Original paper

Altered immunoexpression of SOX2, OCT4 and Nanog in the normal-appearing oral mucosa of tobacco users

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Abstract

Background. Tobacco use is causatively associated with various human cancers, including oral carcinoma. A number of pathways have been delineated to describe its etiopathological link with oral carcinogenesis, including alterations in the expression of stem cell markers. Embryonic stem cell markers, such as sex-determining region Y-box 2 (SOX2), octamer-binding protein 4 (OCT4) and homeobox protein Nanog, which are mainly involved in the maintenance of stemness and pluripotency, have been positively associated with the pathogenesis of oral potentially malignant disorders and oral cancers. In this context, we attempted to explore the subcellular impact of tobacco through examining the expression of these stem cell markers in normal and normal-appearing oral mucosa in non-tobacco users and tobacco users.

Objectives. The aim of the study was to analyze the immunoexpression of SOX2, OCT4 and Nanog in the normal-appearing oral mucosa (NAOM) of tobacco users as compared to the normal oral mucosa (NOM) of non-tobacco users.

Material and methods. The tissue samples of tobacco users and non-tobacco users ($n = 50$ per group) were immunohistochemically stained to assess the expression of SOX2, OCT4 and Nanog.

Results. In the oral mucosa of non-tobacco users, a peculiar parabasal expression pattern of SOX2 and OCT4 was observed, whereas Nanog was non-reactive. The grade of inflammation was found to be a predictive variable influencing the expression of the 2 markers. In tobacco users, variables such as male gender, mixed habit and basilar hyperplasia significantly controlled the basilar and suprabasilar expression of SOX2, OCT4 and Nanog. The expression of SOX2 and OCT4 was higher in tobacco users; in particular, OCT4 positivity was significantly increased ($p < 0.001$) in comparison with non-tobacco users.

Conclusions. The altered expression of the examined stem cell markers could be an indication of early molecular changes in NAOM under the influence of tobacco.

Keywords: tobacco, stem cells, oral mucosa

Cite as
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Introduction

Tobacco use is one of the leading contributors to untimely deaths and associated economic damage globally, as it has been consistently linked with the etiopathogenesis of several human cancers, including oral cancer. Various forms of tobacco, including smoking and smokeless, or some combination of both, are thought to be involved in the multistep process of oral carcinogenesis.[1] Around 60 chemicals, including pro-carcinogens that are present in tobacco products, can cause genetic and epigenetic aberrations in oral mucosal cells through DNA damage and chromosomal instability, and thus contribute to field cancerization. These subcellular changes appear to be early events in multistep progression from clinically normal and histopathologically non-dysplastic mucosa (normal oral mucosa (NOM)), through oral potentially malignant disorders, to oral cancer.[2] The deleterious effects of tobacco at the cellular level need to be detected early through biomarkers so that individuals at risk of developing clinical lesions can be identified and managed accordingly.

A well-characterized transcription factor called sex-determining region Y-box 2 (SOX2) is known to play a crucial role in the maintenance of pluripotency and the self-renewal capability of human embryonic stem (ES) cells.[3] Another transcription factor, octamer-binding protein 4 (OCT4) – a member of the POU-family of DNA-binding proteins, also contributes to the regulation of pluripotency of ES cells through cooperative interactions with SOX2.[4] Homeobox protein Nanog is another member of core pluripotency regulators that acts on the downstream targets of OCT4, and hence plays a key role in cell fate determination, mainly in the differentiation of ES cells.[5] Several clinical studies have reported on the association of these master regulators of ES cell pluripotency at various stages of carcinogenesis and the overall prognosis of various human malignancies.[6–8] The expression of SOX2, OCT4 and Nanog have been studied in combination with or without other stem cell markers as predictors of the risk of malignant transformation in oral potentially malignant disorders and with regard to the clinical outcomes of oral cancer. Despite the conflicting results, the overexpression of these transcription factors has been observed to play a vital role in oral carcinogenesis.[9–11] Furthermore, a number of studies have also noticed an increased expression of these markers in normal-appearing oral mucosa (NAOM) adjacent to the lesional carcinoma tissue, supporting the association of SOX2, OCT4 and Nanog in the field cancerization of oral mucosa under the influence of tobacco.[9,12] Based on this evidence, we evaluated the expression of SOX2, OCT4 and Nanog in the NOM of non-tobacco users in comparison with that of tobacco users by means of immunohistochemistry to assess the influence of tobacco on oral mucosa prior to any evidence of clinically established lesions.

Methodology

Patients and tissue sample collection

The research protocol was reviewed and ethically approved by the institutional Ethical Review Board at the MGM Institute of Health Sciences, Navi Mumbai, India (No. of approval: MGMIHS/RES./02/2018-19/63). Healthy patients, with or without tobacco habits, were selected from among the individuals who visited the dental hospital for periodontal, orthodontic or surgical treatment, such as crown lengthening, ridge augmentation, pericoronitis, or periodontal flap surgery. After obtaining informed consent from the participants, the discarded NAOM samples taken from the abovementioned surgical procedures were collected. A total of 100 tissue samples were obtained from non-tobacco users (n = 50) and tobacco users (n = 50) for the present study.

Histopathological assessment

All tissue samples were fixed in 10% neutral buffered formalin, followed by tissue processing and staining with the use of hematoxylin and eosin (H&E). The histopathological assessment of the samples was carried out by 2 independent oral pathologists for the presence of microscopic changes, such as basilar hyperplasia and inflammation. Inflammation in the samples was scored as follows: 0 – no inflammation; 1 – mild (less than 25 inflammatory cells); 2 – moderate (more than 25 and less than 125 inflammatory cells); and 3 – severe (more than 125 inflammatory cells).[13]

Immunohistochemical staining

From each paraffin-embedded block, tissue sections were cut to a thickness of 3 μm with a rotary microtome (Leica RM 2245; Leica Camera, Wetzlar, Germany). The sections were dewaxed in xylene, and then rehydrated in absolute alcohol, 95% alcohol and 85% alcohol. Antigen retrieval was performed by immersion in Tris-EDTA (0.1 M, pH 9) in a decloaking chamber at 125°C for 10 min. Endogenous peroxidase activity was blocked by using 3% hydrogen peroxide in methanol for 30 min. The slides were then incubated with an anti-SOX2 rabbit monoclonal antibody (prediluted, Clone EP103, Cat. No. PR071; PathnSitu Biotechnologies, Livermore, USA), an anti-OCT4 rabbit monoclonal antibody (prediluted, Clone EP143, Cat. No. BSB2029; Bio SB, Santa Barbara, USA) and an anti-Nanog mouse monoclonal antibody (prediluted, Clone 5A10; Novus Biologicals, Centennial, USA) diluted in Tris-buffered saline (TBS) and 5% bovine serum albumin (BSA) at 4°C overnight in a wet chamber. For the substrate chromogenic reaction, the sections were immersed in a freshly prepared solution of 0.03% diaminobenzidine for 2 min at room temperature, followed by

counterstaining with Mayer's hematoxylin. The samples of colon carcinoma, esophageal carcinoma and seminoma were used as positive controls, whereas the substitution of the primary antibody with TBS served as an internal negative control for each batch of staining.

Immunohistochemical analysis and scoring

Two pathologists independently reviewed the stained slides without any access to clinical or demographic data of the patients. The nuclear expression of SOX2, OCT4 and Nanog was observed in the epithelial cells of both tobacco users and non-tobacco users. A total of 1,000 cells were counted in a ×400 magnification field per specimen, using the ImageJ software (National Institutes of Health (NIH), Bethesda, USA; https://imagej.nih.gov/ij/). The percentage positivity of all markers was calculated by dividing the total number of positively stained cells by the total number of cells in the section (minimum 1,000 cells) in high-power fields ×100%. Staining intensity was observed and graded as weak, moderate or strong. The scoring of percentage positivity (<5% of cells – 0; 5–24% – 1; 25–49% – 2; 50–74% – 3; and >75% of cells – 4) and intensity (no cells – 0; weak – 1; moderate – 2; and strong – 3) was done according to the methodology proposed by Vijayakumar et al.[14] The final scoring of each section was calculated by adding the scores of percentage positivity and intensity (0–3 – low expression; and 4–7 – high expression). If any disagreement occurred (intensity score discrepancy >1), the slides were re-evaluated by a third independent pathologist along with previous observers to obtain a consensus diagnosis.[9]

Statistical analysis

The observations were noted and recorded in a Microsoft® Excel sheet (Microsoft Corporation, Redmond, USA). The results were compared for statistical significance using IBM SPSS Statistics for Windows, v. 24.0 (IBM Corp., Armonk, USA). Student's independent *t* tests were used to analyze the intra- and intergroup variations in SOX2, OCT4 and Nanog expression. Linear regression analysis was applied to explore independent predictor variables influencing the expression of stem cell markers.

Results

The study participants constituted 2 groups: group I comprised healthy controls with NOM, without any tobacco habit (n = 50); and group II consisted of individuals with NAOM with a tobacco habit (n = 50). The association of the expression patterns of the stem cell markers with the demographic data of both groups are depicted in Tables 1–4.

Expression patterns of SOX2, OCT4 and Nanog, and their correlation with clinicopathological factors in non-tobacco users (group I)

The nuclear expression of SOX2 and OCT4 was observed only in the parabasal layer of NOM, with mild to moderate intensity of immunoreactivity observed for both markers. Nanog was found to be negative for all NOM samples (Fig. 1). The association between the percentage expression of SOX2 and OCT4 and the clinicopathological features of this group, including age, gender and the grade of inflammation, was examined with the independent *t* test. The percentage expression of SOX2 and OCT4 was statistically significantly different among the grades of inflammation ($p < 0.001$), whereas age and gender did not seem to influence their expression (Table 1). Linear regression analysis showed the grade of inflammation to be a significant and independent predictor of expression of these 2 stem cell markers (Table 2).

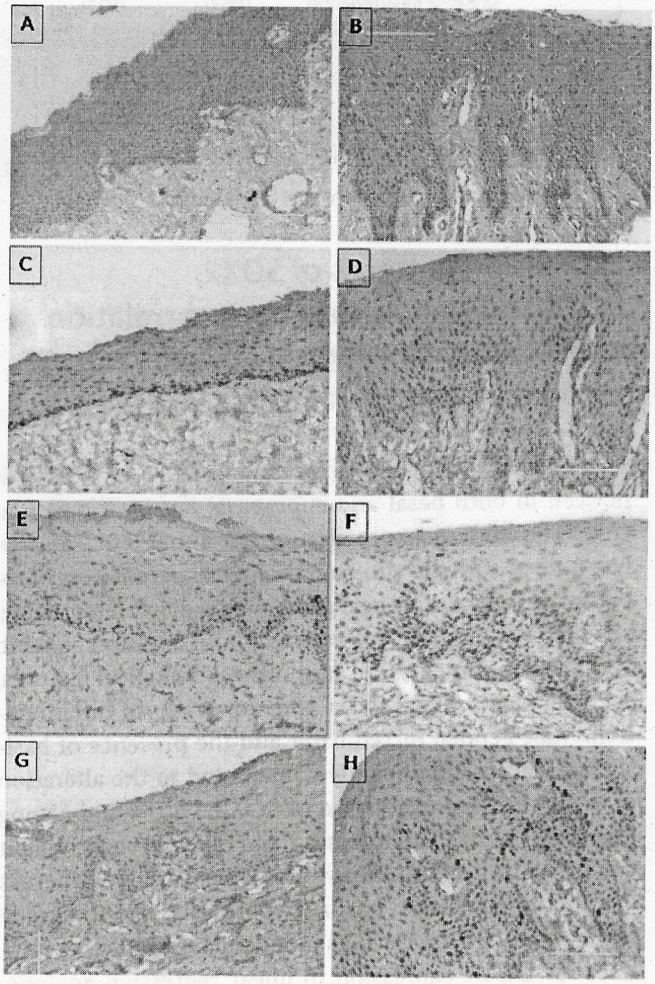

Fig. 1. Histopathological staining of oral mucosa (hematoxylin and eosin (H&E); ×400 magnification) in non-tobacco users (A) and tobacco users (B). In non-tobacco users, the immunohistochemical expression of SOX2 (C), OCT4 (E) and Nanong (G) were found to be limited to the parabasal layer (if positive for staining), whereas the immunoreactivity of all these markers increased up to the basal and suprabasal layers and above in tobacco users (D, F and H for SOX2, OCT4 and Nanong, respectively)

Table 1. Correlation between demographic features and the percentage expression of SOX2 and OCT4 in normal oral mucosa (NOM) in non-tobacco users

Variables		Sample size ($n = 50$)	Percentage expression of SOX2 [%]	p-value	Percentage expression of OCT4 [%]	p-value
Age [years]	≤40	28	3.12 ±1.59	0.842	0.93 ±0.93	0.853
	>40	22	3.03 ±1.72		0.98 ±1.07	
Gender	male	38	2.83 ±1.64	0.053	0.92 ±0.98	0.723
	female	12	3.88 ±1.40		1.04 ±1.07	
Grade of inflammation	no	34	2.58 ±1.43	<0.001*	0.65 ±0.83	<0.001*
	mild	8	3.21 ±1.56		0.93 ±1.03	
	moderate	8	5.06 ±0.93		2.62 ±0.41	

Data presented as mean ± standard deviation ($M \pm SD$). * statistically significant (unpaired two-sample t test).

Table 2. Multiple regression analysis showing the association of predictive variables with the percentage expression of SOX2 and OCT4 in the normal oral mucosa (NOM) of non-tobacco users

Model		Unstandardized coefficients		Standardized coefficient	t	p-value	95% CI for β	
		β	SE	β			lower bound	upper bound
SOX2 expression	(constant)	2.002	0.843	–	2.374	0.022	0.303	3.700
	age	−0.248	0.401	−0.076	−0.618	0.539	−1.055	0.559
	gender	0.622	0.485	0.164	1.284	0.206	−0.354	1.599
	grade of inflammation	0.890	0.331	0.415	2.692	0.010*	0.224	1.556
OCT4 expression	(constant)	0.710	0.527	–	1.347	0.185	−0.352	1.772
	age	0.000	0.242	0.000	0.001	0.999	−0.487	0.488
	gender	−0.286	0.294	−0.125	−0.975	0.335	−0.878	0.305
	grade of inflammation	0.668	0.190	0.513	3.522	0.001*	0.286	1.050

SE – standard error; CI – confidence interval; * statistically significant.

Expression patterns of SOX2, OCT4 and Nanog, and their correlation with clinicopathological factors in tobacco users (group II)

In NAOM, the nuclear expression of all 3 markers was noticed in both basal and suprabasal layers of oral mucosa. The immunoexpression of these markers was analyzed along with the clinicopathological parameters of tobacco users, including age, gender, type of habit (smoking, smokeless and mixed, i.e., with alcohol), duration of habit, and tobacco contact time, and histopathological features, such as basilar hyperplasia and the grade of inflammation. Male gender, mixed habit and the presence of basilar hyperplasia significantly contributed to the alteration of the percentage expression of SOX2, OCT4 and Nanog (Table 3). For SOX2 expression, the type of tobacco habit was found to be a significant predictive variable, whereas age, duration of habit, basilar hyperplasia, and the grade of inflammation emerged as independent predictive variables for OCT4 expression in linear regression analysis. Gender, tobacco contact time and the grade of inflammation were observed to have significantly influenced Nanog immunoexpression (Table 4). The intensity of immunoreactivity of all markers was noticed to increase along with an increase in the grade of inflammation.

Intergroup comparison of SOX2, OCT4 and Nanog expression

The mean value of percentage expression of OCT4 (9.74 ±4.79%) was found to be statistically higher in tobacco users when compared to non-tobacco users (0.95 ±0.99) ($p < 0.001$). A similar increasing trend was observed in SOX2 expression under the influence of tobacco, though the result was not statistically significant. In the case of Nanog, the 2 groups were not compared, as this marker was expressed only in tobacco users and was absent in the NOM of non-tobacco users.

