \exp [-i (\omega t - k_o z)], \quad (1)$$

$$k_o = (\omega/c) \left(1 - \frac{\omega_{po}^2}{\omega^2}\right)^{\frac{1}{2}}, \quad (2)$$

$$\omega_{po}^2 = 4\pi n_o e^2 / m \quad (3)$$

and ω, ω_{po}, c, -e, m and n_o are the frequency of the main beam, the unperturbed plasma frequency of the medium, the velocity of light, the electron charge, the electron mass and the unperturbed concentration of the plasma respectively. In the presence of the field (1), the electrons acquire drift velocity in accordance with the momentum balance equation

$$m \frac{\partial \vec{v}}{\partial t} = -e \vec{E} - m\nu_{ei} \vec{v}, \quad (4)$$

where ν_{ei} is the electron collision frequency. Expressing the variation of $\vec{v}$ as exp [-i(ωt-kz)], we obtain, in the limit $\omega^2 >> \nu_{ei}^2$,

$$\vec{v} = \frac{e\vec{E}}{im\omega}\left(1 - \frac{i\nu_{ei}}{\omega}\right). \quad (5)$$

Besides this, the electrons absorb energy from the wave at the rate of $-e\vec{E} . \vec{v}$. Whose time average is

$$-\frac{1}{2} e \vec{E}^* . \vec{v} = \frac{e^2 A_o A_o^*}{2m\omega^2}. \quad (6)$$

In the steady state the rate of energy gain must balance with the rate of energy loss through collisions and thermal conduction. Hence

$$-\nabla.\left(\frac{\chi}{n}\nabla T_e\right) + \frac{3}{2}\delta\nu_{ei}(T_e - T_o) = \frac{e^2\nu_{ei}A_o A_o^*}{2m\omega^2}, \quad (7)$$

where

$$\frac{\chi}{n} = \frac{v_{th}^2}{\nu_{ei}},$$

$\delta = 2m/m_i$

is the fraction of excess energy lost per electron-ion energy exchange collision, T_e is the nonlinear field – dependent electron temperature and $v_{th} = (2T_o/m)^{\frac{1}{2}}$ is the electron

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thermal speed. For $\nu_{ei} r_o^2 / v_{th}^2 < (\delta\nu_{ei})$ thermal conduction is important, and we solve the energy – balance equation in the perturbation approximation. For a beam of finite extent we express

$$T_e = T_o + \Delta T_e, \qquad (8)$$

where $\Delta T_e << T_o$. Then Eq. (7) can be recast as

$$\nabla^2(\Delta T_e) - \frac{3}{2}\frac{\delta\nu_{ei}^2}{v_{th}^2}(\Delta T_e) = -\frac{e^2\nu_{ei}^2}{2m\omega^2 v_{th}^2}|A_o|^2 \qquad (9)$$

Now we perturb the beam by a perturbation

$$A_1(r, z)\exp[-i(\omega t - kz)], \qquad (10)$$

where $A_1(x,z)$ is not necessarily a slowing varying function of space variables. The total electric vector of the laser may now be written as

$$\vec{E} = A_o + A_1(r, z)\exp[-i(\omega t - kz)], \qquad (11)$$

Where $r = (x^2 + y^2)^{1/2}$ refers to a cylindrical polar co-ordinate, A_o is the amplitude in the absence of fluctuations (polarized in the y direction) and A_1 is the amplitude of the fluctuations, which is a spatially slowly varying function. The combined effect of these two fields is to heat the electrons and exert a pressure-gradient force, causing redistribution of plasma via ambipolar diffusion. The nonlinear field-dependent electron temperature T_e in the steady state may be obtained by solving Eq. (9) only the x and y dependence of A_1 is known. Taking $\mathbf{A_1} \propto e^{i\mathbf{q}.\mathbf{r}}$ with $q_{\|} << q_{\perp}$, where $q = q_{\perp} + q_{\|}$ is the scale length of the perturbation (the subscripts $\|$ and $\perp$ referring to components parallel and perpendicular to the z direction), T_e may be written as

$$T_e - T_0 = \frac{e^2\left[A_0.\left(A_1 + A_1^*\right) + A_0^2\right]}{3m\omega^2\delta'} \qquad (12)$$

Where $\delta' = \delta + \frac{2}{3}\frac{q_{\perp}^2 v_{th}^2}{3\nu_{ei}^2}$

As a result of non-uniformity in heating, the plasma is redistributed so that

$$n(T_e + T_0) = n_0(T_{e0} + T_0), \qquad (13)$$

where $T_{e0} = T_0 + \frac{e^2 A_0^2}{3m\omega^2\delta'}$. (14)

Using Eq. (12), (13) and (14), the modified electron density may be written as

$$n = n_0\left[1 - \frac{e^2 A_0.\left(A_1 + A_1^*\right)}{3T_0 m\omega^2\delta'\left(2 + e^2A_0^2/3m\omega^2T_0\delta'\right)}\right]. \qquad (15)$$

The dielectric constant of the plasma may be written as

$$\in = \in_0 + \in_2 A_0.\left(A_1 + A_1^*\right), \qquad (16)$$

where

$$\in_2 = \frac{\omega_{po}^2}{\omega^2}\frac{\alpha P}{2 + \alpha P A_0^2}$$

$$P = \frac{1}{1 + 2q^2 v_{th}^2/3\nu_{ei}^2\delta}$$

and

$$\alpha = \frac{e^2}{3m\omega^2 T_0\delta}.$$

Substituting E, from Eq. (1) into the wave equation and using $\nabla.(\in E) = 0$ and linearizing in A_1, we obtain the following equation for A_1:

$$2i\,k_0\frac{\partial A_1}{\partial z} + \frac{\partial^2 A_1}{\partial r^2} + \frac{1}{r}\frac{\partial A_1}{\partial r} + \frac{\omega_{p0}^2}{c^2}\frac{\alpha A_o^2 p\left(A_1 + A_1^*\right)}{\left(1 + \alpha p A_o^2\right)} = 0, \qquad (17)$$

following Sodha *et al.*[1] and expressing $A_1 = A_{1r} + iA_{1i}$ Eq.(17) splits into two coupled equations for A_{1r} and A_{1i}:

$$2\,k_0\frac{\partial A_{1r}}{\partial z} + \frac{\partial A_{1i}}{\partial r^2} + \frac{1}{r}\frac{\partial A_{1i}}{\partial r} = 0, \qquad (18)$$

$$-2\,k_0\frac{\partial A_{1i}}{\partial z} + \frac{\partial^2 A_{1r}}{\partial r^2} + \frac{1}{r}\frac{\partial A_{1r}}{\partial r} + \frac{2\omega_{p0}^2}{c^2}\frac{p\alpha A_o^2}{\left(1 + p\alpha A_o^2\right)}A_{1r} = 0$$

For $A_{1r}, A_{1i} \sim J_o(q_{\perp}r)\, e^{\Gamma z}$ Eq. (18) straight way yields the spatial growth rate

$$\Gamma = \frac{q_{\perp}}{2k_o}\left[-q_{\perp}^2 + 2k_o^2\frac{\in_2 A_o^2}{\in_o}\right]^{1/2}. \qquad (19)$$

The spatial growth maximizes to

$$\Gamma_{max} = \frac{\omega_{p0}^2}{2k_o^2c^2}\frac{p\alpha A_o^2}{1 + p\alpha A_o^2} \qquad (20)$$

at

$$q_{opt} = \frac{\omega_{p_o}}{c}\left(\frac{p\alpha A_o^2}{1 + p\alpha A_o^2}\right)^{1/2},$$

where $\alpha A_o^2 = \frac{1}{6}\frac{V_o^2}{c_s^2}$, $V_o = \frac{e|Ao|}{m\omega_o}$, $c_s = \left(\frac{2T_o}{m_i}\right)^{1/2}$ and m_i is the mass of ion. The first zero of J_o occurs at $q_{\perp}r = 2.4$ .The amount of power tends to localize in maximally growing filament can be expressed as

$$P' = \frac{c}{8\pi}\pi r^2 A_o^2$$

$$= 4.3\,\frac{c^3 c_s^2 m^2\omega_o^2}{e^2\omega_{po}^2}\left(1 + p\alpha A_o^2\right). \qquad (21)$$