Co-expression of SOX2, OCT4 and Nanog

The co-expression of SOX2, OCT4 and Nanog was assessed by evaluating the percentage expression in the same area of the tissue specimens. In tobacco users, a higher co-expression of SOX2 and OCT4 (76%) was observed in comparison with non-tobacco users (30%), though the statistical correlation was found to be non-significant (Spearman' coefficient $\rho = 0.120$; $p = 0.408$). The co-expression of all 3 markers was observed in 58% of all tobacco users. The overall intensity of immunoreactivity of all markers was observed to be higher in tobacco users in comparison with non-tobacco users.

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SE – standard error; *CI* – confidence interval; * statistically significant.

Expression patterns of SOX2, OCT4 and Nanog, and their correlation with clinicopathological factors in tobacco users (group II)

In NAOM, the nuclear expression of all 3 markers was noticed in both basal and suprabasal layers of oral mucosa. The immunoexpression of these markers was analyzed along with the clinicopathological parameters of tobacco users, including age, gender, type of habit (smoking, smokeless and mixed, i.e., with alcohol), duration of habit, and tobacco contact time, and histopathological features, such as basilar hyperplasia and the grade of inflammation. Male gender, mixed habit and the presence of basilar hyperplasia significantly contributed to the alteration of the percentage expression of SOX2, OCT4 and Nanog (Table 3). For SOX2 expression, the type of tobacco habit was found to be a significant predictive variable, whereas age, duration of habit, basilar hyperplasia, and the grade of inflammation emerged as independent predictive variables for OCT4 expression in linear regression analysis. Gender, tobacco contact time and the grade of inflammation were observed to have significantly influenced Nanog immunoexpression (Table 4). The intensity of immunoreactivity of all markers was noticed to increase along with an increase in the grade of inflammation.

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Table 3. Correlation between demographic features and the percentage expression of SOX2, OCT4 and Nanog in normal-appearing oral mucosa (NAOM) in tobacco users

Variables		Sample size ($n = 50$)	Percentage expression of SOX2 [%]	*p*-value	Percentage expression of OCT4 [%]	*p*-value	Percentage expression of Nanog [%]	*p*-value
Age [years]	≤40	28	5.06 ±6.20	0.559	9.85 ±7.14	0.908	3.96 ±4.65	0.291
	>40	22	4.13 ±4.62		9.60 ±8.07		2.54 ±4.66	
Gender	male	41	5.38 ±5.82	<0.001*	10.71 ±7.50	0.034*	4.07 ±4.89	0.016*
	female	9	1.37 ±1.37		5.32 ±5.93		0.00 ±0.00	
Type of habit	smoking	15	3.11 ±1.56	<0.001**	12.66 ±7.11	<0.001**	3.71 ±3.58	0.003**
	smokeless	23	2.81 ±4.66		4.54 ±4.04		1.24 ±1.71	
	mixed	12	10.10 ±6.98		15.38 ±6.90		6.72 ±7.21	
Duration of habit [years]	≤5	26	5.94 ±6.20	0.196	12.09 ±7.52	0.060	4.46 ±5.39	0.136
	6–10	16	3.72 ±5.22		7.69 ±6.55		2.71 ±3.90	
	>10	8	2.32 ±1.72		6.22 ±7.35		0.90 ±1.76	
Tobacco contact time [h]	≤1	36	3.84 ±4.37	0.197	9.44 ±7.63	0.662	3.54 ±5.41	0.609
	>1	14	6.73 ±7.57		10.49 ±7.33		2.78 ±1.63	
Basilar hyperplasia	absent	20	1.98 ±1.85	0.001*	5.44 ±5.80	<0.001*	1.45 ±1.72	0.016*
	present	30	6.44 ±6.42		12.60 ±7.17		4.59 ±5.55	
Grade of inflammation	no	9	3.33 ±3.09	0.535	5.96 ±8.01	0.025*	1.91 ±1.92	0.260
	mild	15	4.73 ±6.52		6.09 ±6.11		1.98 ±1.77	
	moderate	24	4.68 ±4.96		12.37 ±7.05		4.63 ±6.27	
	severe	2	9.80 ±13.85		16.30 ±8.02		4.25 ±0.63	

Data presented as mean ± standard deviation ($M \pm SD$). * statistically significant (unpaired two-sample *t* test); ** statistically significant (one-way ANOVA (two-tailed)).

Table 4. Multiple regression analysis showing the association of predictive variables with the percentage expression of SOX2, OCT4 and Nanog in the normal-appearing oral mucosa (NAOM) of tobacco users

Model		Unstandardized coefficients		Standardized coefficient	*t*	*p*-value	95% *CI* for β	
		β	*SE*	β			lower bound	upper bound
SOX2 expression	age	−0.636	1.886	−0.058	−0.337	0.738	−4.448	3.176
	gender	−2.913	2.903	−0.204	−1.004	0.322	−8.781	2.954
	type of habit	3.259	1.254	0.436	2.599	0.013*	0.724	5.793
	duration of habit	0.701	1.451	0.095	0.483	0.632	−2.232	3.633
	tobacco contact time	−0.972	2.078	−0.080	−0.468	0.642	−5.171	3.227
	basilar hyperplasia	2.075	1.710	0.186	1.214	0.232	−1.381	5.531
	grade of inflammation	0.343	1.090	0.052	0.315	0.754	−1.860	2.547
OCT4 expression	age	4.971	2.131	0.333	2.332	0.025*	0.663	9.278
	gender	−4.440	3.302	−0.230	−1.347	0.185	−11.120	2.225
	type of habit	0.552	1.644	0.055	0.336	0.739	−2.771	3.875
	duration of habit	−4.020	1.634	−0.403	−2.463	0.018*	−7.329	−0.722
	tobacco contact time	−5.060	2.676	−0.307	−1.892	0.066	−10.470	0.346
	basilar hyperplasia	4.460	1.937	0.295	2.304	0.027*	0.547	8.378
	grade of inflammation	4.302	1.223	0.477	3.517	0.001*	1.830	6.773
Nanog expression	age	0.694	1.464	0.075	0.474	0.638	−2.265	3.653
	gender	−4.752	2.049	−0.395	−2.320	0.026*	−8.893	−0.612
	type of habit	1.739	1.028	0.276	1.691	0.099	−0.340	3.817
	duration of habit	−0.904	1.125	−0.145	−0.804	0.426	−3.177	1.369
	tobacco contact time	−5.407	1.590	−0.526	−3.400	0.002*	−8.621	−2.193
	basilar hyperplasia	1.089	1.322	0.115	0.824	0.415	−1.582	3.760
	grade of inflammation	2.132	0.839	0.380	2.540	0.015*	0.436	3.829

* statistically significant.

Discussion

The cumulative evidence of a positive correlation between the immunoexpression of SOX2, OCT4 and Nanog and various oral potentially malignant disorders and oral carcinoma indicates the putative role of these proteins in oral carcinogenesis. Though previous research has reported on the presence or absence of these pluripotent stem cell markers in normal mucosa as compared to that of the lesional tissues, an exclusive study targeting their expression in adult stem cells in NOM has not been conducted until now. In this context, the present study observed only SOX2 and OCT4 expression in NOM, which is in agreement with the observations of Qiao et al.,[10] whereas all the samples showed negative immunoreactivity toward Nanog.[10,15] SOX2 is thought to be a guardian of the embryological development of the head and neck region through its expression in neural crest cells. Since most of the oral and orofacial structures are derived from the migrated neural crest cells, the presence of SOX2 in the NOM of non-tobacco users explains its putative role in oral mucosa differentiation through a precise cell flow.[16]

The lower mean value of OCT4 percentage expression as well as the absence of Nanog expression in NOM could be explained by the regulatory and repressing effect of SOX2 on OCT4 and Nanog during specific lineage differentiation.[11,17] We observed the parabasal layer expression of these pluripotent stem cells, in contrast to the basal layer expression reported by many authors. As NOM is thought to have its stem cell population distributed in the quiescent basal cell and active parabasal cell layers, this justifies the peculiar pattern of parabasal layer expression of SOX2 and OCT4 rather than the basal cell layer expression, as observed in previous studies.[18] The presence of inflammation not only was found to be significantly associated with an increased immunoreactivity of these markers, but also emerged as a predictive variable for the same in the NOM of non-tobacco users. Inflammation has an inducible effect on stem cell proliferation through cytokine production. In NOM, even sterile inflammation (non-microbial), which may result from chemical and physical insults, can cause the proliferation of stem cells. At the site of injury, inflammatory cells recognize danger-associated molecular patterns and secrete molecules that prime the tissue restoration via stem cell induction.[19]

In tobacco users, an increased expression of SOX2 and OCT4 and the appearance of Nanog in tobacco-affected oral mucosa were observed. Naini et al. reported similar results in the adjacent non-tumor oral tissue, which indicated the potential role of these markers in the early molecular stages of carcinogenesis.[12] Fu et al. also observed a similar higher expression of Nanog in the corresponding tumor-adjacent normal tissues as compared to their normal counterparts, which supports its underexplored behavior in carcinogenesis.[9] Significant associations between tobacco-related parameters, including the type of habit and tobacco contact time, and an increased immunoreactivity of all these markers indicate a critical role of nicotine in oral carcinogenesis through the regulation of the expression and stemness of SOX2, OCT4 and Nanog.[20] In addition, a mixed habit that includes alcohol use was shown to be associated with an increased expression of all of these ES cells markers, which is in accordance with the experimental findings showing the alcohol-activated induction of cancer stem cells.[21]

Histopathological factors, including basilar hyperplasia, were correlated with an elevated expression of stem cell markers in the present study. Under the influence of tobacco, the shift and expansion of the stem cell niche from the parabasal cell layer (active stem cell population) to the basal cell layer (quiescent stem cell population) was evident from the basal cell hyperplasia observed in histopathological examinations and the criteria depicting architectural changes in epithelial dysplasia. In addition, the grade of inflammation also correlated positively with an increased expression of stem cell markers. Both smokeless and smoke tobacco can cause genetic and proteomic alterations through the dysregulation of inflammation-associated pathways, such as MAPK/ERK as well interferon signaling in oral keratinocytes.[22]

The mean percentage expression values of all of the markers were significantly elevated in tobacco users in comparison with non-tobacco users, which may indicate the cumulative effect of long-term tobacco exposure on oral keratinocytes by inducing the expansion of the embryonic stem cell population. The co-expression of SOX2 and OCT4 was found to be increased in the oral mucosa of tobacco users, similar to the observations made by Qiao et al., who reported an elevated $SOX2^{+}OCT4^{+}$ profile in the transforming oral mucosa of the rat and in precancerous lesions of humans that displayed simple hyperplasia.[10] The co-expression of all 3 markers in over a half of tobacco users could be a result of the mechanical and chemical impact of tobacco on oral keratinocytes.

Conclusions

The use of tobacco and the tobacco habit can induce early molecular changes at the cellular level through the expansion of the stem cell niche, which could be recognized histopathologically as basilar hyperplasia. Increased expression and co-expression of pluripotent stem cell markers SOX2, OCT4 and Nanog in NAOM may indicate another molecular pathway of tobacco-induced oral carcinogenesis. The observations of the present study could be used as a baseline for further research on the impact of tobacco on oral mucosal stem cells in the development of various oral potentially malignant disorders and oral cancer.

Ethics approval and consent to participate

The research protocol was reviewed and ethically approved by the institutional Ethical Review Board at the MGM Institute of Health Sciences, Navi Mumbai, India (No. of approval: MGMIHS/RES./02/2018-19/63). Informed consent was obtained from all participants.

Data availability

All data generated and/or analyzed during this study is included in this published article.

Consent for publication

Not applicable.

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References

1. Chaturvedi P, Singh A, Chien CY, Warnakulasuriya S. Tobacco related oral cancer. *BMJ*. 2019;365:l2142. doi:10.1136/bmj.l2142
2. Giaretti W, Maffei M, Pentenero M, et al. Genomic aberrations in normal appearing mucosa fields distal from oral potentially malignant lesions. *Cell Oncol*. 2012;35:43–52. doi:10.1007/s13402-011-0064-2
3. Zhang S, Xiong X, Sun Y. Functional characterization of SOX2 as an anticancer target. *Signal Transduct Target Ther*. 2020;5:135. doi:10.1038/s41392-020-00242-3
4. Rizzino A. The Sox2–Oct4 connection: Critical players in a much larger interdependent network integrated at multiple levels. *Stem Cells*. 2013;31(6):1033–1039. doi:10.1002/stem.1352
5. Chambers I, Colby D, Robertson M, et al. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell*. 2003;113(5):643–655. doi:10.1016/s0092-8674(03)00392-1
6. You L, Guo X, Huang Y. Correlation of cancer stem-cell markers OCT4, SOX2, and NANOG with clinicopathological features and prognosis in operative patients with rectal cancer. *Yonsei Med J*. 2018;59(1):35–42. doi:10.3349/ymj.2018.59.1.35
7. Van Schaijik B, Davis PF, Wickremesekera AC, Tan ST, Itinteang T. Subcellular localisation of the stem cell markers OCT4, SOX2, NANOG, KLF4 and c-MYC in cancer: A review. *J Clin Pathol*. 2018;71(1):88–91. doi:10.1136/jclinpath-2017-204815
8. Hepburn AC, Steele RE, Veeratterapillay R, et al. The induction of core pluripotency master regulators in cancers defines poor clinical outcomes and treatment resistance. *Oncogene*. 2019;38(22):4412–4424. doi:10.1038/s41388-019-0712-y
9. Fu TY, Hsieh IC, Cheng JT, et al. Association of OCT4, SOX2, and NANOG expression with oral squamous cell carcinoma progression. *J Oral Pathol Med*. 2016;45(2):89–95. doi:10.1111/jop.12335
10. Qiao B, He B, Cai J, Yang W. The expression profile of Oct4 and Sox2 in the carcinogenesis of oral mucosa. *Int J Clin Exp Pathol*. 2013;7(1):28–37. PMID:24427323. PMCID:PMC3885457.
11. Swain N, Thakur M, Pathak J, Swain B. SOX2, OCT4 and NANOG: The core embryonic stem cell pluripotency regulators in oral carcinogenesis. *J Oral Maxillofac Pathol*. 2020;24(2):368–373. doi:10.4103/jomfp.JOMFP_22_20
12. Naini FB, Aminishakib P, Abdollahi A, Hodjat M, Mohammadpour H, Khoozestani NK. Relative expression of OCT4, SOX2 and NANOG in oral squamous cell carcinoma versus adjacent non-tumor tissue. *Asian Pac J Cancer Prev*. 2019;20(6):1649–1654. doi:10.31557/APJCP.2019.20.6.1649
13. Naderi NJ, Semyari H, Elahinia Z. The impact of smoking on gingiva: A histopathological study. *Iran J Pathol*. 2015;10(3):214–220. PMID:26351487. PMCID:PMC4539769.
14. Vijayakumar G, Narwal A, Kamboj M, Sen R. Association of SOX2, OCT4 and WNT5A expression in oral epithelial dysplasia and oral squamous cell carcinoma: An immunohistochemical study. *Head Neck Pathol*. 2020;14(3):749–757. doi:10.1007/s12105-019-01114-1
15. de Vicente JC, Rodríguez-Santamarta T, Rodrigo JP, et al. The emerging role of NANOG as an early cancer risk biomarker in patients with oral potentially malignant disorders. *J Clin Med*. 2019;8(9):1376. doi:10.3390/jcm8091376
16. Mandalos NP, Remboutsika E. Sox2: To crest or not to crest? *Semin Cell Dev Biol*. 2017;63:43–49. doi:10.1016/j.semcdb.2016.08.035
17. Zhang S, Cui W. Sox2, a key factor in the regulation of pluripotency and neural differentiation. *World J Stem Cells*. 2014;6(3):305–311. doi:10.4252/wjsc.v6.i3.305
18. Dunaway S, Rothaus A, Zhang Y, Kadekaro AL, Andl T, Andl CD. Divide and conquer: Two stem cell populations in squamous epithelia, reserves and the active duty forces. *Int J Oral Sci*. 2019;11(26):26. doi:10.1038/s41368-019-0061-2
19. Michael S, Achilleos C, Panayiotou T, Strati K. Inflammation shapes stem cells and stemness during infection and beyond. *Front Cell Dev Biol*. 2016;4:118. doi:10.3389/fcell.2016.00118
20. Pedregal-Mallo D, Hermida-Prado F, Granda-Díaz R, et al. Prognostic significance of the pluripotency factors NANOG, SOX2, and OCT4 in head and neck squamous cell carcinomas. *Cancers (Basel)*. 2020;12(7):1794. doi:10.3390/cancers12071794
21. Xu M, Luo J. Alcohol and cancer stem cells. *Cancers (Basel)*. 2017;9(11):158. doi:10.3390/cancers9110158
22. Rajagopalan P, Patel K, Jain AP, et al. Molecular alterations associated with chronic exposure to cigarette smoke and chewing tobacco in normal oral keratinocytes. *Cancer Biol Ther*. 2018;19(9):773–785. doi:10.1080/15384047.2018.1470724

Original Article

Identification of genetic instability in peripheral blood lymphocyte of oral squamous cell carcinoma patients assess by comet assay

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Abstract

Context: Studies established that human cancer is principally a genetic disease; it arises as accumulation of a set of genetic changes. In the pathogenesis of cancer, genetic instability is the sequential event to a carcinogenic stimulus resulting in various genomic changes including DNA damage.

Aims: To assess genetic instability, as susceptibility to DNA damage, we used single-cell gel electrophoresis (comet assay) to study double strand breaks in associated with the risk of oral squamous cell carcinoma (OSCC).

Materials and Methods: We used comet assay to measure double strand break in individual peripheral blood lymphocytes from 50 individuals with OSCC and 30 healthy control subjects. All personal information was gathered from subjects including tobacco history. DNA damage was visualized as comet assay and quantified by movement of damaged strands as length of tail.

Results: Study results of OSCC patients were observed in relation to clinical staging and histological grading of carcinoma. On the basis of clinical observation, cases were grouped in to Stage I, Stage II, Stage III and Stage IV. No stage I cases were in study sample. The mean DNA damage migration length was observed 4.600 ± 0.4613 μm in stage II, whereas in Stage III and Stage IV, it was observed to be 4.961 ± 0.5620 μm and 4.883 ± 0.410 μm, respectively. The DNA damage length in histological grades of squamous cell carcinoma patients in Grade I was 4.6437 ± 0.3061 µm and Grade II was 5.3533 ± 0.3831 µm. In comparison with control group and squamous cell carcinoma group, it was observed in the range of 0.02–0.36 µm and varied from 4.04 to 5.84 µm range, respectively. Thus, the results were statistically significant with the histological grading of OSCC. Statistical Analysis: Unpaired' test and "ANOVA" test are used for statistics.

Conclusion: The amount of DNA strand breaks in peripheral lymphocytes are measured by comet assay which is associated with relative risk of OSCC.

Keywords: Comet assay, DNA damage, DNA strands, gel electrophoresis, lymphocyte, squamous cell carcinoma

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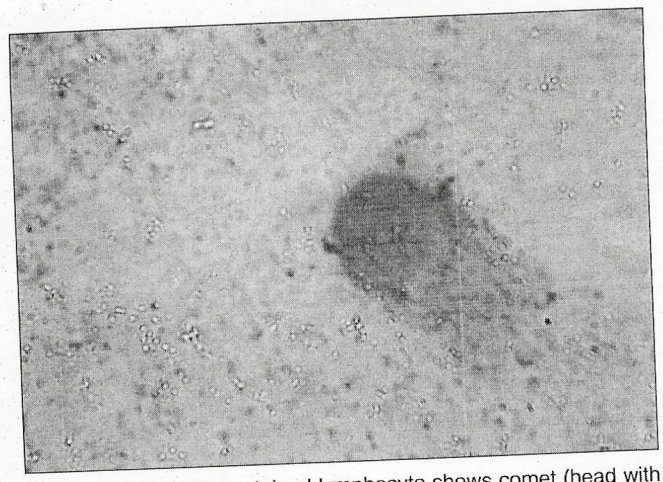

Figure 1: Silver nitrate-stained lymphocyte shows comet (head with tail) appearance of DNA strand breaks of oral squamous cell carcinoma patient

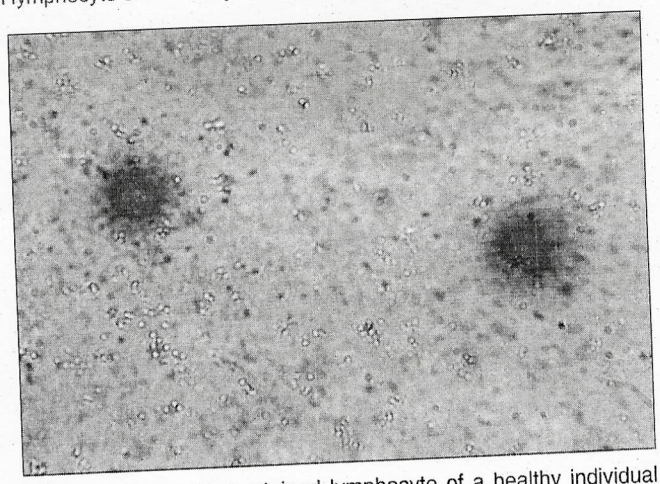

Figure 2: Silver nitrate-stained lymphocyte of a healthy individual shows no tail of DNA strand break

the image (nuclear) diameter were measured using an oculometer fitted to the eyepiece after standardization with a stage micrometer. The DNA tail lengths were measured by subtracting the diameter from the total length.

RESULTS AND OBSERVATIONS

The present study on the basis of clinical staging grouped as 3 cases (6%) of Stage II, 20 cases (40%) of Stage III and 27 cases (54%) of Stage IV. Not a single case was observed in Stage I. Histological grading was done of all 50 patients as 32 cases (64%) of Grade I and 18 cases (36%) of Grade II squamous cell carcinoma. There was no case of Grade III carcinoma.

The DNA damage length was observed in the control group [Figure 2] which was in the range of 0.02–0.36 μm, whereas it varied from 4.04 to 5.84 μm range in squamous cell carcinoma group. The mean DNA damage length observed in the control group was found to be 0.0987 ± 0.0546 μm, whereas in the squamous cell carcinoma group, it was observed to be 4.8992 ± 0.4781 μm, which were statistically significant [Table 1].

In squamous cell carcinoma group, DNA-SSB in different clinical stages was observed as follows. There were no cases in Stage I. In Stage II, the mean DNA damage length was observed 4.600 ± 0.4613 μm, whereas in Stage III and Stage IV, it was observed to be 4.961 ± 0.5620 μm and 4.883 ± 0.410 μm, respectively [Table 1].

The DNA damage length in histological grades of squamous cell carcinoma patients was as follows: The mean DNA damage length in Grade I was 4.6437 ± 0.3061 μm, whereas in Grade II, it was 5.3533 ± 0.3831 μm. There was no case in Grade III squamous cell carcinoma patients [Table 1].

Table 1: Comparison of length of DNA damage in various parameter

Parameter	Number of cases	Mean±SD (μm)	*P*
Clinical stages			
Stage I	Nil	Nil	0.47
Stage II	3	4.600±0.4613	
Stage III	20	4.961±0.5620	
Stage IV	27	4.883±0.410	
Histological grade			
Grade I	32	4.6437±0.3061	<0.0001
Grade II	18	5.3533±0.3831	
Grade III	0	Nil	
Study group			
SCC group	30	4.8992±0.4781	<0.0001
Control group	50	0.0987±0.0546	

SD: Standard deviation, SCC: Squamous cell carcinoma

The difference between mean DNA damage length values of different histological grades was calculated and statistical evaluation was done using an unpaired *t*-test. The DNA damage length was found to increase from Grade I and Grade II, and the difference was extremely statistically significant [$P < 0.0001$; Table 1].

The values of DNA damage length were also compared within different clinical stages of squamous cell carcinoma, the mean difference was calculated and the statistical analysis was done using one-way "ANOVA" test, the values were observed, but the difference was not statistically significant [$P = 0.47$; Table 1] since there was no case in Stage I.

DISCUSSION

Oral cavity cancer is one of the most lethal cancers, ranking as the sixth-leading cause of cancer mortality

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Gupta N, Gupta R, Acharya AK, Patthi B, Goud V, Reddy S, *et al.* Changing trends in oral cancer – A global scenario. Nepal J Epidemiol 2016;6:613-9.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
3. Zini A, Czerninski R, Sgan-Cohen HD. Oral cancer over four decades: Epidemiology, trends, histology, and survival by anatomical sites. J Oral Pathol Med 2010;39:299-305.
4. Sunny L, Yeole BB, Hakama M, Shiri R, Sastry PS, Mathews S, *et al.* Oral cancers in Mumbai, India: A fifteen years perspective with respect to incidence trend and cumulative risk. Asian Pac J Cancer Prev 2004;5:294-300.
5. Abreu LP, Kruger E, Tennant M. Oral cancer in Western Australia, 1982-2006: A retrospective epidemiological study. J Oral Pathol Med 2010;39:376-81.
6. Bettendorf O, Piffkò J, Bànkfalvi A. Prognostic and predictive factors in oral squamous cell cancer: Important tools for planning individual therapy? Oral Oncol 2004;40:110-9.
7. Boyd NM, Reade PC. Mechanisms of carcinogenesis with particular reference to the oral mucosa. J Oral Pathol 1988;17:193-201.
8. Nieburgs HE, Goldberg AF. Changes in polymorphonuclear leukocytes as a manifestation of malignant neoplasia. Cancer 1968;22:35-42.
9. Romsdahl MM, Valaitis J, Mcgrath RG, Mcgrew EA. Cytological changes of megakaryocytes in patients with carcinoma. Acta Cytol 1964;8:343-7.
10. Valverde M, Ostrosky-Wegman P, Rojas E, Fortoul T, Meneses F, Ramírez M, *et al.* The application of single cell gel electrophoresis or Comet assay to human monitoring studies. Salud Publica Mex 1999;41 Suppl 2:S109-13.
11. Lee RF, Steinert S. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutat Res 2003;544:43-64.
12. Taussig MJ. Processes in pathology. In: An Introduction for Students of Medicine. 1st ed. Oxford, London: Blackwell Scientific Publications; 1979.
13. Collins AR, Dusinská M, Gedik CM, Stětina R. Oxidative damage to DNA: Do we have a reliable biomarker? Environ Health Perspect 1996;104 Suppl 3:465-9.
14. Astley SB, Hughes DA, Wright AJ, Elliott RM, Southon S. DNA damage and susceptibility to oxidative damage in lymphocytes: Effects of carotenoids *in vitro* and *in vivo*. Br J Nutr 2004;91:53-61.
15. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, *et al.* Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.
16. Chen SC, Lai YH, Liao CT, Lin CC, Chang JT. Changes of symptoms and depression in oral cavity cancer patients receiving radiation therapy. Oral Oncol 2010;46:509-13.
17. Rao GV, Kumar GS, Ahuja YR. Single cell gel electrophoresis on peripheral blood leukocytes of patients with oral squamous cell carcinoma. J Oral Pathol Med 1997;26:377-80.
18. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184-91.
19. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, *et al.* Single cell gel/comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ Mol Mutagen 2000;35:206-21.
20. Kizilian N, Wilkins RC, Reinhardt P, Ferrarotto C, McLean JR, McNamee JP. Silver-stained comet assay for detection of apoptosis. Biotechniques 1999;27:926-30.

worldwide.[15,16] Majority of new cases of intraoral cancer are discovered only when patients become symptomatic. More than 80% of these cancers are in advanced stages.

Alterations in peripheral blood cells in a diverse range of malignancies are well known. The occurrence of DNA damage in these cells is a recent finding. The increased frequency of DNA-SSB was observed in peripheral blood leukocytes in the patients with precancerous and cancerous lesions of the uterine cervix.[9,17]

Lymphocytes play an important role in defense mechanism. Hence, the above observations lead us to study the DNA-SSB in lymphocytes of OSCC patients. A solitary study has been done in OSCC by Rao *et al.*[17] where DNA damage in peripheral blood leukocytes was examined.

The single-cell gel electrophoresis assay utilizes nucleated cells, which are sandwiched between different agarose layers and subsequently subjected to lytic treatment at high salt concentrations. After electrophoresis under alkaline conditions and staining with fluorescent dyes or silver nitrate, nuclei with damage can be quantified under a microscope using an ocular micrometer.

DNA-specific dyes used for comet visualization depend on largely investigator-specific needs and presumably have little effect on assay sensitivity or reliability.[11] The fluorescent dyes used most frequently are ethidium bromide,[18] propidium iodide, 4, 6-diamidino-2 and phenylindole (Lee and Steinert, 2003),[11] and SYBR Green (Singh *et al.* 1998).[18] The common nonfluoresent staining technique for visualization of commet assey is by silver nitrate staining technique.[19,20] In the present study we used silver nitrate stain due to its simple staining technique and ready availability. The stained slides were observed in monocular microscope in 100X to identify DNA damage.

In the present study, the squamous cell carcinoma cases were distributed in the 3rd, 4th, 5th, 6th and 7th decades. The maximum number of cases were observed to involve alveolar ridge (20 cases) followed by buccal mucosa (15 cases), which was the actual placement of tobacco quid in most of the patients.

In the present study, DNA damage index in peripheral blood lymphocyte of the control group was observed in the range of 0.02–0.36 μm with a mean damage index of 0.0987 ± 0.0546 μm. The study done by Rao *et al.*[17] showed quite similar results in healthy control groups as 0.4900 ± 0.1323.

In the present study, the DNA damage index was evaluated according to histological grades of squamous cell carcinoma. The mean levels observed were 4.643 and 5.353 μm for Grade I and Grade II, respectively. These levels were similar to those obtained by Rao *et al.*[17] which were 4.352 and 5.131 μm for Grade I and Grade II squamous cell carcinoma, respectively. The overall level of DNA damage index was observed to be in the range of 4.04 to 5.84 μm, which was in the range of those obtained by Rao *et al.*[17] which were 3.72 to 6.84 μm.

Another observation in the study of Rao *et al.*[17] was that the DNA damage length increased significantly from Grade I, Grade II and Grade III respectively in squamous cell carcinoma group. Thus, the DNA damage detected by single-cell gel electrophoresis or comet assay correlated well with the histological grading of squamous cell carcinoma. In the present study, similar results were obtained.

The patients were also grouped according to clinical stages. The DNA damage length was observed to be more in Stage III (40961 ± 0.562 μm) as compared to Stage IV (4.883 ± 0.410 μm) and Stage II (4.60 ± 4.61 μm). The results were not in increasing order as in clinical stages. Hence, the difference was not statistically significant. There were no cases in Stage I, and hence, a comparison could be done between I and II and III and IV stages. The results obtained by Venkateswara R *et al.*[17] were highly significant between Stage I and Stage II, Stage I and Stage IV, Stage II and Stage IV and Stage III and Stage IV, while between Stage I and III and II and III, it was not very significant.

CONCLUSION

Genetic instability in the form of DNA damage appears to be associated with an increased relative risk of oral cancer. Measuring single strand break with comet assay is a reliable approach for determining susceptibility and genetic instability in an individual toward cancer. Patients suffering from OSCC show a remarkable difference in the degree of single strand breaks as a result of genetic instability. Control subjects show a constant degree of SSB in comparison with OSCC patients.