Following Sodha et. al. [1] the temperature and density profile in the filament can be written as

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$$T_e = T_o \left[1+2\alpha p A_o^2\right] \left[1+2\alpha_1 p E_o^2(r)\right],$$

$$n_o^{/} = \frac{n_o}{\left[1+\alpha_1 p E_o^2(r)\right]},$$

$$\nu = \nu_o \left(\frac{T_e}{T_o}\right)^{-3/2} \left(\frac{n_o^{/}}{n_o}\right), \qquad (22)$$

and

$$\alpha_1 = \frac{\alpha}{1+2\alpha p A_o^2}, \qquad (23)$$

where $\vec{E}_o(r)$ is the total electric field of filament at r and ν_o is collision frequency corresponding to n_o and T_o, Expressing $\vec{E}_o(r)$ for cylindrically symmetric beam, as $\vec{E}_o = \vec{A}(r, z)$ exp {-i(ω_o t – k_o z)}and neglecting $\frac{\partial^2 A}{\partial z^2}$ in wave equation which implies that the characteristic distance (in the z directions) of the intensity variation is much greater than the wavelength and following Sodha et al.[1] a self-consistent solution of the wave equation under the modified density and temperature profile turns out to be

$A = A_0(r,z)$ exp (-ikS),

$$A_0^2(r,z) = \frac{E_{00}^2}{f^2}\exp\left(-r^2/r_0^2 f^2\right),$$

$$S(r,z) = \frac{r^2}{2}\beta(z) + \Psi(z), \qquad (24)$$

$$\beta = \frac{1}{f}\frac{df}{dz},$$

where f is the ratio of the beam diameter to its value at z = 0, β corresponds to the inverse radius of the curvature of the wave front. The beam width parameter, f (z), scales the beam radius. As f (z) decreases, the intensity increases as $1/f^2(z)$ in order to conserve energy. The eikonal gives an ordinary differential equation for f(z) if it is expanded to order r^2. This expansion is known as the paraxial ray approximation since it emphasizes the importance of the paraxial (those near r = o) rays. The aberration less and paraxial ray approximations are essentially synonymous since they yield a set of solutions characterized by a single parameter that scales the shape of the beam. We obtain equation for the beam width parameter f:

$$\frac{d^2 f}{dz^2} = \frac{1}{R_d^2 f^3} - \frac{E_{00}^2}{r_0^2 f^3}\phi^{/}\left(\frac{E_{00}^2}{f^2}\right),$$

$$\frac{d^2 f}{dz^2} = \frac{1}{R_d^2 f^3} - \frac{1}{R_n^2 f^3} \qquad (25)$$

where $\phi^{/}$ is the derivative of ϕ with respect to its arguments and terms of order higher than r^2 have been neglected. Employing paraxial ray approximation, the radius of nonlinear steady state self-trapped cylindrical filament propagating through a homogeneous plasma can be obtained from Eq. (25) balancing diffraction and self-focusing terms,

$$R_d^2 = R_n^2 \qquad (26)$$

where

$R_d = k_o r_o^2$

and

$$R_n = \frac{\omega_o^2}{\omega_{po}^2} r_o^2 \frac{\left(1+\alpha_1 p E_{oo}^2\right)^2}{\alpha_1 p E_{oo}^2}.$$

Equation (24) determines the radius r_o of a self-trapped filament,

$$r_o = \frac{c}{\omega_{po}} \frac{\left(1+\alpha_1 p E_{oo}^2\right)}{\left(\alpha_1 p E_{oo}^2\right)^{1/2}}, \qquad (27)$$

where E_{oo} is the amplitude of the filament of radius r_o, in the nonlinear state, on the axis. The corresponding power in nonlinear steady state is

$$P = \frac{c}{8\pi}\pi r_o^2 E_{oo}^2 = \frac{c^3}{8\omega_{po}^2}\frac{\left(1+\alpha_1 p E_{oo}^2\right)^2}{\alpha_1 p}.$$

Equating the power contained in the filament p to $p^{/}$ one obtains

$$\alpha_1 p E_{oo}^2 = \left[2.4\frac{\left(1+\alpha p A_o^2\right)^{1/2}}{\left(1+2\alpha p A_o^2\right)^{1/2}} - 1\right]. \qquad (28)$$

Thus the radius and field intensity in a self-trapped filament are dependent of the initial power density of the incident beam. The modified density, temperature and collision frequency variation near the axis of the filament can be obtained by expanding $n_o^{/}, T_e$ and ν around r ≅ 0

$$n_o^{/} = n_o^o\left(1+\frac{r^2}{a^2}\right), \qquad (29)$$

$$T_e = T_o^o\left(1-\frac{r^2}{b^2}\right), \qquad (30)$$

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$$v = v_o^o\left(1+\frac{r^2}{d^2}\right), \quad (31)$$

where

$$a^2 = \frac{r_o^2\left(1+\alpha_1 pE_{oo}^2\right)}{\alpha_1 pE_{oo}^2}, \quad (32)$$

$$b^2 = \frac{r_o^2\left(1+2\alpha_1 pE_{oo}^2\right)}{2\alpha_1 pE_{oo}^2}, \quad (33)$$

$$d^2 = \frac{2a^2b^2}{3a^2+2b^2} \quad (34)$$

$$n_o^o = \frac{n_o}{\left(1+\alpha_1 pE_{oo}^2\right)}, \quad (35)$$

$$T_o^0 = T_o\left(1+2\alpha pA_o^2\right)\left(1+2\alpha_1 pE_{oo}^2\right) \quad (36)$$

and

$$v_o^o = v_o\left(\frac{n_o^o}{n_o}\right)\left(\frac{T_o^o}{T_o}\right)^{-3/2}. \quad (37)$$

The self –trapped laser decays into a low frequency Langmuir wave with scalar potential

$$\phi = \phi(r)\, e^{-i(\omega t - kz)} \quad (38)$$

and a backscattered electromagnetic wave

$$\vec{E}_1 = \vec{E}_1(r)e^{-i(\omega_1 t - k_1 z)} \quad (39)$$

and

$$\vec{B}_1 = \frac{c\vec{k}_1 \times \vec{E}_1}{\omega_1},$$

where

$$\vec{k}_1 = \vec{k} - \vec{k}_o$$

and

$\omega_1 = \omega - \omega_o$.

The pump and sideband waves exert a low frequency Ponderomotive force $\mathbf{F_p}$ on the electrons [1]

$$\vec{F}_p = e\nabla\phi_p = -\frac{m}{2}\left[\vec{v}_o.\nabla\vec{v}_1 + \vec{v}_1.\nabla\vec{v}_o\right] - \frac{e}{2c}\left[\left(\vec{v}_o \times \vec{B}_0\right)\right] \quad (40)$$

where ϕ_p is the Ponderomotive potential. Solving (40), one obtains

$$\phi_p = \frac{e\vec{E}_o.\vec{E}_1}{2m\omega_o\omega_1}, \quad (41)$$

driving the Langmuir wave

$$\frac{\partial^2\phi}{\partial r^2} + \frac{1}{r}\frac{\partial\phi}{\partial r} + \left[\left\{\frac{\omega^2 - \omega_{po}^2 - k^2v_{tho}^2 + i\omega v_o^o}{v_{tho}^2}\right\} - \left\{r^2\frac{\omega_{po}^2}{a^2v_{tho}^2}\left(1 - \frac{iv_o^o\omega}{\omega_{po}^o}\frac{a^2}{c_1^2}\right)\right\}\right]\phi$$

$$= -\frac{\omega^2|v_o}{2\omega_o v_{th}^2}E_1 \quad (42)$$

where

$$v_{tho} = \left(\frac{2T_o^o}{m}\right)^{1/2},$$

$$v_o = v_{osc}\exp\left(-\frac{r^2}{2a^2}\right), \quad (43)$$

$$v_{osc} = \frac{eE_{oo}}{m\omega_o},$$

$$c_1^2 = \frac{b^2d^2}{b^2+d^2},$$

$$\omega_{po} = \left(\frac{4\pi n_o^o e^2}{m}\right)^{1/2}$$ and we have assumed only collisional damping.

The current density at the side band frequency can be written as

$$\vec{J}_1 = -n_o^o e\vec{v}_1 - \frac{1}{2}ne\vec{v}_o \quad (44)$$

$$= \left[\left\{-\frac{n_o^o e^2\vec{E}_1}{im\omega_1}\right\} - \left\{\frac{k^2}{4\pi e}\frac{e^2\vec{E}_o\phi}{2im\omega_o}\right\}\right], \quad (45)$$

Using Eq. (45) in the wave equation we get

$$\frac{\partial^2E_1}{\partial r^2} + \frac{1}{r}\frac{\partial E_1}{\partial r} + \left(\frac{\omega_1^2 - \omega_{po}^2 - k_1^2c^2}{c^2} - \frac{\omega_{po}^2}{a^2}\frac{r^2}{c^2}\right)E_1$$

$$= -\frac{k^2\omega_o|v_o|}{2c^2}\phi. \quad (46)$$

It is considered that the sideband wave is not affected by Landau damping. However, it may suffer damping due to collisions.

One obtains

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$$\frac{\partial^2 E_1}{\partial r^2}+\frac{1}{r}\frac{\partial E_1}{\partial r}+\left[\left\{\frac{\omega_1^2-\omega_{po}^2\left(1-i\frac{\nu_o^o}{\omega_1}\right)-k_\ell^2c^2}{c^2}\right\}-\left\{\frac{\omega_{po}^2}{a^2}\frac{r^2}{c^2}\left(1-\frac{i\nu_o^o a^2}{\omega_1 d^2}\right)\right\}\right]E_1$$

$$=-\frac{k^2\omega_o|v_o|}{2c^2}\phi \qquad (47)$$

Equations (42) and (47) are coupled nonlinearly. When nonlinear coupling is ignored, the solutions of (42) and (47) are written as [17, 18]

$$\phi=\phi_l=\Gamma_\ell L_\ell\left(\frac{r^2}{b_1^2}\right)\exp\left(-\frac{r^2}{2b_1^2}\right)\exp\left(\frac{ir^2}{4b_1^2}\frac{\nu_o^o\omega}{\omega_{po}^2}\frac{a^2}{c_1^2}\right),$$

$$E_1=E_{1m}=\Gamma_m L_m\left(\frac{r^2}{b_2^2}\right)\exp\left(-\frac{r^2}{2b_2^2}\right)\exp\left(\frac{ir^2}{4b_2^2}\frac{\nu_o^o}{\omega_1}\frac{a^2}{d^2}\right), \qquad (48)$$

where

$$b_1=\left(\frac{av_{tho}}{\omega_{po}}\right)^{1/2},$$

$$b_2=\left(\frac{ca}{\omega_{po}}\right)^{1/2},$$

$$L_\ell(\xi)=e^\xi\frac{d^\ell}{d\xi^\ell}\left(\xi^\ell e^{-\xi}\right)$$

where l= 0,1,2,.... , m = 0,1,2... and Γ_l and Γ_m are normalization constants. Since the pump field (hence v_o) scales as $\exp\left(-\frac{r^2}{2a^2}\right)$, the most unstable backscatter mode would correspond to m = 0. In the presence of nonlinear coupling terms, it is possible to express ϕ in terms of an orthogonal set of wave functions ϕ_l where as E_1 in terms of E_{10} .Where as E_1 can be taken to be dominant mode:

$$\phi=\sum_l s_l\phi_l,$$

and

$$E_1=TE_{10}. \qquad (49)$$

Substituting for ϕ and E_1 in (42) and (47) and multiplying the resulting equation by ϕ_l and E_{10}, respectively, and integrating over rdr, one obtains

$$\left[\left\{\frac{\omega^2-\omega_{po}^2-k^2v_{tho}^2+i\omega\nu_o^o}{v_{tho}^2}\right\}-\left\{2(\ell+1)\frac{\omega_{po}}{av_{tho}}\left(1-\frac{1}{2}\frac{\nu_o^o\omega}{\omega_{po}^2}\frac{a^2}{c_1^2}\right)\right\}\right]s_\ell$$

$$=-\frac{\omega^2T}{2\omega_oV_{tho}^2}\int rdr|v_o|\phi_\ell E_{lo}\left(1+\frac{r^2}{b^2}\right), \qquad (50)$$

$$\left[\left\{\frac{\omega_1^2-\omega_{po}^2\left(1-\frac{i\nu_o^o}{\omega_1}\right)-k_1^2c^2}{c^2}\right\}-\left\{\frac{2\omega_{po}}{ac}\left(1-\frac{1}{2}\frac{i\nu_o^o}{\omega_1}\right)\right\}T\right]$$

$$=-\frac{k^2\omega_o}{2c^2}\sum_\ell S_\ell\int rdr|v_o|\phi_\ell E_{lo} \qquad (51)$$

leading to a nonlinear dispersion

$$\left[\left\{\omega_1^2-\omega_{po}^2\left(1-\frac{i\nu_o^o}{\omega_1}\right)\right\}-\left\{k_1^2+\frac{2\omega_{po}}{ac}\left(1-\frac{1}{2}\frac{i\nu_o^o}{\omega_1}\frac{a^2}{d^2}\right)\right\}c^2\right]$$

$$=4\Gamma_o^2\omega\omega_o\times$$

$$\sum\frac{\left(I_l^2+\frac{I_l.I_l(1)}{b^2}\right)}{\left[\left(\omega^2-\omega_{po}^2+i\omega\nu_o^o\right)-\left\{k^2+\frac{(2l+1)\omega_{po}}{av_{tho}}\left(1-\frac{1}{2}\frac{\nu_o^o\omega a^2}{\omega_{po}^2c_1^2}\right)\right\}v_{tho}^2\right]} \qquad (52)$$

where

$$I_\ell=\int_0^\infty rdr\phi_\ell E_{lo}\exp\left(-\frac{r^2}{2a^2}\right), \qquad (53)$$

$$I_\ell(1)=\int_0^\infty r^3dr\phi_\ell E_{lo}\exp\left(-\frac{r^2}{2a^2}\right), \qquad (54)$$

$\Gamma_o=\frac{1}{4}(k\nu_{osc})(\omega/\omega_o)^{1/2}$ is uniform medium growth rate and we have used $v_o\cong v_{osc}e^{-r^2/2a^2}$. Since ϕ_1 is localized in a narrow region around $r\le_1 <<a$. $I_1(1)$ may be simplified to become

$$I_1\cong\frac{\sqrt{2}}{b_2}\int_0^\infty r^3\,dr\phi_\ell \qquad (55)$$

and

$$I_l(1)\cong\frac{\sqrt{2}}{b_2}\int_0^{oo}r^3dr\phi_\ell. \qquad (56)$$

Expressing $\omega=\omega+i\Gamma$, the maximum growth rate can be expressed as [21]

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$$\Gamma = \Gamma_o \frac{2b_1}{b_2}\left(1+\frac{2b_1^2}{b^2}\right) \tag{57}$$

and the uniform growth rate

$$\Gamma'_{oo} = \frac{1}{4} k V_o \left(\frac{\omega}{\omega_o}\right)^{1/2}$$

one obtains

$$\frac{\Gamma}{\Gamma'_{oo}} = \frac{v_{OSC}}{V_o} 2\left(\frac{v_{tho}}{c}\right)^{1/2}\left(1+\frac{2b_1^2}{b^2}\right).$$

The threshold intensity can be expressed as .