Single-cell gel electrophoresis is a simple method to detect single strand breaks in the DNA of individual cells through peripheral blood lymphocyte. OSCC is known to have DNA damage in oral epithelial cells and in peripheral blood leukocytes. Although this study may not enough to demonstrate the progression and prognosis of disease, large population-based studies are required to validate comet assay utility in clinical practice.

INTRODUCTION

Oral cancer globally is a serious growing problem and occupies the sixth most common cancer worldwide.[1-3] New cases of oral cancer exceed 410,000 annually and account for between 1% and 5% of all cancer worldwide, but in India may be as high as 15%–40% of all cancer registered.[4,5] Oral squamous cell carcinoma (OSCC) accounts for 3%–5% of all malignancies. The World Health Organization predicts a continuing worldwide increase in the incidence of oral cancer.[6]

A number of factors contribute to the cause of oral cancer. In country like India where the standard of living is low, oral cancer has the highest rate and is the most common type of cancer in males. Various irritants such as tobacco, (chewed, inhaled, or swallowed) and alcohol are clearly important.

In carcinogenesis, the initial step involves more or less alteration in the DNA of the cell.[7] Malignancy-associated changes were also noted in blood leukocytes, megakaryocytes and monocytes.[8-10]

A variety of methods have been developed for detecting DNA strand damage such as micronuclei, sister-chromatid exchange assay and snapshot of DNA strand break single-cell gel electrophoresis.

Single-cell gel electrophoresis or comet assay is a more recent method to detect DNA single strand breaks (SSB) and alkali-labile sites by measuring the migration of DNA from immobilized nuclear DNA and have an advantage over other methods like it can be done on any eukaryotic cell, economical, simple and fast.[11,12]

The aim of this study was to find out the DNA damage index in peripheral blood lymphocytes in patients with OSCC and to compare the DNA damage index between patients of OSCC and the healthy control group.

MATERIALS AND METHODS

The study consisted of 50 diagnosed patients of OSCC and 30 healthy individuals without any tobacco-related habit with a detailed case history. All participants enrolled with prior consent and detailed case history filters the prerequisite for study.

Under all aseptic conditions, 2 ml of venous blood was drawn from OSCC patients and healthy subjects with the help of a disposable needle and syringe. It was then transferred into sterile glass bottles containing 3.8% sodium citrate, used as an anticoagulant.

The clinical staging of patients suffering from OSCC was done based on site, size and extent of the lesion and involvement of lymph nodes as per the TNM classification given by the American Joint Committee for cancer staging and end result reporting (AJCCS) in 2018. Histopathological grading of squamous cell carcinoma was done as Grade I (well differentiated), Grade II (moderately differentiated) and Grade III (poorly differentiated) according to the malignancy grading system.[13]

Preparation of slides for single-cell gel electrophoresis

Lymphocytes were isolated from the sample of blood by standard centrifugation at 400 × g at room temperature for 30 min with lymphocyte separating medium[14] (HiSep™ LSM 1077).

Lymphocyte cell suspension was added to 140 µl of 0.5% low melting agarose (26°C–30°C).

Plane microscopic slides were covered with 220 µl of 0.5% normal melting agarose (melting point 36°C ± 1.5°C) in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline and immediately covered with a coverslip and placed in the freezer at 4°C for 3 min to solidify. After gently removing the coverslip, 140 µl of 0.5% melting agarose in which blood sample is loaded at 37°C was rapidly pipetted on the first layer of agarose and kept at 4°C for 4 min to solidify. The second layer was overlaid. Similarly, third layer of low melting agarose (140 µl of 0.5%) placed on slide and kept in freezer at 4°C to solidify. Then, the slides were immersed in cold (5°C–8°C) lysing solution (2.5 M Nacl, 100 mM disodium EDTA, 10 mM Tris at pH 10) to which the freshly prepared 1% Titron × 100 and 10% dimethyl sulfoxide were added and the slides were left over night at 4°C.

The slides were then removed from the lysing solution and left in alkaline buffer for 20 min prior to electrophoresis in order to allow unwinding of DNA.

Then, the slides were placed in a horizontal gel electrophoresis system. The tank was filled with fresh electrophoresis buffer (1 mM disodium EDTA and 300 mM NaOH).

After electrophoresis, the slides were washed by flooding with neutralizing buffer (0.4 M Tris, pH 7.5) and left for 10 min so as to neutralize the charged ions. This procedure was repeated twice. After neutralization, slides were stained with silver nitrate.

Cells with DNA damage appeared as comets [Figure 1]. Total image length (nuclear and migrating DNA) and

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ORIGINAL RESEARCH

Association of Premenstrual Syndrome with Adiposity and Nutrient Intake Among Young Indian Women

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Abstract: Premenstrual syndrome (PMS) refers to a heterogenous group of symptoms occurring in luteal phase of the menstrual cycle. Women of childbearing age are affected by PMS, and it may impact their quality of life. Various factors related to the biology of menstruation, hormones, and lifestyle are associated with PMS.

Purpose: To explore the incidence and severity of PMS among students in India and its correlation with nutrient intake, adiposity, and lifestyle factors.

Methods: A semi-structured questionnaire was used to collect data on menstrual pattern, nutrient intake, dietary habits, and physical activity. Moose's Menstrual Distress Questionnaire and Premenstrual Symptoms Screening Tool were employed for the identification and classification of PMS. Anthropometric indices included height, weight, body mass index, waist circumference, hip circumference, waist-to-hip ratio, and four-site skinfold thickness—tricep, bicep, subscapular, and suprailiac.

Results: Of the 330 participants, 71.3% reported to have experienced at least one symptom of PMS. Furthermore, 46.9% had mild PMS, 31.5% had moderate PMS, 8.3% had strong PMS, and 13.3% had no symptoms. Anxiety and irritability were the most observed symptoms. The mean body mass index (BMI) of the participants was within the normal range (21.76 ± 4.81 kg/m^2); however, body fat percentage was above the normal range ($33.95\% \pm 4.89\%$). PMS severity was significantly correlated with body fat percentage and BMI. Nutrient intake was significantly lower than the recommended dietary allowance (RDA), but dietary fat consumption was higher than the RDA. Protein intake was higher in participants with mild PMS than those with moderate and severe PMS ($p<0.05$). An inverse association between oilseed consumption and PMS was observed.

Conclusion: PMS was associated with anthropometric parameters, nutrient intake, and dietary preference. PMS showed correlation with the intake of calorie-rich foods, sweets, and fried salted snacks, whereas consumption of oilseeds alleviated its incidence.

Keywords: premenstrual syndrome, adiposity, dietary habits, nutrient intake, lifestyle factors

Introduction

Premenstrual syndrome (PMS) is a condition in which certain symptoms arise during the late luteal phase of menstruation and disappear when menstruation commences. The World Health Organization (WHO) has classified PMS under the 10th revision of the International Classification of Diseases (ICD-10).[1] This condition can be experienced by any woman of childbearing age. The most experienced symptoms include irritability, anxiety, breast pain, and body aches. The symptoms are well defined and have been studied widely.[2] The American College of Obstetrics and Gynecology has defined PMS as the cyclic occurrence of symptoms that are sufficiently severe to interfere with some aspects of life, and that appear with a consistent and predictable relationship to the menses.[3] Approximately 80–95% of women of childbearing age experience some form of PMS. However, 3–8% of women may experience a severe form of PMS, known as premenstrual dysphoric disorder (PMDD).[4–6] PMDD is an intense form of PMS with predominantly psychological

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symptoms. The symptoms of PMS and PMDD have been well described by many researchers. The interaction of ovarian hormones with brain neurotransmitters could be one of the pathophysiologies.[7] Many experts have opined that PMS is caused by a combination of genetic, hormonal, psychological, dietary, and behavioral factors.[8] Irritability, nervousness or anxiety, mood changes, reduced concentration, and sleep problems are the common psychological signs of PMS. The common physiological symptoms include abdominal bloating, breast pain or tenderness, weight gain, tiredness, appetite changes, and food cravings.[3,4,9] Nutraceutical therapy and other natural approaches have been documented to alleviate the symptoms.[10,11] The inclusion of probiotic sources has been researched for its role in other gynecological diseases, such as polycystic ovarian syndrome (PCOS).[12] Researchers have surveyed the impacts of dietary habits, nutritional supplementation, and lifestyle modifications on PMS.[5,13] Although menstruation is a natural biological phenomenon, it is considered as "unclean" or as a "problem," and several taboos associated with it prevail in many parts of India. Health issues related to the menstrual cycle, such as irregular menses, painful menses, and premenstrual syndrome, cannot be discussed openly under these circumstances. Nutritional status, diverse anthropometric indices indicating adiposity, and several lifestyle factors impact the overall health of women. Moreover, issues linked to the menstrual cycle, including PMS, affect women's health. Most research on PMS has been conducted in the West or Far East, and very few studies have been performed in India. Cultural variations influence the disease phenomena and lifestyle. Therefore, this observational study was aimed at understanding PMS in the context of body composition, adiposity, nutrient intake, and lifestyle.

Materials and Methods

This observational study was conducted among women in the age group of 18–24 years who were studying at the Symbiosis International (Deemed) University. The sample size was determined based on the prevalence from a similar study.[9] For the power of the study to be 0.9 with a 5% level of significance, the sample size calculated was 330 women aged 18–24 years, unmarried, and apparently healthy. Women who were diagnosed with hypertension, diabetes mellitus, PCOS, or any disorder related to systems and glands were excluded. Moreover, women students who were on medications or hormonal therapy, as advised by a medical practitioner, were excluded.

The study was conducted in agreement with the principles indicated in the Declaration of Helsinki. The research proposal and protocol were approval by the Research Advisory Committee and the Independent Ethics Committee of the Symbiosis International (Deemed) University (SIU/IEC/02122015). Written informed consent was obtained from the willing participants prior to their enrolment in the study. The participants were enrolled from July 2018 to August 2019.

A pilot study was performed to assess the reliability and validity of the data collection instruments and semi-structured questionnaires. The pilot study also aided in ensuring the standardization of the adopted techniques. The questionnaire consisted of the following categories:

Sociodemographic Information

This questionnaire was based on the Kuppuswamy's Scale for Socioeconomic Status (SES) in India and included information on age, educational status, place of residence, type of accommodation, and family income.[14] These variables were scored as per the scale, and the participants were grouped into SES categories.

Menstrual History and PMS Assessment

Age at menarche, days of bleeding during the menstrual cycle, interval between two cycles, and problems related to the length of the menstrual cycle were included in the menstrual history. PMS was assessed as per the diagnostic criteria recommended by ICD-10. Rudolf Moose's Menstrual Distress Questionnaire (MDQ) comprising 46 PMS symptoms grouped under eight subscales—pain, water retention, autonomic reaction, negative effect, impaired concentration, behavioral changes, arousal, and control—was used.[15] The symptoms were rated according to their severity in the luteal phase of the menstrual cycle, and the total raw score was calculated. The scores were compared with the intermenstrual symptom scores to confirm PMS. Steiner's Premenstrual Symptoms Screening Tool (PSST) was utilized to categorize the participants based on the severity of PMS symptoms.[16]

Anthropometric Measurements

The participants were instructed to wear light and comfortable clothing for measuring the anthropometric parameters.

Height – A stature meter was used for measuring the height. The participants were made to stand on the footboard of the stature meter without shoes, with their heels, back of knees, buttocks, shoulder blades, and back of head touching the back of the board and the head held in the Frankfurt plane. The height was documented to the closest 0.1 cm.

Weight – Weight was measured using a digital weighing scale. It was ensured that the participants wore light clothing and that the footwear was removed while measuring the weight.

Body mass index (BMI) – BMI was calculated using the Quetlet's formula: BMI = Weight (kg)/Height $(m)^2$, and the participants were classified into BMI categories for Asians.[17]

Waist and hip circumference – For measuring the circumferences, a non-stretchable fiberglass tape was used. The waist circumference is one of the indicators of central obesity, and it was measured at the midpoint between the lower rib cage and iliac crest. The hip circumference was measured at the level of maximum extension of the buttocks while the participant stood upright, with the feet held together. The waist-to-hip ratio (WHR) was calculated as waist circumference (cm) divided by hip circumference (cm).[18] Similarly, waist-to-height ratio (WHtR) was computed as waist circumference (cm) divided by height (cm).

Skinfold thickness (SFT) – The skinfold thickness was measured at four sites: triceps, bicep, subscapular, and suprailiac. SFT was measured using the Harpenden skinfold caliper. First, body density was calculated using the sum of the skinfolds and Durnin and Womersley's equation. Body fat percentages were derived from the body density using Siri's equation.[19,20]

Dietary Habits and Nutrient Intake

A 3-day-diet recall was recorded, including one weekend, which included the consumption of foods and beverages for 3 consecutive days. Time of meal intake, total meals consumed per day, and quantity of each food item were documented. Food Frequency Questionnaire (FFQ) was also used to assess nutrient intake. The questionnaire consisted of 70 food items grouped as fruits, green leafy vegetables, pulses, sprouts, milk and milk products, Indian sweets, confectionaries, fried and savory snacks (common street foods), and fast foods.[21] Macronutrient and micronutrient intake was calculated using the DietCal software version 5.0.

Physical Activity Level (PAL)

Modified International Physical Activity Questionnaire was used to assess the duration of each physical activity per day. Energy expenditure over a period of 24 hours was calculated using the appropriate metabolic equivalent (MET) for each activity. PAL was calculated as the ratio of total energy expenditure (TEE) and basal metabolic rate (BMR).[22]

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software v. 16.0 for MS Windows. The STROBE guidelines were followed to report the analysis. Descriptive statistics was applied to present the data in the form of mean, standard deviation, number of counts, and percentages, as appropriate. Pearson's correlation was employed to assess the linear relationship between the variables, and Spearman rank–order correlation was used for associations between ordinal/categorical and continuous variables. Paired *t*-test and analysis of variance were applied for comparisons between variables. Statistical significance was set at $p < 0.05$.

Results

The mean age of the study participants was 20.08 ± 1.25 years (range: 18–24 years). Of the total participants, 237 (72%) were pursuing undergraduate studies and the remaining 93 (28%) were studying at the postgraduation level. The detailed characteristics of the study participants are presented in Table 1.

The time spent on sedentary activities was 162.2 ± 70.7 minutes/day, moderate activities was 18.01 ± 8.7 minutes/day, and household chores was 33.79 ± 6.15 minutes/day. Significant differences were not seen in the time spent on various

Table 1 Characteristics of the Participants (n = 330)

Characteristics	n (%)
Field of study	
Technology	203 (61.5)
Arts	91 (27.5)
Social sciences	36 (10.9)
Type of accommodation	
University hostel	109 (32.9)
Rented apartment	55 (16.8)
Own house	68 (20.6)
With family	98 (29.6)
Socioeconomic status	
Upper	37 (11.1)
Upper middle	129 (39.3)
Lower middle	121 (36.5)
Lower	43 (13)
Tobacco use/smoking	
Yes	44 (13.3)
No	286 (86.7)
Alcoholic beverages	
Yes	98 (29.6)
No	232 (70.4)
Interval between menstrual cycle	
21 to 35 days	218 (66.1)
< 21 days	55 (16.6)
> 35 days	57 (17.3)
Menstrual problems	
Oligomenorrhea	117 (35.3)
Polymenorrhea	28 (8.5)
Amenorrhea	9 (2.8)
None	176 (53.3)
Type of diet	
Lactovegetarian	99 (29.8)
Ovolactovegetarian	62 (19)
Nonvegetarian	169 (51.2)
Usual mode of transport	
Walking	123 (37.2)
Car/motorbike	183 (55.5)
Bus	19 (5.7)
Cycle	65 (1.7)
Physical activity level	
Sedentary (1.40–1.69)	290 (87.9)
Moderately active (1.70–1.99)	33 (10)
Very active (2.00–2.40)	7 (2.1)

activities among the participants belonging to the three PMS categories. Furthermore, alcohol consumption and smoking were not associated with the occurrence or severity of PMS symptoms.