$$P_{th}^{//} = \left(\frac{v_{osc}}{c}\right)^2_{SRS-th} = \frac{1}{4}\left(\frac{\omega_{po}}{\omega_o}\right)^2 \frac{v_o^{o2}}{\omega_o \omega_{po}} \frac{yz}{x} \tag{58}$$

where

$$y = \left(1+\frac{v_{tho}}{a\omega_{po}}\frac{a^2}{c_1^2}\right),$$

$$z = \left(1+\frac{c}{a\omega_{po}}\frac{a^2}{d^2}\right), \tag{59}$$

and

$$x = \frac{b_1^2}{b_2^2}\left(1+\frac{2b_1^2}{b^2}\right).$$

The threshold condition for B-SRS, when background plasma and intensity of laser beam is uniform is written as [21]

$$P_{th}^{/} = \left(\frac{V_o}{c}\right)^2_{SRS-th} = \frac{1}{4}\left(\frac{\omega_p^2}{\omega_o^2}\right)\frac{v_o^2}{\omega_o \omega_p}. \tag{60}$$

[III] RESULTS AND DISCUSSION

A uniform–laser beam propagating through collisional plasma is unstable to a transverse perturbations, and break up into filaments. An optimum value of $q_\perp$ of the perturbation is required for a maximum growth rate. A uniform plane wave does not cause redistribution of carriers. However, as a result of perturbations in the intensity distribution along the wave front, electrons do become redistributed. The process of B-SRS in a filament is aided by the enhancement of power density over its initial value but it is inhibited by the localization of Langmuir wave and hence of the interaction region. The Process of B- SRS is inhibited by thermal conduction and it is observed that the power density inside the filament is much greater than the initial power density of the laser beam. Hence, the enhanced intensity in laser filament reduces collisional damping of backscatter light wave, diminishing the threshold power for B-SRS. The onset of B-SRS is strongly correlated with intensity threshold of the filamentation instability that depends on thermal conduction. The growth rate of SRS is reduced by a geometrical factor I_l and $I_l(1)$ depending on the mode structure of the pump and decay waves. The growth rate scales linearly with the amplitude of the laser wave.. In a plasma filled FEL, the Raman instability would limit the growth of FEL instability via diverting FEL wave energy into the Langmuir and the sideband waves.

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RESEARCH ARTICLE OPEN ACCESS

RELATIONSHIP BETWEEN HSP-65 AND IL-2, IL-10 PROFILE IN TB PATIENTS. IMPLICATION FOR VACCINE DESIGN: A PRELIMINARY STUDY

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ABSTRACT

Tuberculosis (TB) is a serious infectious disease. Bacillus Calmette Guerin (BCG) only vaccine currently available has shown to offer limited protection against incidence of TB. Hence novel vaccines instead of BCG are required for protection against TB worldwide. Mounting evidence suggest that ideal vaccine against TB should drive efficient Th1 immune response. Hsp65 is a major immunoreactive protein and is supposed to be an important target for subunit vaccine development. In present study serum samples of TB patients were analyzed for Hsp 65 expression and also IL-2 and IL-10 cytokine profile was studied. Our preliminary results suggest that with an increase in Hsp 65 expression, level of IL-2 increased while that of IL-10 was found to get decreased. This relationship between Hsp65 and IL-10 and IL-2 cytokine profile suggest us that Hsp65 may modulate Th1 immune response and thus have a potential for vaccine design in near future. However, there are lots of future studies needed in in-vitro and animal models to prove the present concept.

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KEY WORDS

Hsp 65; IL-10, IL-12; Tuberculosis; vaccine

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[I] INTRODUCTION

Tuberculosis (TB) is a largest single infectious cause of human mortality [1-2]. The incidence of TB has remained high in most of the developing world and the disease is a public health problem. Bacillus Calmette Guerin (BCG) is among the worlds most widely used and only vaccine currently available against TB for use in humans. However due to lack of consistent protective efficacy in different parts of the world, it remains the most controversial vaccine in current use [3]. The development of a new improved TB vaccine is thus, a highly prioritized international research area. Vaccine potential of various novel antigens of *Mycobacterium tuberculosis* (MTB) including antigen 85 complexes and ESAT-6 have been tried by various investigators with encouraging results [3] However, no novel vaccine for clinical use has yet been developed in the world.

Other proteins, which have been proposed to have important immune target for subunit vaccine development are, heat shock protein (Hsp) family [4]. Hsp(s) are well-conserved and immunodominant antigens which elicit a cellular and humoral immune response and may play an important role in host defense against invading microorganisms and autoimmune disorders [5-6]. Among Hsp, 65 kD Hsp protein, is present in a wide range of mycobacterial species and has been most intensively studied [7-9]. It is one of the major immunoreactive proteins of the mycobacteria [10-11]. It has been shown that Hsp(s) are potent activators of the innate immune system, capable of inducing proinflammatory cytokine production by the monocyte-macrophage system, and the activation and maturation of dendritic cells (antigen-presenting cells).which play an important role in host defense against TB.

Hsp 65 protein has been mapped at epitope level for both T and B cells recognition. MHC Class II specific CD4+, Th 1 cells recognize a single epitope in Hsp 65 indicating it to be an important candidate with immunotherapeutic and preventative vaccine potential [10-11]. The vaccination potential of this protein has also been explored in M. habana and MTB and was seen to confer protection against experimental challenges in mice [12].

Cytokines are important hormonal mediators, produced in tissues undergoing defense, growth and repair processes [12]. It is believed that immunity to TB is mediated by Th1 lymphocytes which activate antimicrobial macrophage

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functions via the release of proinflammatory cytokines like IL-2, IL-12 and IFN-γ [13]. Human monocytes, generally, respond to microbial infection by increasing the expression and secretion of such cytokines, which are involved in inflammatory responses. They play an important role in the modulation of the immune response to infection with MTB. tuberculosis. Thus current attempts to find a vaccine for TB are based on the assumption that it must drive Th1 immune response.

In the present study to justify our above hypothesis we examined expression of Hsp 65 in serum samples of TB patients and its co relationship with proinflammatory (IL-2) and anti-inflammatory (IL-10) cytokines.

[II] MATERIALS AND METHODS

2.1. Study Subject

Serum samples of forty-two cases were analyzed in the present study for cytokine expression with respect to 65kD Hsp. All the TB patients (24 female; 18 males, age 15–65 years) had history and clinical findings compatible with diagnosis of TB. TB was confirmed if AFB staining and / or culture of sputum specimens were positive for MTB. Out of these, fourteen were both sputum smear and culture positive cases (Grade2+). Remaining twenty-eight patients were clinically suspected TB cases, where both AFB and culture tests were negative and diagnosis was made on the basis of clinical findings like, low grade fever, loss of appetite, abnormal chest X-ray, weight loss, night sweats, chronic cough with or without hemoptysis, chronic abdominal pain, past history of TB. Serum samples were obtained from all the patients before initiation of anti-kotch treatment (AKT) and were stored at -20°C until they were tested. All patients included in the study were vaccinated with BCG. Samples were collected from all study groups for which patient's consent was obtained. The Central India Institute of Medical Sciences Ethical Committee, Nagpur, India approved the study. All the patients were grouped as follows:

2.2. Diagnostic Criteria

2.2.1. Confirmed TB

TB was confirmed if AFB staining and /or culture of sputum specimens were positive for MTB.

2.2.2. Clinically Suspected TB

When AFB staining and culture tests were negative, the diagnosis of TB was made on the following clinical observations:
a) Low grade fever, b) Loss of appetite, c) Abnormal Chest X-ray, d) Weight Loss, e) Night sweats, f) Chronic cough with or without hemoptysis, g) Chronic abdominal pain, h) Past history of TB

2.3. Specimens

Sputum specimens obtained from patients were digested and decontaminated with N-acetyl cystein (NALC) and 2% sodium hydroxide (NaLc-NaOH) solution and then processed for further investigations. Ziehl-Nielson acid fast staining was used to confirm the presence of acid-fast bacilli. Venous blood was collected from all the patients as well as control subjects. Blood was allowed to clot and after centrifugation (1000×g, 10 min) serum was separated and stored at -20°C until use.

2.4. Antibodies

The 65kD monoclonal antibody was obtained from Colorado State University, USA through the TB Research Materials and Vaccine Testing Contract (NO1-AI-40091) derived from MTB, strain H37Rv, designated IT 13.The secondary antibody was rabbit anti rat IgG peroxidase conjugate obtained from Genei, Bangalore, India. IL-2 and IL-10 module Set were obtained from Bender Med Systems.

2.5. Estimation of 65kD Hsp antigen

Prior to patients sampling, the assay was standardized using different concentration of 65kD antigen (1-1000ng/ml) in Phosphate buffered saline pH 7.2 with 0.05%Tween 20 (PBS-T). After standardization, wells of flat-bottom microtiter plates were coated with 100 ùl of serum samples (1:200 dilutions in PBS-T) of selected groups and incubated for 90 min at 37°C. The wells were then washed with PBS-T and blocked with 100 ùl of 0.5% BSA in PBS-T at 37°C for 60 min. After blocking, monoclonal antibody against 65 kD Hsp antigen was added to all the wells (1:5,000 dilution in PBS-T) and incubated at 37°C for 60 min. The wells were washed with the PBS-T followed by addition of, 100 ùl of affinity purified anti-rat IgG-HRP conjugate (Genei, Bangalore, India) with 1:10,000 dilution in PBS-T, and incubated at 37°C for 60 min. After incubation the wells were washed extensively with PBS-T followed by addition of 100ul of TMB/H_2O_2 substrate and incubated at room temperature for 10 min. The reaction was stopped with addition of 100 ùl of 2.5 N H_2SO_4. The absorbance of each well was read at 450 nm. Each sample was tested in triplicates.

2.6. Estimation of IL-2 and IL- 10

The serum samples were subjected to cytokine assay, IL-2 and IL-10 concentrations were measured by quantitative ELISA (IL-2 and IL-10 Module Set, Bender Med Systems). Flat-bottom micro titer plates were coated with 100 µl of coating antibody solution and incubated at 2-8^{0}C overnight. The wells were then washed once with 300 µl PBS-T. 250 µl of blocking buffer was added to each well and incubated at 37°C for 2 hrs. This was followed by washing and sample addition. 100µl sample and 50 µl of diluted biotin conjugate (1 µl/ml) were added to all the wells. The plates were incubated at room temperature (18ºC - 25ºC) on shaker for 2hrs. The wells were washed thrice with wash buffer followed by addition of 100 µl diluted Streptavidin-HRP (0.2µl/ml) and incubation at room temperature for 1hr. After incubation the wells were washed thrice, 100µl TMB substrate was added to each well. After 10 min stop solution (100ul of 4N sulphuric acid) was added and absorbance was taken at 450nm.

2.7. Statistical Analysis

The Pearson's correlation amongst these along with their statistical significance is presented in **Table–1** and **Table–2**. The correlation between IL-2 and 65kD in TB patients was found to be significant ($p<0.05$). Also the correlation between IL-10 and 65kD was found to be significant ($p<0.05$). Graphs were designed using Regression Scatter chart for IL-2, IL-10 and 65kD Hsp.

[III] RESULTS

Serum samples of 42 TB cases were collected. All the samples were subjected to ELISA for detection of concentration of 65kD Hsp antigen in serum. The samples from the same group were then tested for IL-2 and IL-10 levels following the method given by Bender Med Systems. Relationship between 65kD Hsp, IL-2 and IL-10 was checked by plotting Regression

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Table: 1. Demonstrates Pearson's Correlation Coefficient for IL-2 and IL-10 with 65kD Hsp in serum samples of TB patients

Variable X	Variable Y	Correlation Coefficient r	Significance Level	95% Confidence interval for r	Positive/ Negative Correlation
65kD	IL2	0.9602	<0.0001	0.9268 to 0.9786	Positive
65kD	IL10	-0.4358	0.0426	-0.7243 to -0.0174	Negative

graphs and Pearson's Correlation Coefficient was also found in each case. P value less than 0.05 was considered to be significant.

Figure–1 shows the relationship between IL-2 and 65kD Hsp in serum samples of TB patients. The regression graph shows a significant increase in IL-2 with the increase in 65kD Hsp. A significant positive correlation between IL-2 and 65kD Hsp was found by Pearson's Correlation Coefficient with $P < 0.05$ **[Table–1]**. On the other hand significant decrease in IL-10 level with the increase in 65kD Hsp expression was noted in serum samples of TB patients as shown in **Figure–2**. A significant negative correlation was noted in this case and P value was found to be < 0.05**[Table-1]**.

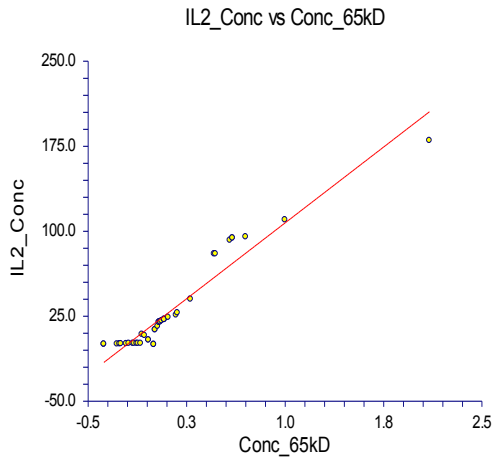

Fig: 1. Regressions between IL-2 and 65kD Hsp concentration in TB patients.

[IV] DISCUSSION

Mycobacterium bovis Bacillus Calmette Guerin (BCG) is the only vaccine available for clinical use but it has shown variable levels of efficacy against pulmonary TB, as demonstrated by several clinical trials. Given the increased public health importance of TB, the development of improved TB vaccines is an area that has been given priority by the WHO in its call for TB as a 'Global Health Emergency' [14].

Many antigens have been tried by various investigators for development of new TB vaccine candidate however still all are under experimental investigations. In the present study we have tried to establish a relationship between Hsp 65 and the increased level of IL-2 with decreasing level of IL-10, and postulated that it may have a role for vaccine design in future.

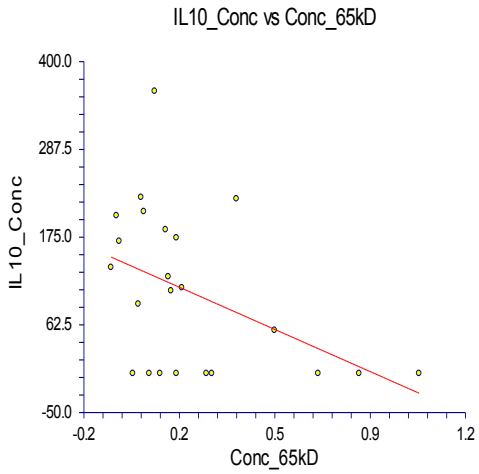

Fig: 2. Regressions between IL-10 and 65kD Hsp concentration in TB patients

As stated earlier, protective immunity against MTB depends on production of Th1 arm of immune response producing proinflammatory cytokines [15-16]. Our results demonstrate an increase in IL-2 levels with an increase in 65kD Hsp in tuberculosis. Thus indicating that 65kD Hsp enhance the expression of IL-2. IL-2 stimulates expansion and enhanced functional capacity of natural killer cells, which can eliminate intracellular MTB [17-18]. IL-2 strongly induces IFN-gamma production by murine splenocytes exposed to *M.bovis* BCG [19], and is a potent growth factor for CD_4+ and CD8+ T cells, both of which contribute to immunity against TB [17].

In case of anti-inflammatory IL-10, our results demonstrated a decrease in IL-10 concentration with the increasing 65kD Hsp in patients infected with M.TB. Earlier Murray et al, demonstrated that IL-10 is an inhibitor of early mycobacterial clearance suggesting that IL-10 negatively regulates numerous macrophage functions as well as playing a role in down-

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regulating the general inflammatory response, especially in conditions where an infection must be controlled through macrophage activity [19-20]. Similarly in another study done by Gloria et al, elevated pulmonary steady-state protein levels of IL-10, IFN-gamma, and bioactive TGF-ß were found in TB patients versus those in other lung disease patients and healthy volunteers. This observation suggests that the combined production of the immunosuppressant IL-10 and TGF-ß, as well as co expression of TGF-ß RI and RII (required for cellular response to TGF-ß), may act to down-modulate host anti-MTB immunity and thereby allow uncontrolled bacterial replication and overt disease [21].