Menstrual History and PMS

The findings showed that in 66.1% of the participants, the menstrual cycle interval was 21–35 days; 17.3% had longer menstrual cycles, and 16.6% had shorter cycles. The mean age of menarche was 13.0 ± 1.25 years. Moreover, 236 (71.39%) participants experienced at least one PMS symptom. With regard to the MDQ scores, 155 (46.9%) had mild PMS, 104 (31.5%) had moderate PMS, 27 (8.3%) had strong PMS, and 44 (13.3%) participants had no symptoms at all.[15] Table 2 lists the frequencies of PMS symptoms of varied severity.

Table 2 Frequency of PMS Symptoms and Severity (n = 330)

Symptoms	None	Mild	Moderate	Severe
	n (%)			
Psychological symptoms				
Depression/mood swings	45 (13.6)	130 (39.3)	100 (30.3)	55 (16.7)
Hopelessness	123 (37.3)	108 (32.7)	76 (23)	23 (6.9)
Anxiety	95 (28.8)	106 (32.1)	72 (21.8)	57 (17.3)
Anger feeling	34 (10.3)	123 (37.2)	98 (29.7)	75 (22.7)
Irritability/agitated	70 (21.2)	97 (29.4)	101 (30.6)	62 (18.8)
Lack of interest	81 (24.5)	106 (32.1)	98 (29.7)	45 (13.6)
Difficulty concentrating	141 (42.7)	118 (35.7)	48 (14.5)	23 (6.9)
Loss of control	93 (28.2)	102 (20.9)	79 (23.9)	56 (16.9)
Feeling overwhelmed	98 (29.7)	106 (32.1)	87 (26.4)	39 (11.8)
Physiological symptoms				
Lethargy/fatigue	55 (16.7)	105 (31.8)	106 (31.1)	64 (19.4)
Increased appetite	112 (33.9)	85 (25.7)	79 (29.9)	54 (16.4)
Food cravings	75 (22.7)	103 (31.2)	89 (26.9)	63 (19.1)
Sleep problems	99 (30)	84 (25.4)	89 (26.9)	58 (17.6)
Breast tenderness	136 (41.2)	95 (28.8)	67 (20.3)	32 (9.7)
Weight gain	170 (51.5)	79 (23.9)	54 (16.3)	27 (8.2)
Headache	127 (38.5)	116 (35.1)	57 (17.3)	30 (9.1)
Pain (muscle/join/back/abdomen)	69 (20.9)	85 (25.7)	98 (29.7)	78 (23.6)
Acne	91 (27.6)	101 (30.6)	73 (22.1)	65 (19.7)
Behavioral symptoms				
Symptoms interfere with relationship	179 (54.2)	98 (29.7)	37 (11.2)	16 (4.9)
Symptoms interfere with study	193 (58.5)	96 (29.1)	34 (10.3)	7 (2.1)
Symptoms interfere with routine	124 (37.6)	140 (42.4)	50 (15.1)	16 (4.8)

Anthropometric Indices

The mean body fat percentage was significantly different among the three PMS categories of mild, moderate, and severe.[15,16] Similarly, significant differences were noted in weight, BMI, tricep, subscapular, and suprailiac skinfold thicknesses, and a sum of skinfolds. Leaner participants with lower circumferences and skinfold thicknesses experienced milder PMS symptoms compared with those in the severe PMS category. The anthropometric measurements of the participants are described in Table 3.

Figure 1 illustrates the BMI categories of the participants. One third of the participants (132, 39.9%) had normal BMI, 65 (19.9%) were underweight, 85 (25.6%) were overweight, 25 (7.6%) had grade I obesity, and 23 (7.1%) had grade II obesity according to the BMI cutoffs for Asians.[17]

Dietary Habits and Lifestyle Factors

Assessment of the dietary habits revealed that 169 (51.2%) were nonvegetarians, 98 (29.9%) were lactovegetarians, and 63 (19%) were ovolactovegetarians. Significant differences were not observed in the occurrence or severity of PMS across the types of diet consumed. However, the type of diet was negatively correlated with some symptoms of PMS individually, such as decreased interest in home activities ($p < 0.01$), decreased interest in social activities ($p < 0.05$), hypersomnia ($p < 0.05$), and anxiety and fearfulness ($p < 0.05$). This observation indicated that lactovegetarians and ovolactovegetarians participants experienced milder PMS symptoms than those who consumed a nonvegetarian diet.

Figure 2 depicts the actual nutrient intake of the participants with reference to the RDA. The energy consumption was 1494 ± 466 Kcal per day, which was less than the RDA. Similarly, the intakes of proteins, dietary fibers, calcium, iron, vitamin C, and vitamin B12 were significantly deficient. Carbohydrate consumption was slightly higher than the RDA, probably because cereals and millets are the staple foods in India. However, fat consumption was significantly higher

Table 3 Anthropometric Measurements of the Participants (n = 330)

Measurement	Mean (SD)
Height (cm)	155.42±6.20
Weight (Kg)	53.6±11.93*
BMI (Kg/m^2)	21.76±4.81*
Waist circumference (cm)	74.31±10.3
Hip circumference (cm)	95.57±9.52
WHR	0.79±0.64
WHtR	0.47±0.06
Mid upper-arm circumference (cm)	25.59±3.97
Tricep skinfold (mm)	17.60±6.31*
Bicep skinfold (mm)	9.38±4.08
Subscapular skinfold (mm)	22.26±8.24*
Suprailiac skinfold (mm)	33.04±8.56*
Sum of skinfolds (mm)	82.23±24.35*
Body fat percentage (%)	33.36±4.89*

Note: *Significant difference among PMS categories ($p < 0.05$).
Abbreviations: BMI, body mass index; WHR, waist to hip ratio; WHtR, waist to height ratio.

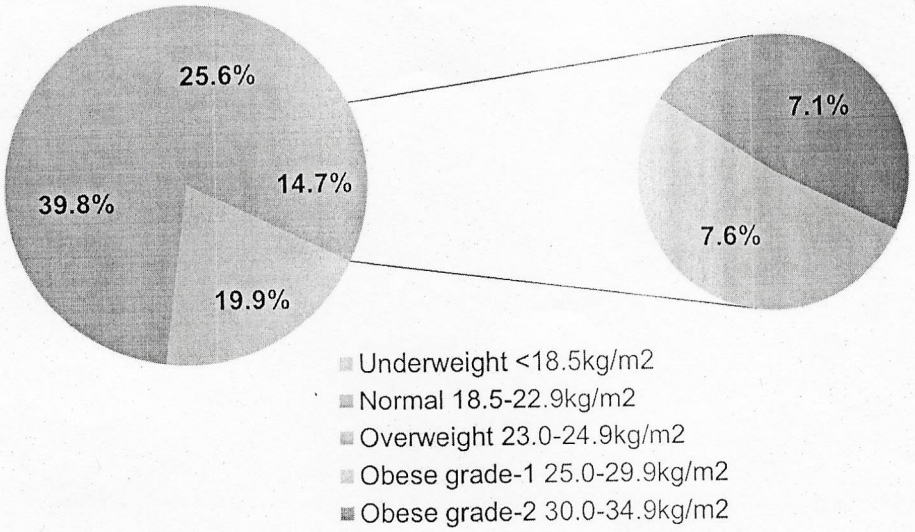

Figure 1 Participants across the BMI categories (n = 330).
Abbreviation: BMI, body mass index.

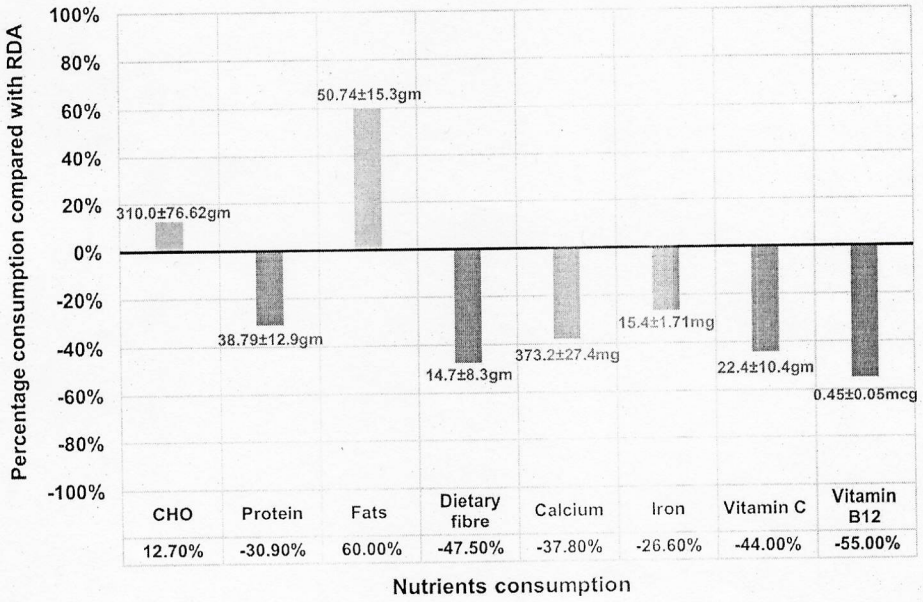

Figure 2 Nutrient intake compared with RDA (n = 330).
Abbreviations: RDA, recommended dietary allowance; CHO, carbohydrates.

than the recommended value ($p < 0.01$). No difference was observed in energy intake between the weekdays and weekends, but the intakes of fat and carbohydrate were significantly higher on the weekend than on the weekdays ($p < 0.01$). Protein intake was significantly higher in participants with mild PMS than in those with moderate and severe PMS ($p < 0.05$). However, in all three categories of PMS, protein intake was less than the RDA.

Table 4 describes the Pearson's correlation, which indicated the significant correlation of PMS with the consumption of sweets, fried savory foods, and fast foods.

Table 4 Correlation Analysis Between Lifestyle/Food Intake and PMS Score (n = 330)

Lifestyle/Food Intake	R value	P value
Indian sweets (mithais)	0.163	0.003**
Chocolates/candies	0.116	0.035*
Fried snacks (samosa/kachori)	0.190	0.001**
Wafers/chips	0.128	0.020*
Fast foods	0.113	0.040*
Instant noodles	0.270	0.001**
Bakery and confectionary	0.180	0.001**
Salads	−0.294	0.001**
Oilseeds (sesame/flax seeds)	−0.117	0.007**
Physical activity level	−0.270	0.001**

Notes: *Significant correlation at the 0.05 level; **significant correlation at the 0.01 level.

Physical Activity Level

Physical activity levels were examined, which included various factors, such as the duration of sleep, screen time, mode of transport to college/institute, time spent on sedentary and moderate activities, and active participation in sports. As seen in Table 1, most of the participants (290, 87.9%) were sedentary, only few (33, 10%) were moderately active, and only a negligible number of women (7, 2.1%) were highly active. The computed PAL showed significant negative correlation across PMS scores ($r = -0.270$, $p < 0.01$), as presented in Table 4. The participants with low PAL displayed high scores in the MDQ subscales.

Discussion

Anxiety, nervousness, irritability, mood swings, and fatigue were the symptoms most frequently reported by the study participants. These findings are consistent with those from other Indian studies in which psychological symptoms were prevalent.[23–25] However, minimal work has been done on the prevalence of PMS in India, particularly in the age group of 18–24 years. In our study, at least 71% of the participants experienced PMS symptoms. Comparable findings have been reported in a study from Thailand involving women of a similar age group.[26] Weight, WHR, and body fat percentage were significantly correlated ($p < 0.01$) with the PMS score of the participants. Parallel findings have been observed in a study from Japan.[27] Body fat percentage was higher than the cutoffs for adiposity even though the mean of BMI was normal. WHR was at the borderline of cutoffs (WHR < 0.08) as per the recommendations of WHO.[18] On the MDQ scale, higher scores were noted for participants with higher body fat. Body fat percentages differed significantly across the PMS categories ($p < 0.05$). Additionally, BMI and the sum of the four skinfold thicknesses were significantly different among the PMS categories. The participants in the mild PMS category had smaller circumferences and skinfolds compared with those in the moderate and severe categories. Leptin, which is synthesized by the adipose tissues, plays a role in the regulation of gonadotropins. Overweight/obese women may have a higher level of leptin because of the greater number of fat cells, which could explain the role of adiposity in PMS. Nurse's Health Study – 2 on PMS and adiposity has predicted a higher risk for PMS with an increase in BMR among women in the USA.[28] These findings have also been supported by work done in Pakistan, Iran, and Korea.[29–34]

Traditionally, exercise is considered to be one of the nonpharmacological treatments for PMS. Many researchers have observed that the frequency of PMS is higher among women with a sedentary lifestyle. In this study too, similar findings were obtained, and the physical activity level was inversely associated with the PMS symptom scores. Physical activity

may alleviate the PMS symptoms via several biological mechanisms, such as secretion of endorphins and improvement in mental and physiological health. Muscle contractions during exercise reduce back pain and pelvic discomfort and ease PMS symptoms by lowering the local concentrations of prostaglandins and other inflammatory substances.[35–37] However, a Japanese study has reported that high-intensity exercises and sedentary activities contribute equally to the risk for PMS.[36] Lifestyle changes and traditional relaxation methods have been shown to reduce pain and enhance the quality of life.[38,39]

The important role of nutrition in the reproductive health of women has a strong theoretical foundation. Various studies have established the association of certain nutrients with PMS and PMDD.[40] A trend of eating street foods, which are generally high in sugars, salt, and oil, is commonly seen among youths. In our study, a significant association was observed between PMS score and the intake of foods high in sugar, fat, and salt. Higher consumption of these foods was linked to higher MDQ scores. These findings are consistent with reports by Houghton et al and Hussein et al, who have shown that the consumption of foods rich in simple carbohydrates and fats was associated with a higher risk for PMS.[41,42] The positive association of the PMS score with high-calorie foods, sweets, and fried and salty snacks noted in our study is consistent with a study on the Iranian population.[43] Participants who were lactovegetarians or ovolactovegetarians reported milder PMS symptoms than those who preferred a nonvegetarian diet. Protein intake is related to PMS via several potential physiological mechanisms. An increased intake of proteins from food sources of animal origin is linked to raised estrogen/estradiol levels, which may be a precursor for certain PMS symptoms. Substitution of proteins with carbohydrates during the luteal phase may increase the risk for the incidence of PMS.[44] Consumption of salads and oilseeds (sesame and flax seeds) is inversely associated with the PMS score. Oilseeds, particularly sesame and flax seeds, are consumed widely in most regions of India in the form of chutneys or dips. Our study is probably the first to describe the inverse association of oilseed consumption with PMS symptoms among Indian women. Nutrient intake and dietary practices play a key role in PMS, which has been supported by findings from various studies. Less incidence and reduced severity of PMS symptoms has been associated with healthy food choices and adherence to a balance diet.[13,45]

Conclusions

The present study has attempted to explore PMS and its determinants among university students, thus emphasizing the prevalence of the condition. Nutrient intake, body composition parameters, and adiposity influence women's health. Anthropometric indices demonstrated that despite normal BMI, body fat percentage and WHR were higher than the recommended values. The studied population showed deficient intake of vital nutrients, such as protein, iron, calcium, and vitamin B12. Lactovegetarians and ovolactovegetarians experienced milder symptoms compared with the nonvegetarians. An association of the severity of PMS symptoms with the consumption of high-calorie foods, sweets, and fried snacks with high sodium was observed, whereas the consumption of oilseeds was linked to a reduced incidence of PMS. These findings highlight the importance of awareness on a balanced diet and healthy lifestyle. The use of oilseeds in the treatment of PMS should be further explored in future studies. Our observations are limited to young women in the age group of 18–24 years. Prospective studies with middle-aged women would add to the existing knowledge. Our findings have established the influence of the analyzed factors on PMS and could serve as a valuable resource to suggest lifestyle modifications as an interventional program to treat PMS in young women.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Yonkers KA, Simoni MK. Premenstrual disorders. *Am J Obstet Gynecol*. 2018;218:68–74. doi:10.1016/j.ajog.2017.05.045
2. Akbarzadeh M, Moshfeghy Z, Dehghani M, et al. Comparison of the effect of Melissa officinalis capsule and care educational programs on the intensity of physical, mental and social symptoms of premenstrual syndrome in high school female students. *Int J Womens Health Reprod Sci*. 2018;16:18–26.
3. American College of Obstetrics and Gynaecology. Premenstrual Syndrome FAQ057; 2011.
4. Bansal M, Goyal M, Yadav S, et al. Premenstrual syndrome-A monthly menace. *Indian J Clin Pract*. 2012;10:491–494.
5. Bendich A. The potential for dietary supplements to reduce premenstrual syndrome (PMS) symptoms. *J Am Coll Nutr*. 2000;19(1):3–12. doi:10.1080/07315724.2000.10718907
6. Biggs WS, Demuth RH. Premenstrual syndrome and premenstrual dysphoric disorder. *Am Fam Physician*. 2011;84(8):918–924.
7. Chocano-Bedoya PO, Manson JE, Hankinson SE, et al. Dietary B vitamin intake and incident premenstrual syndrome. *Am J Clin Nutr*. 2011;93 (5):1080–1086. doi:10.3945/ajcn.110.009530
8. Choudhary A, Mishra J. Effect of 16 weeks yogic intervention in premenstrual syndrome. *Int J Pharm Bio Sci*. 2013;4:207–212.
9. Deuster PA, Adera T, South-Paul J. Biological, social, and behavioral factors associated with premenstrual syndrome. *Arch Fam Med*. 1999;8 (2):122–128. doi:10.1001/archfami.8.2.122
10. De Franciscis P, Conte A, Schiattarella A, et al. Non-hormonal treatments for menopausal symptoms and sleep disturbances: a comparison between purified pollen extracts and soy isoflavones. *Curr Pharm Des*. 2020;26(35):4509–4514. doi:10.2174/1381612826666200721002022
11. De Franciscis P, Colacurci N, Riemma G, et al. A nutraceutical approach to menopausal complaints. *Medicina*. 2019;55(9):1–17. doi:10.3390/medicina55090544
12. Schiattarella A, Riemma G, La Verde M, et al. Polycystic ovary syndrome and probiotics: a natural approach to an inflammatory disease. *Curr Womens Health Rev*. 2021;17(1):14–20. doi:10.2174/1573404816999200601162506
13. Gold EB, Bair Y, Block G, et al. Diet and lifestyle factors associated with premenstrual symptoms in a racially diverse community sample: Study of Women's Health Across the Nation (SWAN). *J Womens Health*. 2007;16(5):641–656. doi:10.1089/jwh.2006.0202
14. Gururaj M. Kuppuswamy's socio-economic status scale–A revision of income parameter for 2014. *Int J Recent Trends Sci Technol*. 2014;11:1–2.
15. Moos RH. The development of a menstrual distress questionnaire. *Psychosom Med*. 1968;30(6):853–867. doi:10.1097/00006842-196811000-00006
16. Steiner M, Macdougall M, Brown E. The premenstrual symptoms screening tool (PSST) for clinicians. *Arch Womens Ment Health*. 2003;6 (3):203–209. doi:10.1007/s00737-003-0018-4
17. Consultation WE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*. 2004;363 (9403):157–163. doi:10.1016/S0140-6736(03)15268-3
18. World Health Organization. Waist circumference and waist-hip ratio: report of a WHO expert consultation. Geneva; 8–11 December, 2008.
19. Durnin JV, Rahaman MM. The assessment of the amount of fat in the human body from measurements of skinfold thickness. *Br J Nutr*. 1967;21 (3):681–689. doi:10.1079/BJN19670070
20. Kuriyan R. Body composition techniques. *Indian J Med Res*. 2018;148(5):648–658. doi:10.4103/ijmr.IJMR_1777_18
21. Longvah T, Anantan I, Bhaskarachary K, Venkaiah K, Longvah T Indian food composition tables. Hyderabad: national Institute of Nutrition. Indian Council of Medical Research. 2017.
22. Anjana RM, Sudha V, Lakshmipriya N, et al. Reliability and validity of a new physical activity questionnaire for India. *Int J Behav Nutr Phys Act*. 2015;12(1):1–2. doi:10.1186/s12966-015-0196-2
23. Anandha Lakshmi S, Saraswathi I, Saravanan A, et al. Prevalence of premenstrual syndrome and dysmenorrhoea among female medical students and its association with college absenteeism. *Int J Biol Med Res*. 2011;2:1011–1016.
24. Brahmbhatt S, Sattigeri BM, Shah H, et al. A prospective survey study on premenstrual syndrome in young and middle aged women with an emphasis on its management. *Int J Res Med Sci*. 2013;1(2):69–72. doi:10.5455/2320-6012.ijrms20130506
25. Garg P, Matreja PS, Khosla PP, et al. Correlation of symptoms of premenstrual syndrome in Indian set-up. *Am J Health Res*. 2014;2(3):102–105. doi:10.11648/j.ajhr.20140203.15
26. Buddhabunyakan N, Kaewrudee S, Chongsomchai C, et al. Premenstrual syndrome (PMS) among high school students. *Int J Womens Health*. 2017;9:501–505. doi:10.2147/IJWH.S140679
27. Takeda T, Tasaka K, Sakata M, et al. Prevalence of premenstrual syndrome and premenstrual dysphoric disorder in Japanese women. *Arch Womens Ment Health*. 2006;9(4):209–212. doi:10.1007/s00737-006-0137-9
28. Bertone-Johnson ER, Hankinson SE, Willett WC, et al. Adiposity and the development of premenstrual syndrome. *J Womens Health*. 2010;19 (11):1955–1962. doi:10.1089/jwh.2010.2128
29. Tabassum S, Afridi B, Aman Z, et al. Premenstrual syndrome: frequency and severity in young college girls. *Anxiety*. 2005;45:4–5.
30. Tariq N, Hashim MJ, Jaffery T, et al. Impact and healthcare-seeking behaviour of premenstrual symptoms and dysmenorrhoea. *Br J Med Prac*. 2009;2:40–43.
31. Song JE, Chae HJ, Jang WH, et al. The relationship between life style, menstrual attitude and premenstrual syndrome in nursing students. *Korean J Women Health Nurs*. 2013;19(2):119–128. doi:10.4069/kjwhn.2013.19.2.119
32. Masho SW, Adera T, South-Paul J. Obesity as a risk factor for premenstrual syndrome. *J Psychosom Obstet Gynaecol*. 2005;26(1):33–39. doi:10.1080/01443610400023049
33. Ashfaq R, Jabeen S. Association between the prevalence of premenstrual syndrome and weight status of adolescent girls (11–21 years). *Adv Obes Weight Manag Control*. 2017;6:1–4.
34. Shahrjooye Haghighi E, Koushkie Jahromi M. The correlation between some body composition indices and premenstrual syndrome in young females. *Womens Health Bull*. 2019;6:1–4.

35. Arruda LM, Martins LS, Moreira TR. Assessment of the symptoms of premenstrual syndrome in physically active and sedentary adult women. *J Nutr Therapeut.* 2016;5:93–102.
36. Morino S, Egawa M, Hirata H, et al. Association between premenstrual syndrome and daily physical activity levels. *J Womens Health.* 2016;5:1–4.
37. Nam SJ, Cha C. Effects of a social-media-based support on premenstrual syndrome and physical activity among female university students in South Korea. *J Psychosom Obstet Gynaecol.* 2020;41:47–53.
38. Doğan H, Eroğlu S, Akbayrak T. The effect of kinesio taping and lifestyle changes on pain, body awareness and quality of life in primary dysmenorrhea. *Complement Ther Clin Pract.* 2020;39:101120. doi:10.1016/j.ctcp.2020.101120
39. Doğan H, Caltekin MD, Taylan ON, et al. Approaches of dealing with primary dysmenorrhea and relationship between kinesiophobia and pain severity. *Konuralp Med J.* 2020;12(3):551–556.
40. Chalasani S, Ortayli N. Reproductive health and nutrition. In: *Nutrition and Health in a Developing World.* Cham: Humana Press; 2017:469–485.
41. Houghton SC, Manson JE, Whitcomb BW, et al. Carbohydrate and fiber intake and the risk of premenstrual syndrome. *Eur J Clin Nutr.* 2018;72 (6):861–870. doi:10.1038/s41430-017-0076-8
42. Hussein RA, Hafiz M, Bin-Afif S, et al. Premenstrual syndrome prevalence, and correlation with carbohydrate intake in young women. *Health Med.* 2012;6:774–780.
43. Hashim MS, Obaideen AA, Jahrami HA, et al. Premenstrual syndrome is associated with dietary and lifestyle behaviors among university students: a cross-sectional study from Sharjah, UAE. *Nutrients.* 2019;11(8):1–18. doi:10.3390/nu11081939
44. Houghton SC, Manson JE, Whitcomb BW, et al. Protein intake and the risk of premenstrual syndrome. *Public Health Nutr.* 2019;22 (10):1762–1769. doi:10.1017/S1368980018004019
45. Yonkers KA, O'Brien PS, Eriksson E. Premenstrual syndrome. *Lancet.* 2008;371(9619):1200–1210. doi:10.1016/S0140-6736(08)60527-9

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REVIEW ARTICLE

Plant based proteins: Sustainable alternatives

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Abstract

Proteins can be procured from both plants and animals. Plant proteins are more preferable as the animal proteins may cause adverse health effects to human life. Proteins derived from plant sources are less expensive and hence are more cost effective. Quality of proteins relies on several factors and biological value is one such factor. Proteins with major essential amino acid are with high biological value. Every plant source is deficient in one or more essential amino acids so it is recommended to include multiple plant-based diets. Also proteins obtained from plant sources are less palatable so it is important to add flavor in order to make it more palatable. The quality and quantity of the proteins also depend on the techniques used for isolation and purification of proteins. Elucidation of the structure of proteins involves the use of techniques like nuclear magnetic resonance, X-ray crystallography and spectroscopy. Apart from the structural analysis the functioning of the protein could be determined by amino acid sequencing which could be performed using mass spectroscopy. Ultrasound assisted extraction, enzyme assisted protein extraction and electro activation method are few of the isolation and purification method which can be used in isolation and purification of these proteins. Owing to the vast availability of plant-based proteins it has various industrial applications like, plant based protein can be used as a dairy substitute, plant based meat analogue and its use as bioactive peptides which have been briefly discussed in the review.

Keywords

Biological value, essential amino acids, legumes, plant proteins, pseudocereals

Introduction

With the rise in population there is increase in demand of food supply. In such condition fulfilling the nutritional requirement not just involves the adequate calorie intake but also intake but also intake of proper macronutrient. Proteins are one of the important macronutrient, however the production of proteins on such largescale is of major concern. Proteins derived from animal source are considered to be of superior quality, but the resources required to sustain the continuous requirement is not adequate (1) In order to overcome this problem finding an alternative protein source as become necessary. Plant based proteins represent a promising solution as it could be cultivated easily at lower productivity cost and are also environmentally sustainable.

The quality of the protein depends on its constituent amino acids

and the human body contain 20 amino acids which are further classified as essential and nonessential amino acids. Nonessential amino acids, are synthesized in our body and are not required essentially through the external diet; which includes alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine, and tyrosine. On the other hand, essential amino acids are not made by our body and should be supplied through the external diet. This group includes histidine, leucine, isoleucine, valine, lysine, phenylalanine, methionine, threonine and tryptophan (2).

Table 1. Comparison between plant and animal source of protein

Animal protein	Plant protein	Reference
Less environmentally sustainable	Environmentally sustainable	(5)
Negative health impacts as it can cause various health hazards	Positive health impacts as it provide greater food safety	(6)
High cost due to which it is not affordable	Low cost easily affordable	(7)
Rich in all essential amino acids and provide complete protein	Deficient in essential amino acids as it provide incomplete protein	(6)
It consists of saturated fat and may contain harmful toxins and low in antioxidants	It consist of unsaturated fats and fiber also rich in various antioxidants and useful bioactive compounds and minerals	(8)

The nutritive value of protein depends on their amino acid content, bioavailability, purity, digestibility, antinutritional factor (substances that when present affects the availability of proteins by themselves or by some metabolic reactions) etc. Animal protein such as egg, meat, poultry, milk and fish contain complete and high quality proteins as they provide all the essential amino acids. However, they also include fats and cholesterol which are threat to human health and may cause heart disease, high blood pressure, stroke etc (3). Consuming more animal proteins are also associated with depletion of natural resources, damage to biodiversity, climate crisis, freshwater depletion etc (3). Therefore, there is shift towards plant-based proteins in recent times. However, these proteins are deficient in one or more essential amino acids.

Replacing plant proteins with animal proteins put forth various challenges. It is very important for protein content and quality should be intact while it is undergoing various processing. The cost of processing and raw material should be affordable. Plant based protein food should be tasteful flavors can be added to make it palatable. It should be easily digestible and available. The effects of anti-nutrients and allergens should be minimized (4).

Individuals who depend completely on plant proteins should have variation in their plant based diet i.e., they should have all types of plant diet which include legumes, cereals, seeds, nuts, variety of fruits and vegetables etc. to meet their protein demands. Plant proteins provide excellent source of protein with reduced fats and cholesterol.

As the world population is growing day by day and to provide safe and nutritious food to the current population without having any negative impact to the environment and maintain healthy ecosystem, it is important to replace traditional animal proteins and develop proteins with better digestibility and bioavailability. Plant proteins are inferior to animal proteins and are often recognized as incomplete. Table 1. Shows comparison between plant and animal sources of proteins. Therefore, protein products are developed with the help of advanced techniques by blending various plant proteins. While developing new formulations of protein, it is important to note that the protein should remain stable and active during transportation, storage and administration inside the human body, as these proteins are prone to degradation (3).

Biological value

There are various approaches to determine the quality of protein one such approach is to determine its biological value. Protein quality or its biological value relies on the amount of essential amino acids consumed through diet (9). Biological value of protein is defined on the basis of number of amino acids present in the particular protein sources that can be digested, absorbed and are utilized by the body to form new proteins (10).

Proteins of superior quality are those which have the most essential amino acid content in them i.e., they are with high biological value whereas proteins of inferior quality have low biological value since they lack one or more amino acids especially the essential amino acids (11). Table 1 represents a summary of biological value of important sources of proteins.

The biological value of protein is calculated by estimating the amount of nitrogen consumed and eliminated by the body as nitrogen is the main component of amino acids. Experimental animals with 2 dietary condition groups are considered; one by feeding the nitrogen free diet and the test sample are kept on protein diet. The amount of nitrogen lost in urine and fecal matter are calculated in both the groups.