Progress in TB research has advanced as a result of the great amount of information obtained from the completion of the M.tb genome sequence. This has led to the rapid characterization and expression of MTB antigens with vaccine potential and Hsp 65 is one among them. Hsp 65 can elicit a strong delayed type hypersensitivity reaction in experimental animals infected with MTB [22]. Hsp 65 produced by M.*leprae* has been proved to be an efficient vaccine antigen against several pathologies including TB, leishmaniasis [23], diabetes, arthritis and cancer [24, 25, 26, 27]. The success of Hsp 65 vaccination in these different diseases reflects its effectiveness as an immunodominant antigen,which can induce Th1 response and CTL response against TB epitopes [26, 31, 32, 33]. Although Hsp are intracellular protein as they lack leader signal sequences that direct their secretion, still, they have been identified extracellularly in body fluids and suggested to play physiological roles [28, 29, 30]. Earlier in our laboratory also we have reported increased level of Hsp 65 in serum and CSF sample of TB patients than in NTB patients, which suggest the increased expression of Hsp in response to stress induced by host defense during disease state. [10, 11].

Overall the present study reveals that 65kD hsp increases the expression of IL-2 with decreasing IL-10 levels, indicating that 65kD Hsp can modulate immune response in TB and may be further evaluated as a candidate for vaccine development in near future. However actual potential of Hsp 65 as a vaccine candidate can only be stated only after proper studies in in *vitro* and animal models by establishing its role in induction of Th1 and CTL response generation There are a few misconceptions stating that, the antigen will appear only in late infection and will not be released by live bacteria and also that the phenotype of the T-cells generated at the later stage will not be appropriate for protection. But experiments by Celio L. Silva reveals the prominence of CD8+ Hsp 65-reactive T cells in BCG vaccinated mice and state that the Hsp 65 protein is produced in substantial amounts by intracellular mycobacteria [22].

Our results showed that increase in Hsp 65 antigen increases IL-2 cytokine levels with significant decrease in IL-10 in serum samples, suggesting its role in immunity against TB. We thus suggest that 65kD Hsp may have significant implications for vaccination against TB infection. However, lot of animal study is needed prior reaching to any conclusion.

[IV] CONCLUSION

In conclusion, relationship between Hsp 65 and IL-10 and IL-2 cytokine profile suggest us that Hsp 65 may modulate Th1 immune response and thus have potential for vaccine design in near future.

FINANCIAL DISCLOSURE

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RESEARCH ARTICLE OPEN ACCESS

STRAIGHT VEGETABLE OIL: AN ALTERNATIVE FUEL FOR COOKING, LIGHTING AND IRRIGATION PUMP

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ABSTRACT

SVO (Straight Vegetable oil) of Jatropha was de-waxed and de-gummed as per the standard procedure, and was tested in irrigation pump set, kerosene cook stove and kerosene lamp. It was found that Jatropha oil could be successfully used as irrigation pump fuel, however at interval of every 25 - 30 hours of operation, fuel injection nozzle and head needs to be cleaned in spite of maintaining the Jatropha oil temperature between 80 to 90 °C. Out put of the irrigation pump is almost similar to diesel fuelled irrigation pump set, however fuel consumption rate in case of Jatropha oil was more compared to diesel. Recorded air pollutants were found to be higher in case of Jatropha oil as compared to diesel, except NOx. No significant, wear and tear in the engine component such as liner, piston, and piston rings, except bore gauge was observed. Trials taken with kerosene stove and kerosene lamp also reveals that Jatropha oil could be used as cooking fuel in pressurized stoves with a blend of 30% Jatropha oil and 70% kerosene without any problems. However for kerosene lamp it can be used with a blending of 10 % Jatropha oil and 90% kerosene.

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[I] INTRODUCTION

As per the Government of India report, published by planning commission, a large part of India's population, mostly in the rural areas which does not have access to commercial energy sources will have to be provided hydrocarbon fuels and power by December 2012. The country faces problems of effectively meeting the growing energy demand and at the same time curb the resulting environmental degradation. In this background bio-fuels offer an attractive alternative for providing energy with less pollution.

All properties of plant oils are close to diesel except the viscosity. High viscosity of the plant oils is considered to be the major constraint although high acid value and presence of wax/gums etc. also adversely affects the engine performance. To reduce the viscosity number of method has been tried by researcher such as; cracking of plant oil, blending of the plant oil with appropriate additives like alcohol, chemically transforming the plant oils to convert them into esters (bio-diesel) by alcoholysis and de-waxing, de-gumming and heating the plant oils before injecting them into the combustion chamber. Considering the economic constrain last option seems more effective. Moreover plant oils, straight has several advantages as combustion fuel. Due to relatively simple and low-cost technology for expelling and filtering, the plant oil can be processed on the farm itself, thus saving the transport cost, time and energy [5, 6, 7].

Literature indicates that up to 75 % of diesel requirement could be replaced by plant oils with satisfactory engine performance [1, 2, 3 10, 12]. As mentioned above, diesel are still required (25 to 30 % of the specific fuel consumption) to supplement plant oil. Short-term engine tests of less than 10 hour duration carried out by author itself indicate that plant oils performed quite well. Problems occur only after the engine is operated on plant oil for longer duration of time [7]. In this study, effort has been made to utilize 100 % Jatropha oil with minor treatment for different applications.

[II] MATERIALS AND METHOD

2.1. Characterization of fuels

Non-edible oil generally contains about 3-4 %wax and gum. De-waxing and de-gumming of plant oils is required not only for smooth running of the CI engine but also to prevent engine failure even if plant oils are blended with diesel [13, 14]. It is therefore necessary to remove wax and gum from the fresh oil before it could be used in CI engine.

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Characterization of diesel and Jatropha oil was done as per the ASTM standards (ASTM, 1983. The results obtained are given in **[Table–1].**

2.2. Modification of characteristics of Jatropha oil

Jatropha oil procured from the local markets was de-waxed & de-gummed as per standard procedure [11].

2.3. Experimental setup and measuring devices used

A 5 hp Field Marshal engine as per specifications given in **Table–2** was used. Performance parameters planned to be studied include: Fuel consumption rate, operating efficiency at fixed level of suction height, engine exhaust temperature and emissions, lube oil contamination, smoke opacity, sound level, visible engine wear (bore diameter etc).

Table: 1. Characteristic of fuels

Fuel Type	Viscosity at 38 ºC, cSt	Density at 38ºC, g/cc	Cetane Index	Flash point, ºC	Cloud point, ºC	Carbon residues, %	Free fatty acid, %	Calorific value, kcal/kg	Wax & gum, %
Diesel	4.44	0.789	45	66	13	0.03	-	10404	-
DDJO	48.96	0.892	43 ± 2	242	16	0.22	1.14	8765	2.3

DDJO- De-waxed & de-gummed Jatropha oil 1 Kcal/kg ≈ 0.0041868 MJ/kg

Table: 2. Engine Details of 5 hp irrigation pump set

Name and Model of engine	Field Marshal, Gf 1
General Details	Constant speed, single cylinder, water cooled naturally aspirated, direct injection
Bore x Stroke	100 x 120 mm
Compression ratio	17: 1
Specific fuel consumption	236 g/kWh
Rated output	3.7 kW/5 bhp at 1500 rpm
Fuel injection opening pressure	175-180 kg/cm^2
Injection timing	26° BTDC
Engine conditions	New engine

The fuel flow rate was measured on volumetric basis. Efforts were made to use waste heat of engine exhaust to preheat the Jatropha oil to minimize operation and maintenance problem due to higher viscosity. A microprocessor based engine exhaust gas analyzer was used for the measurement of emissions levels. Smoke meter, sound pressure level meter and K type thermocouple and indicator were used to measure smoke intensity, sound pressure at a distance of 1 meter from the engine fly wheel, exhaust gas and oil temperature respectively.

Experiments were carried at a fixed suction head (5.2 m) of water column. A sampling port was provided in the exhaust pipe for measuring flue gas temperature and to collect flue gas samples. The test was conducted as per BIS Code No.13018 (1990), however due to limitation of sources/ facility all the parameters as mentioned in BIS Code No.13018 could not be measured.

2.4. Heating of Jatropha oil

A 20-litre capacity mild steel container (300 mm diameter & 350 mm height) was designed and developed to heat Jatropha oil through engine exhaust gas. Container consists of copper coil, temperature gauge and manual agitator **[Figure–1]**. The copper coil was made with 22 mm outer diameter and 20 mm inner diameter copper pipe having 7 rounds. One end of the copper coil was attached with exhaust gas pipe and the other end to the container releasing exhaust gas outlet. The mild steel container is supported using a proper stand. About 16-18 litres of Jatropha oil was poured in the container and it was observed that it took about 30 minutes to attain a temperature of 90 to100 °C from an ambient temperature of 30 °C.

It was also noted that although Jatropha oil temperature in the container was in the range of 90-100 °C, still when it reaches the nozzle, oil temperature drops down to room temperature due to capillary action. Later it was decided that the fuel pipe carrying Jatropha oil to the nozzle could be wrapped on the exhaust pipe. Number of rounds wrapped was decided by trial and error method with an objective to obtain about 80-90 °C oil temperature at the nozzle. The desire effect was achieved by wrapping 7 to 8 rounds of the fuel pipe on the exhaust pipe **[Figure–2]**. Both the exhaust pipe and the fuel pipe were insulated to maintain the desire temperature.

2.5. Test conditions and variables

Test conditions and variables involved in the trials have been listed in **[Table–3]**. The irrigation pump set was tested at original conditions as per the specifications provided by the manufacturer. However, minor changes in the engine variables during the test period due to environmental change, carbon deposition and wear of components were beyond control.

[III] RESULTS AND DISCUSSION

3.1. Performance of irrigation pump set

The performance of 5 hp irrigation pump set fuelled by de-waxed & de-gummed and heated Jatropha oil was determined at a constant engine speed of 1500 rpm which was controlled within a

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Fig.1 Heating arrangement for JO

Fig 2 Modified heating arrangement for JO

range of ±15 rpm. Simulated condition was created by keeping the irrigation pump set at about 5.2 m height from ground level and a 1000 litre water tank was used to store and re-circulate water. The test was repeated three times to verify the out put and engine exhaust data. Specific energy consumption in diesel and Jatropha oil was calculated from the fuel consumption and calorific value of diesel and Jatropha oil. Calorific value of diesel and Jatropha oil was determined using bomb calorimeter.

Engine exhaust temperature was always higher in case of Jatropha oil compared to diesel. The increase in exhaust gas temperature may be due to delayed combustion because of slower combustion characteristics of Jatropha oil. Moreover almost all plant oils have slow burning characteristic compared to diesel. The increase in exhaust gas temperature may be responsible for reducing the brake thermal efficiency. Batt et al., 2001[4] also have reported similar result while working with karanja oil in a single cylinder engine. Engine was also run at 1200 rpm maintaining other engine parameter constant, however no improvement in the engine performance was observed. Similarly fuel injection pressure was also increased from 175-180 kg/cm^2 to 190 kg/cm^2, again no improvement in the engine performance was observed. Performance of irrigation pump set using both fuels is shown in **Table–3**. The effect on pollutants using diesel as well Jatropha oil are shown in **Figures–4, 6**.

3.1.1. Fuel consumption rate

Fuel consumption rate increased by almost 15-20% compared to diesel at the 5.2 m suction height of water. **[Figure–3]** shows a comparison of the fuel consumption rate obtained for diesel and de-waxed & de-gummed and heated Jatropha oil.

3.1.2. Wear and tear in the engine component

Since the engine was run on pure Jatropha oil heated to about 70-80°C, it was considered desirable to study the effect of Jatropha oil on the wear and tear of various engine components. The effect was studied in terms changes that took place in different components (e.g. change in shape, diameter, thickness, weight and length etc) after 100 hours cumulative operation of irrigation pump set with heated Jatropha oil. No significant change was observed in the liner diameter, piston diameter, and thickness of piston ring, except bore gauge, however about 2.985 g carbon deposition on different components of the engine was recorded.

3.1.3. Effect on engine oil

Engine oil used in irrigation pump set was characterised for viscosity, flash point, TBNE, sediment and water content before putting into the engine and after 100 hours running for pumping of water. Study reveals that Kinematics Viscosity, TBNE value and sediments increased after 100 hours running for pumping water. It may be due to dilution of engine oil by Jatropha oil.

Table: 3. Effect on specific energy consumption and efficiency with change of fuel in irrigation pump set

Fuel	Fuel consumption rate, Kg/hr	SFC, g/kWh	SEC, MJ/kWh	Brake thermal Efficiency, %
Diesel	0.644	302	13.18	7.59
DDJO	0.8678	407	14.87	6.72

DDJO- De-waxed & de-gummed Jatropha oil; Specific Fuel Consumption; SEC- Specific Energy Consumption

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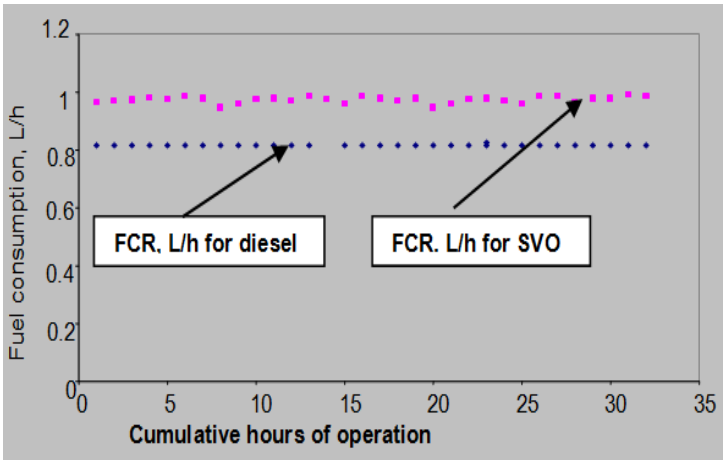