The number of amino acids absorbed and retained by the body represents the biological value. Through the formula given below, the biological value of plant or animal sources can be calculated (12). Table 2 gives information about biological value of some plants and animal sources.

$$\text{Biological Value (BV)} = \frac{\text{retained N}}{\text{absorbed N}} = \frac{[\text{N intake}] - [\Delta \text{ fecal N}] - (\Delta \text{ urinary N})}{[\text{N intake}] - (\Delta \text{ fecal N})} \quad \text{.....Eqn.1}$$

(where N = nitrogen content)

Table 2. Biological value of various protein sources (12)

Sources of Protein	Biological value
Egg	94
Fish	76
Beef	74
Sunflower seed	70
Casein	80
Oats	65
Rice	65
Peanuts	55
Soybeans	73
Wheat	65
Maize	59
Lentils	45

There are some other methods by which protein quality can be assessed such as net protein utilization (NPU) and protein efficiency rate (PER). Some researchers find NPU more suitable as it also considers the digestibility of the protein. In case of PER, it is assumed that all the protein are used for growth.

Sources of proteins

Animals, microorganisms and plants are the different sources of proteins. Major animal proteins include milk, egg, poultry, meat and fish which provide complete proteins with major essential amino acids. Egg proteins are divided among yolks and egg white. They also contains fats and cholesterol due to which it is considered as unhealthy and its consumption is declining over a past few decades especially for elderly people (13). Milk proteins are classified in to 2 major proteins i.e. whey and casein both are complete protein and also contain minerals like calcium and phosphorus (14). Meat proteins are associated with fatty acids mostly saturated fatty acids as well as cholesterol. There are many health-related problems associated with meat proteins. Heart disorders due to high cholesterol and fat, type-2 diabetes, bone health due to absorption of sulphur containing amino acids in animal proteins, high animal protein leads to more urinary excretion of calcium which affects bone density and leads to osteoporosis, high blood pressure etc (15). Fishes are rich source of proteins and also contains polyunsaturated fatty acids mainly omega-3 fatty acids; vitamins; minerals like calcium, zinc and iron. However, due to bioaccumulation of heavy metal like mercury, lead, nickel etc. fishes have become a matter of concern for human health (16).

Major plant protein sources

Legumes

Legumes belong to the family *Leguminosae*. They play an important role in human diet due to their rich protein content as well as certain minerals, vitamins and calories. Legumes are considered as poor man's meat as they are rich source of protein at low cost (17,18). Pulses which belong to the family of legumes are major contributors of protein in African and Asian diets. Some common pulses shown in Fig 1. include beans, peas, pigeon peas, chickpeas, peanuts (ground nuts), faba beans, soybeans, lentils, mung, kidney beans (also known as common or dry beans), cowpeas, and black gram (19).

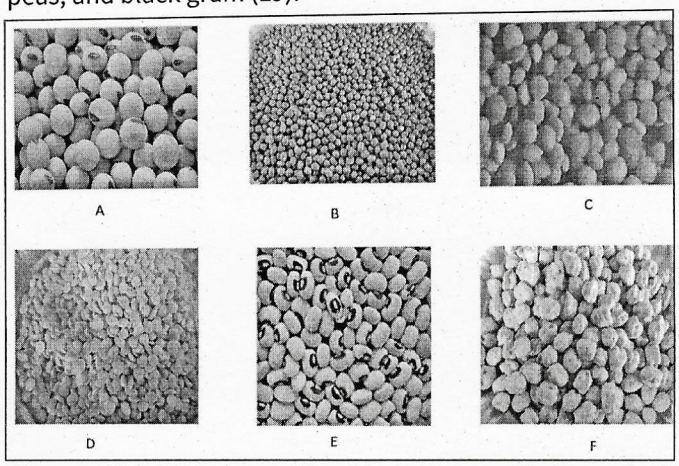

Fig. 1. Lentils and legumes: The given figure represents different sources of lentils and legumes. (**A**) Soy beans; (**B**) Green gram beans; (**C**) Red lentil; (**D**) Bengal gram; (**E**) Cow peas; (**F**) Chickpeas.

Soy proteins

Soy beans are legumes grown as pulses and for extracting oils. Soy beans contain approximately 34 to 37% of protein which is highest in comparison to other cereals and legumes. They also contain carbohydrates and dietary fibre. Human consumption needs a little more processing after which various soy products are manufactured, which includes soy milk for kids, soy flour, soy concentrates, tofu and soy isolates. Soy proteins include storage proteins like glycinin and β-conglycinin which contain most of the essential amino acids but low in sulfur containing amino acids like i.e., cysteine and methionine. Other minor protein includes lectins, lipooxygenase etc. but these proteins are removed as they may disturb the nutritional quality of proteins and also affects the taste (20).

Lentils

Lentils are the legumes belongs to the *Leguminosae* family. They are called "lentil" due to their lens shaped structure. Various types of lentils are available in market for consumption. Some of the very commonly used are green gram beans, red lentils, yellow pigeon pea, green and white peas, bengal gram, black gram etc. Lentils are rich in essential amino acids like phenylalanine, leucine, threonine, lysine, but contain low amount of sulfur containing amino acids like cysteine and methionine. Lentils also contain minerals like iron, potassium, phosphorus, zinc etc. They are also rich sources of vitamin B. Lentils are often consumed with cereals as they make complete protein source together (21).

Cereals

Cereals are edible seeds, which are often referred as grains and are belonging to grass family Gramineae. Cereals shown in Fig. 2. are staple food that provide nutrients like starch, proteins, vitamins and minerals. Wheat, barley, maize, rice, oat etc. are some of the major cereals.

Rice is the major staple food consumed in major

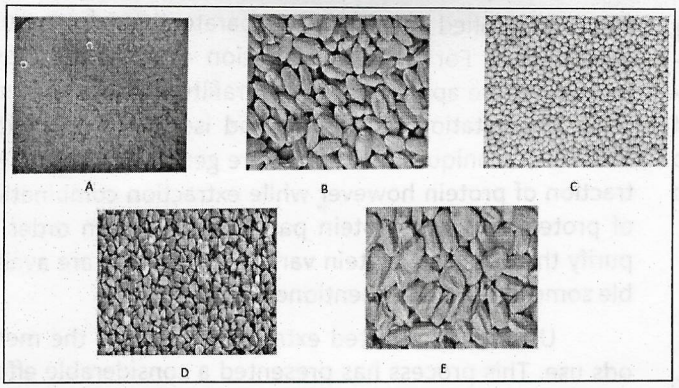

Fig. 2 Cereals and Pseudocereals: The given figure represents different cereals and Pseudocereals (**A**) Rice; (**B**) Wheat; (**C**) Amaranth; (**D**) Buckwheat; (**E**) Maize.

part of the world and especially in Asian countries. In market rice is available in 2 forms: brown rice and white rice. Various storage proteins are present in rice like albumin, globulin, gluteins and prolamins. Brown rice is comparatively more nutritious than white rice as it contains higher content of cereals, proteins minerals and vitamins. Aleuron layer and germ gets removed from brown rice due to polishing and provide us with white rice which contains low proteins, vitamins, minerals and large amount of fibre as compared with brown rice. The amino acid composition of prolamins include alanine, glutamic acid, arginine, hydrophobic amino acids like glutamine, valine and leucine but low sulphur containing amino acids and also deficit in lysine. Both gluteins and globulins are rich in sulphur containing amino acids with disulphide cross-linkage (22).

Another important cereal is wheat which is consumed in the form of chapati, bread, noodles, pasta etc (23). Gluten is the main storage protein present in wheat other proteins present are albumin, gliadins, globulin. Lysine is present in lower content but other sulphur containing amino acids are leucine, valine and isoleucine. L- glutamine is the readily available essential amino acid, which helps in immunity boost up and are considered important for athletes (24).

Pseudo cereals are gluten free and are recommended for patients with celiac disease and are used as food for infants. Pseudo cereals includes amaranth, buckwheat, quinoa. Pseudo cereals are rich in their protein content with high essential amino acids (25). Amaranth has the highest source of protein among all pseudo cereals i.e. approximately 13 to 16% whereas buckwheat contains 11 to 19 % and quinoa 12 to 14 %. Amaranth is rich in essential amino acids like methionine, arginine, cysteine, lysine, tryptophan as compared with cereals (26). Quinoa contain high amount of methionine, cysteine and lysine and are deficit in aromatic amino acids like tyrosine and phenylalanine. Buckwheat are nutritionally superior than cereals. It contains cereals like glutamic acid, arginine, lysine and aspartic acid but are limiting in amino acids like threonine, methionine, cysteine, phenylalanine and tyrosine (27). Buckwheat is unsuitable as staple food due to its low nutritional value but it can be have in combination with other sources to compensate the amino acids content (25).

Seeds and nuts as protein source

Peanuts

Peanuts are considered as highest source of plant based proteins. They also contain other nutrients like carbohydrates, fats, vitamins and minerals. Carbohydrates with high dietary fiber and monounsaturated and polyunsaturated fatty acids like omega fatty acids are present. Though peanuts contain all the major essential amino acids the other amino acids can get supplemented by including other plant based diets (28).

Flax seeds

Flax seeds are rich in proteins and oil content which consists approximately 73% of PUFA and 9% of saturated fatty acids. Flax seed protein includes amino acids like aspartate, glutamic acid, glutamine, asparagine whereas lysine, threonine and tyrosine are limiting amino acids of flax seeds (29).

Chia seeds

Chia seeds include approximately 25 to 41% carbs, 14 to 25% of protein, and 25 to 40% of fat. Chia seeds includes globulin as higher protein fraction and also contain aromatic and sulfur containing amino acids. Amino acids composition of chia protein includes aspartic acid, glutamic acid, threonine, histidine, leucine, isoleucine, lysine, valine, methionine, tryptophan, cysteine and phenylalanine. Amino acids present in chia seeds plays an important role in metabolic pathways i.e. plays an important role in hormonal regulation, immunity boost up, sulfur containing amino acids helps in maintaining structure of proteins, protection from cardiovascular disease (30).

Canola seeds

Canola seed occupies second rank in oil extraction after soybean. They are also rich in protein and provide a beneficial food source due to high levels of essential amino acids and sulfur based amino acids. All the essential amino acids are present but due to various processing method during oil extraction and increased temperature lysine contents gets reduced.

Various other seed and nuts rich in proteins include sunflower seeds, pumpkin seeds, cashews, almonds etc. Fig. 3. represents some of the seeds and nuts. Researchers are still looking for various plant-based protein sources

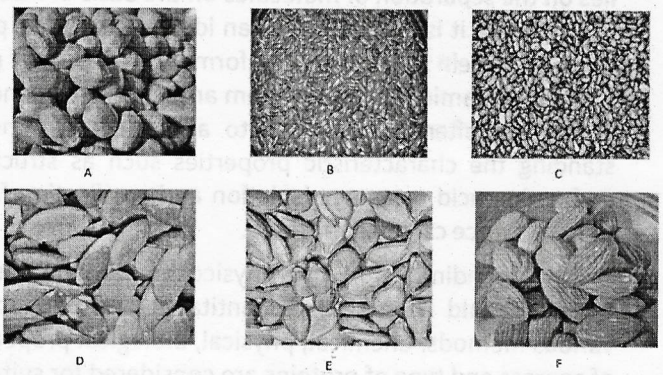

Fig. 3. Seeds and Nuts: The following are the seeds and nuts as source of plant based proteins (**A**) Peanuts; (**B**) Flax seeds; (**C**) Chia seeds; (**D**) Sunflower seeds; (**E**) Pumpkin seeds; (**F**) Almonds.

and their composition to meet the demand of growing world population. Processing methods performed to obtain plant based products also affects the quality of protein. Therefore, though plant protein contains majority of amino acids they are still deficient in one or more amino acid so it is recommended to include multiple plant based diets (31).

Protein alternatives as supplements

Animal based food products are considered to be the conventional source of proteins. Meat makes up a considerable portion of diet over the globe. However various health concerns have led to replacement of these animal based proteins with plant based proteins. Table 3. Given below mentions various plant protein based alternative along with the advantage and disadvantage.

Table 3. List of plant based protein alternatives

Product	Plant based alternative	Advantages	Disadvantages	Reference
Cheese	Protein from peanut, soy, vegetable fat	Good potential to lower the lipid profile Low cost of production	Amount of nutritional content may be low	(32)
Egg	Proteins from flax seed, pea and sunflower seed	Functional properties (emulsification, foaming) similar to egg Low fat content	Contains anti nutritional substance	(33)
Milk	Soy, almonds, coconut	Efficient for digestive disorder (lactose intolerance), Ideal as vegan source of milk	Less palatable if not flavoured	(34)
Microalgae	*Chlorella* sp. *Spirulina* sp.	Requires less land High protein content	Microalgae produced through genetic modification can have regulatory issues.	(35)

Tremendous research has been carried out in order to obtain plant based products which is palatable nutritious and most importantly has texture identical to meat. Consumers demand products that are sustainable, palatable, safe, nutritious, available and affordable.

Isolation and purification of plant based proteins

In order to understand the functionality of protein it is important to know the amino acid sequence and structure of particular protein. Methods like nuclear magnetic resonance, X-ray crystallography and spectroscopy are widely used for analysis of 3D structure of proteins. Apart from knowing the structure of protein it is also important to know the amino acid sequence. As mass spectroscopy relies on the separation of molecules on the basis of mass to charge ratio, it is considered as an ideal method for, peptide and protein sequencing, conformation analysis of protein and dynamics (36). Apart from amino acid sequencing is selected afterward taking into account. After understanding the characteristic properties such as structure and amino acid sequence isolation and purification from desired source can be performed.

Depending upon the physicochemical properties proteins could be isolated, quantitated and purified by various methods. Chemical, physical, biological properties of sources and type of proteins are considered for suitable isolation methods (36). While using these techniques various parameters like temperature, pH and type of solvent need to be carefully controlled (37). Proteins after isolation must be purified in order to separate them from non-protein part. For better purification of protein various techniques are applied such as ultrafiltration, dialysis, micellar precipitation techniques and isoelectric precipitation. The techniques mentioned are generally used for extraction of protein however while extraction combination of protein and non-protein part is obtained. In order to purify the obtained protein various techniques are available some of which are mentioned below (38).

Ultrasound assisted extraction is one of the methods use. This process has presented a considerable effect on the rate of different method in the food industry (39). The advantage of ultrasound assisted extraction has been reported earlier wherein they have shown that UAE in combination with micelles is effective in increasing the yield of wheat germ protein. Along with the increase in extraction, the overall time required was also reduced (40). In UAE milled soybean slurry used was subjected to ultrasound using laboratory probe for various time interval 0, 0.5, 1, 5 and 15 min. From this it was found that 1 min treatment gave approximately 10% improved yield of proteins. Further studies done by using Confocal laser scanning microscopy revealed the effect of ultrasound which showed the presence of undisrupted intact cells. From this it was found that the improved extraction of soya proteins was due to improved solubility. Thus, ultrasound assisted extraction is considered to be a reliable method because it gave improved yield in short time interval with lower energy consumption (40).

Solvent extraction is mostly used in initial step for separation and extraction of proteins. Various solvents could be employed for this purpose. Alcohol extraction method used for extraction of zein from maize (41). On commercial scale zein is extracted from corn gluten meal in small amount. 70% ethanol is used for extraction of zein which was then diluted using 40% ethanol and subjected to centrifugation. About 70-80 % protein was extracted. It also allowed removal of other components prior to dilution of extract (42).

Peanuts are found to have a wide industrial application as they have a superior content of oil and protein. But separation and isolation of oil and protein could be a tedious process. In order to separate the protein from the peanut seed enzyme assisted protein extraction technique was can be used. Through the use of various proteases this

plants as source of proteins has now led to their use in various field. The application of plant-based protein is depicted in Fig. 4. Given below.