Fig: 3. Effect on fuel consumption rate with respect to time

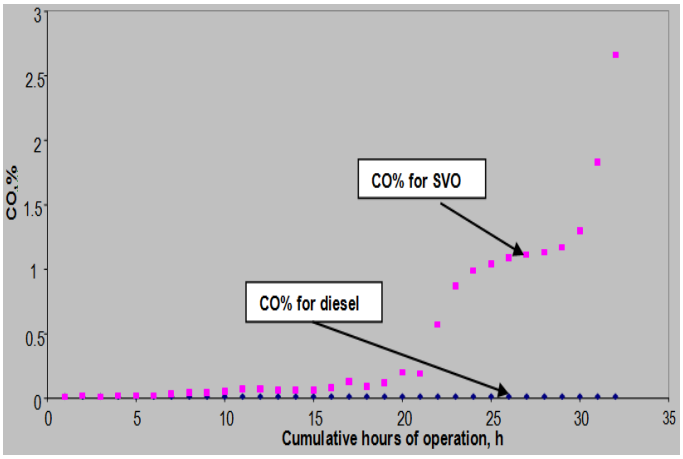

Fig: 4. Effect on CO with respect to time

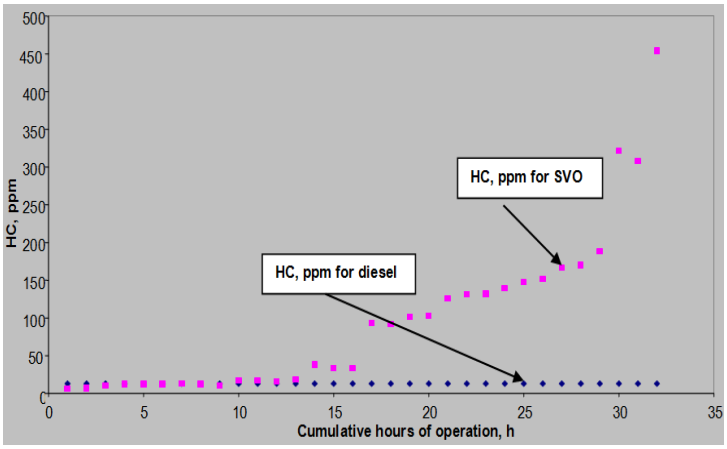

Fig: 5. Effect on HC with respect to time

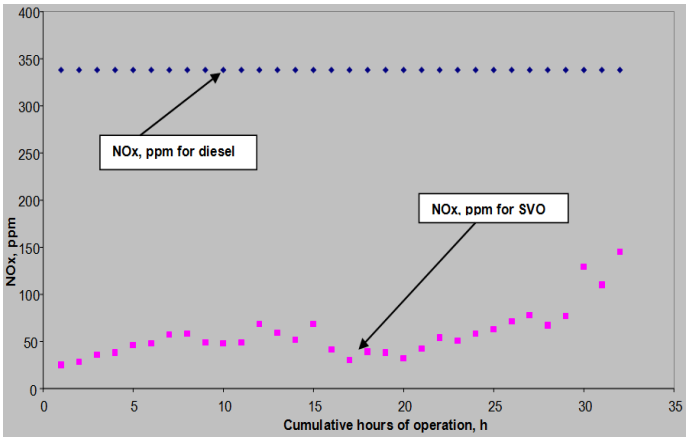

Fig: 6. Effect on NOx with respect to time

3. 2. Jatropha oil for cooking

3.2.1. Cooking

To check the maximum possible blending of Jatropha oil with Kerosene, without sacrificing stove performance, blends of Jatropha oil and Kerosene in the ratio of 10:90, 20: 80, 30:40, 40:60, and 50:50 on volumetric basis were prepared. These blends were tested using commercially available pressure stoves **[Figure–7]**. The stove performance was found to be satisfactory up to 30 % of Jatropha oil blends with 70 % kerosene; however after 25 - 30 minutes interval soot deposited on the nozzle has to be removed. With the blends having mixture ratio of more than 30:70 Jatropha oil and kerosene following observations were made:

1) Although at the blend ratio of 50:50, Jatropha oil & Kerosene stove was working smoothly, however frequent pulses with higher flame length was observed at intervals of 20-30 seconds.
2) Flame temperature was more than 954 °C.
3) From the nozzle of stove burner dripping of Jatropha oil was observed
4) Viscosity of freshly prepared 50: 50 (Jatropha oil & kerosene) blend was compared with used 50: 50 (Jatropha oil & kerosene) blend and was observed as 17.2 and 18.3 cS respectively.

3.2.2. Fuel consumption rate (FCR)

The stove was filled with the blended fuel up to three-fourth of its capacity. The stove was lighted and brought up to a working pressure of 140 kN/m2 (1.4 kgf/cm^2) within 5 minutes. Immediately the lighted stove was kept on a sensitive balance with an accuracy of 1 g for recording the fuel consumption rate. The stove was allowed to burn for 1 hour with an aluminium pan having sufficient water in it. At the end of 1 hour, weight of the burning stove was noted and weight of stove without aluminium pan was recorded. The difference in the initial and final weight of the burning stoves divided by time gave the fuel consumption rate. **Table 4** shows the performance of pressure stove at different blending of Jatropha oil with kerosene. For cooking application wick (thread) stove was also tried, however it could not work even at 10 % blending of Jatropha oil with 90 % kerosene.

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Fig: 7. Kerosene pressure stove

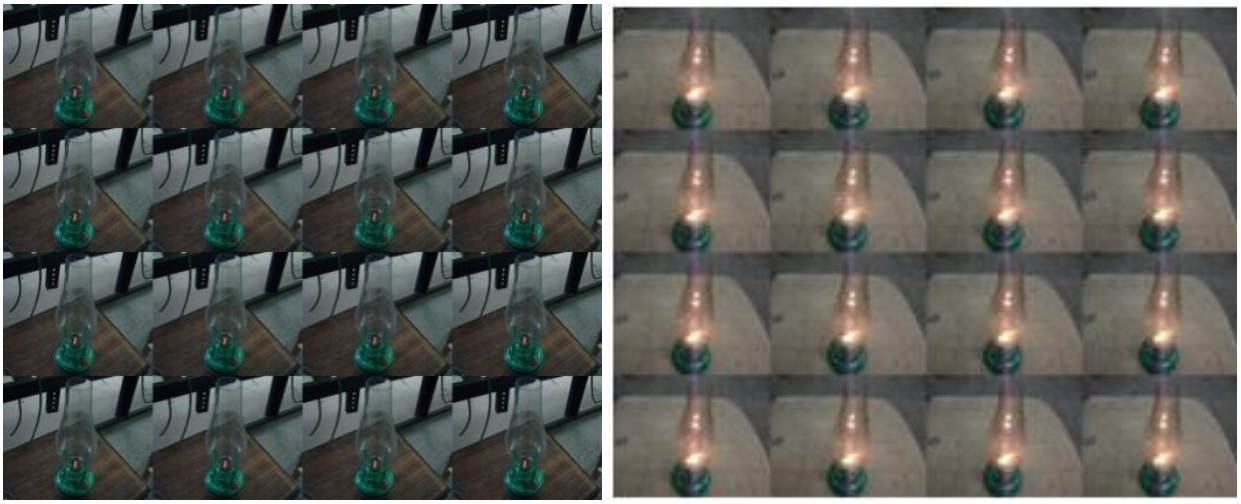

Fig: 8. Performance of Kerosene lamp with blended Jatropha oil

3.3. Jatropha oil for lighting

To check maximum possible blending of Jatropha oil with Kerosene, without sacrificing the performance of kerosene lamp, blends of Jatropha oil and Kerosene in the ratio of 10:90, 20: 80, 30:40, 40:60, and 50:50 on volumetric basis were prepared. These blends were tested at commercially available Kerosene lamp **[Figure–8]**. It was observed that with less than or equal to 25% blending of Jatropha oil with kerosene worked satisfactorily only for a period of one hour, due to very poor capillary action of Jatropha oil, however 10% blending of Jatropha oil with kerosene worked satisfactorily for more than 4 hours continuously without any problems. Air pollution, which include % CO, % CO_2, % O_2, ppm HC, ppm NOx and smoke intensity were also measured and found to be within limits.

Table: 4. Performance of pressure stove at different blending of Jatropha oil with kerosene

Sr. No.	Blend Ratio (JO:K)	Initial weight of stove+ kerosene	Final weight of stove+ kerosene	Duration, minutes	Fuel consumption rate (FCR), kg/h
1	0:100	1.367 kg	1.312 kg	20	0.165
2	30:70	1.549 kg	1.493 kg	20	0.168
3	40:60	1.678	1.853	20	0.175
4	50:50	1.678 kg	1.587 kg	20	0.30

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[V] CONCLUSION

Following conclusions were drawn from the study:

- Jatropha oil could be used as irrigation pump fuel, however for every 25 - 30 hours of operation the fuel injection nozzle and head have to be cleaned even though Jatropha oil temperature was maintained between 80 to 90 °C.
- Discharge of water was about 24658 L/hr for diesel as well heated Jatropha oil.
- Out put of the irrigation pump is similar for both diesel & Jatropha oil; however fuel consumption rate in case of Jatropha oil is more compared to diesel.
- All the pollutants released by using Jatropha oil are higher as compared to diesel, except NOx.
- No significant, wear and tear in the engine components such as liner, piston, and piston ring, except bore gauge was observed after 100 cumulative hours of operation, however about 3 g carbon deposition was found on different engine components.
- Jatropha oil could be used as fuel in cooking pressure stove with a blend of 30% Jatropha oil and 70% kerosene without any problems.
- Jatropha oil could be used as lighting fuel with blend of 10% Jatropha oil and 90% kerosene without any problems.

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