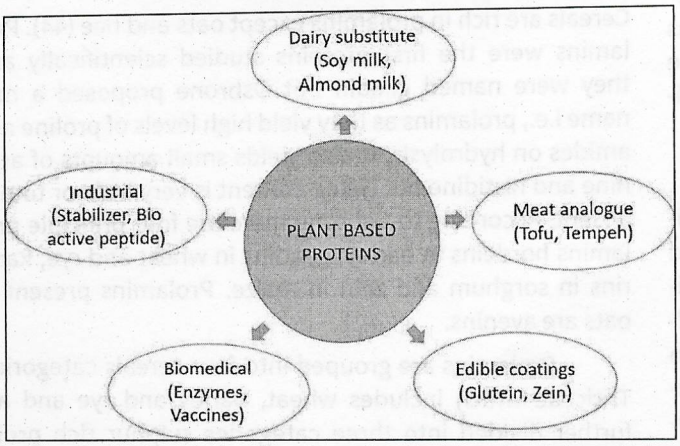

Fig. 4. Application of plant based proteins: Plant based protein could be beneficial means asa protein source.

Application of plant based protein in biomedical industry

Emergence of plant-based proteins has led to their commercialization and its production at industrial scale. The emergence of various molecular biology techniques has proved very useful in production of recombination proteins using plants. Monoclonal antibodies is one such products. As per one study, it has shown that the use of monoclonal antibodies produced from plants which has their use as immunotherapeutic agent for cancer (45). The production of monoclonal antibodies for various viral diseases such as rabies is possible using plant systems. Apart from monoclonal antibodies various other pharmaceutical products such as enzymes, hormones, vaccines etc. could be produced and considered to be of prime importance (46).

Neem leaf glycoprotein (NLGP) is an active ingredient present in neem leaf extract. NLGP enhance immune system in tumor bearing host by increasing amount of various immune cells like T helper cells, cytotoxic T cell, macrophage, monocyte, dendritic cell etc. (47). In cancer cells there are various suppressor like Tregs, myeloid derived suppressor cells (MDSCs) and tumor associated macrophages (TAMs) impairs the immune functions. Function of such suppressor cells is amplified in cancer cells (48). NLGP upregulates the immune system by downregulating the suppressor cells.

HIF1α regulates vascular endothelial growth factor (VEGF). VEGF is important for angiogenesis in cancer as it fulfills the nutrient and oxygen requirement for the cancer development and growth. NLGP reduces VEGF secretion by preventing the binding of HIF1α to VEGF. NLGP shows antimetastaic role by inhibiting VEGF by reducing angiogenesis (49).

Plant based protein as edible coatings

Plant based proteins are also used as edible films. Environmental concerns have led to replacement of coating material like plastic with edible plant protein based film. Plant protein based films provided an option of using a renewable resource. Various cereals, pulses could be used for production of edible films example soya, wheat etc. Gluten protein present in wheat is found to have high elastic properties which is used as a film or coating (50). Apart from gluten various other protein sources like zein, rice bran protein etc can also be used. Zein has proved to show excellent result as edible film due to its hydrophobic nature and higher amount of disulfate and hydrogen linkage.

Application of plant based protein as dairy substitute

Increased population and demand of food has led to consumption of plants as protein source which has various health effects (51). Plant based proteins could be used as an alternate source in disease and disorders Lactose intolerance is one of the increasing disorders which involves inability of an individual to digest milk due to lack of enzymes lactase (52) . Plant sources are thus used to obtain analogues of milk known as plant based non-dairy milk. Also, the milk obtained from plant source could be subjected to fortification with vitamins, minerals etc. Apart from lactose intolerance various diseases like chronic kidney disease could be cured by addition of plant-based proteins in diet (53). Including plant protein in various diet is nutritionally adequate and have pleiotropic effects which may be useful for chronic kidney disease patient (54).

Plant based protein as meat analogue

Plant-based meat alternatives (PBMA) have been used as a substitute to conventional animal based meat product. These meat analogues are derived through processing of different legumes, vegetable and fruits, examples of different plant based meat analogues are mentioned in the Fig. 4. (55). Vegetarianism and Veganism are the phenomenon which though sound similar but has a minor difference. Vegetarianisms is exclusion of animal meat (56). Whereas Veganism is referred to as complete exclusion of animal meat as well animal based products like milk. With the exclusion of animal and other non veg source of protein plant based protein is considered to be the most appropriate source of protein. A vegan diet is considered to have various health related benefits which is one of the reasons in global increase in trend of vegan diets.

Other industrial application of plant based proteins

The advances in food technology and search for renewable source of food material have led to the development of alteranative sources. Bioactive peptides are organic substances formed by amino acids joined by covalent bonds also known as amide or peptide bond. Studies perfomed by Samaei et al., depict the use of faba bean Faba bean based bioactive peptides in obtaining fortified fruit juice and other functional food. Apart from BAP plant based protein also as has good potential as stabilizers (57). Peanut protein Pea protein concentrate and soy protein isolate have shown resistance to lipid oxidation and reduced droplet flocculation.

Conclusion

Out of the four macromolecules protein is one of the important macromolecules which is building block of human

method has proved to give a considerable increase in yield of protein (36). Apart from enzyme assisted method various studies show the use of electro activation method wherein proteins from canola were isolated. Higher protein extractability with improved quality of protein was found through this technique. It can be observed that use of these advance techniques is supposed to be very efficient for isolation of proteins from plant sources.

Composition of plant proteins

Proteins are classified into various system due to their diverseness. Some are classified based upon biological and chemical properties and some on the basis of technological applications. Osbrone classification system is the oldest classification system based on proteins solubility in various solutions. Osbrone on the basis of solubility classified plant protein into four major fractions; albumin, prolamins, globulin, glutelins mentioned in Table 4. (43). Albumins are soluble in water and diluted salt solutions whereas globulins are soluble in salt solutions but insoluble in water. Prolamins are soluble in water alcohol mixture but insoluble if only water or alcohol is present and glutelins are insoluble in salt, alcohol or neutral water solutions but soluble in dilute acids or alkali solutions.

Table 4. Composition of plant proteins: major and minor fractions of plant proteins present in various sources

Components	2S Albumins	7-8S Globulins	11-12S Globulins	Prolamins	Glutelins
Major components	Legumes	Legumes	Legumes	cereals	wheat
	Cruciferae	Cotton seed	Compositae		
	Compositae	Palms	Cucurbitaceae		
	Castor beans	cocoa	Oats and rice		
	Cotton seed		Cruciferae		
	Brazil nut		Cannabis		
			Brazil nut		
Minor components		Cereals	French bean	Oats and rice	

Albumins

According to one report (44), albumins are storage proteins majorly present in legumes, oilseeds, and pulses rather than cereals. On ultra-centrifugation of various plant sources 3 fractions of albumin were isolated; 2S and 11S mainly present in all types of plant sources and 7S expect few sources present in all. Out of 3 fractions 2S is the major storage albumin protein in many sources. This globular small 2S albumin proteins are high in sulphur containing amino acids in various plant sources like legumes, lupines and peas though they occupy only 10 to 30% of total proteins.

Globulins

The major globulin content is present in legumes. They are present in both dicot, monocot and are also present in gymnosperms. As compared with albumin, globulins are deficient in sulphur based amino acids. The globulin fractions obtained after ultra-centrifugation is 7S and 11S. Some globulin proteins in various plant sources includes β-conglycinin (7S) and glycinin (11S) in soybean, β-conglutin (7S) and α- conglutin (11S) in lupins, leguminin (11S), convicilin (7S) and vicilin (7S) in peas, helianthnin (11S) in sunflower seeds, cucurbitin (11S) in *Cucurbita* spp. etc. (37).

Prolamins

Cereals are rich in prolamins except oats and rice (44). Prolamins were the first proteins studied scientifically and they were named gliadin but Osbrone proposed a new name i.e., prolamins as they yield high levels of proline and amides on hydrolysis. It also yields small amounts of arginine and histidine but lysine content is very little or totally absent. According to Osbrone there are four principle prolamins hordeins in barley, gliadins in wheat and rye, karfirins in sorghum and zein in maize. Prolamins present in oats are avenins.

Prolamins are grouped into four cereals categories. Triticeae which includes wheat, barley and rye and are further divided into three categories sulphur rich prolamins (S-rich) mainly includes amino acids proline, glutamine and sulphur containing amino acid cysteine with inter and intrachain disulphide bonds, High molecular weight prolamins (HMW) are rich in glycine and glutamine and sequence similar to S-rich prolamins and Sulphur poor amino acids. Prolamins in oats include avenins which occupies 10% of the seed protein; Prolamins in rice are divided into four class out of which class 1 to 3 are major prolamins and class 4 is minor prolamins but rich in sulphur amino acids methionine and cysteine (44). Prolamins in maize are zein which includes various fractions α, β, γ and δ. Sorghum includes karfirins are with disulfide bond with high cysteine residues which are further divided into subclasses α, β, γ and δ.

Glutelins

Glutens are mixture of two proteins i.e., prolamins and glutelins present in various grass varieties. Wheat contains gluten which are further categorized into two groups prolamins are called gliadin and glutelins are called glutenin. Gliadin are monomeric proteins and glutenins are polymeric proteins. Glutenins are with intrachain disulfide bonds present between two subunits i.e., HMW and LMW (i.e., high molecular weight and low molecular weight subunits) (44).

Application of plant based proteins

Plant based protein could be beneficial means as a protein source due to their composition, availability and other techniques available for isolating them. The availability of

body. The availability of protein is distributed in various sources out of which plant-based protein is one such source which has increase in demand. The review gives information about various sources of plant-based proteins with its composition. From the information mentioned about the composition of plant-based proteins it is clear that to get all the essential amino acids one protein source needs to be complimented with other. This is because one of the drawback of plant based protein over animal based protein source. The techniques used for purification discussed above are most widely used for extraction, however in order to improve the functionality of extracted protein advanced techniques are to be considered to be isolated. Some advance isolation techniques like ultrasound assisted extraction, enzyme assisted method and electro activation method provides efficient isolation of desired proteins however, these advanced techniques could be expensive. Thus, advance techniques which are more cost effective must be employed. The application of plant-based proteins in various aspects state the abundance availability of plant-based proteins. The usage of plant-based protein in various form should thus be considered as a replacement for animal and other sources which would help in overcoming the increase in demand of the protein in nutritional and other aspects. Thus, more research on plant based proteins need to be conducted.

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Authors contributions

KM and SDS searched, collected and wrote the first draft. HA, and NP reviewed and edited the manuscript. MT and DJ critically reviewed and edited the same. AA analysed the manuscript, provided the regular assistance to revise and finalize it. All authors read and approved the final manuscript.

Compliance with ethical standards

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References

1. Alcorta A, Porta A, Tárrega A, Alvarez MD, Vaquero MP. Foods for plant -based diets: Challenges and innovations. Foods. 2021 Feb 1;10 (2):293. https://doi.org/10.3390/foods10020293
2. Hoffman JR, Falvo MJ. Protein - Which is Best? J Sport Sci Med. 2004;3 (3):118-30.
3. Almeida AG, Franco Moreno YM, Mattar Carciofi BA. Plant proteins as high-quality nutritional source for human diet. Trends Food Sci Technol, 2020; 97:170-84. https://doi.org/10.1016/j.tifs.2020.01.011
4. Hertzler SR, Lieblein-Boff JC, Weiler M, Allgeier C. Plant proteins: Assessing their nutritional quality and effects on health and physical function. Nutrients. 2020; 12(12): 3704. https://doi.org/10.3390/nu12123704
5. Langyan S, Yadava P, Khan FN, Dar ZA, Singh R, Kumar A. Sustaining protein nutrition through plant-based foods. Frontiers in Nutrition. 2021;8. https://doi.org/10.3389/fnut.2021.772573
6. Fathi M, Mohebbi M, Razavi SM. Application of image analysis and artificial neural network to predict mass transfer kinetics and color changes of osmotically dehydrated kiwifruit. Food and Bioprocess Technology. 2011 Nov;4(8):1357-66. https://doi.org/10.1007/s11947-009-0222-y
7. Sabaté J, Soret S. Sustainability of plant-based diets: back to the future. The American journal of clinical nutrition. 2014 Jul 1;100 (suppl_1):476S-82S. https://doi.org/10.3945/ajcn.113.071522
8. Pojić M, Mišan A, Tiwari B. Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. Trends in Food Science and Technology. 2018 May 1;75:93-104. https://doi.org/10.1016/j.tifs.2018.03.010
9. Allison JB. Biological Evaluation of Proteins. Adv Protein Chem.1949; 5:155-200. https://doi.org/10.1016/S0271-5317(99)00117-7
10. Moore DR, Soeters PB. The biological value of protein. Nestle Nutr Inst Workshop Ser. 2015; 82:39-51. https://doi.org/10.1159/000382000
11. Friedman M. Nutritional value of proteins from different food sources. A review. J Agric Food Chem.1996;44(1):6-29. https://doi.org/10.1021/jf9400167
12. Brody T. Protein. Nutritional Biochemistry 2nd ed. University of California, Berkely, Academic Press.1998; 421-87. https://doi.org/10.1016/B978-012134836-6/50011-1
13. Abeyrathne ED, Lee HY, Ahn DU. Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents-A review. Poult Sci. 2013; 92(12):3292-99. https://doi.org/10.3382/ps.2013-03391
14. Bar C, Mathis D, Neuhaus P, Dürr D, Bisig W, Egger L, Portmann R. Protein profile of dairy products: Simultaneous quantification of twenty bovine milk proteins. Int Dairy J. 2019; 97:167-75. https://doi.org/10.1016/j.idairyj.2019.01.001
15. Picota L, Bordenavea S, Didelota S, Fruitier-Arnaudina I, Sanniera F, Thorkelssonb G et al. Antiproliferative activity of fish protein hydrolysis on human breast cancer cell lines. Proc Bio. 2006; 41(5):1217-22. https://doi.org/10.1016/j.procbio.2005.11.024
16. Maurya PK, Malik DS, Yadav KK, Kumar A, Kumar S, Kamyab H. Bioaccumulation and potential sources of heavy metal contamination in fish species in river Ganga basin: Possible human health risks evaluation. Toxicol Rep. 2019;6:472-81. https://doi.org/10.1016/j.toxrep.2019.05.012
17. Jabborova DP, Enakiev YI, Davranov KD, Begmatov SA. Effect of co-inoculation with *Bradyrhizobium japonicum* and *Pseudomonas putida* on root morph-architecture traits, nodulation and growth of soybean in response to phosphorus supply under hydroponic conditions. Bulg J Agric Sci. 2018;1;24(6):1004-11.
18. Jabborova D, Wirth S, Kannepalli A, Narimanov A, Desouky S, Davranov K et al. Co-inoculation of rhizobacteria and biochar application improves growth and nutrientsin soybean and enriches soil nutrients and enzymes. Agronomy. 2020;10(8):1142. https://doi.org/10.3390/agronomy10081142
19. Singh U, Singh B. Tropical grain legumes as important human foods. Econ Bot. 1992; 46(3):310-21. https://doi.org/10.1007/BF02866630
20. Thrane M, Paulsen PV, Orcutt MW, Krieger TM. Soy Protein. Nadathur S et al. Sustainable Protein Sources 1st ed. Academic press. 2017;23-45. https://doi.org/10.1016/B978-0-12-802778-3.00002-0
21. Samaranayaka A. Lentil: Revival of poor man's meat. In: Nadathur S, Wanasundara JPD, Scanlin L. Sustainable protein sources 1st ed. Academic press. 2017;185-96. https://doi.org/10.1016/B978-0-12-802778-3.00011-1
22. Hoogenkamp H, Kumagai H, Wanasundara, JPD. Rice protein and rice protein products. In: Nadathur S, Wanasundara JPD, Scanlin L.

Sustainable protein sources 1st ed. Academic press. 2017; 47-65. https://doi.org/10.1016/B978-0-12-802778-3.00003-2

23. Jabborova D, Narimanov A, Enakiev Y, Kakhramon D. Effect of *Bacillus subtilis* 1 strain on the growth and development of wheat (*Triticum aestivum* L.) under saline condition. Bulg J Agric Sci. 2020;26(4):744-47.
24. Flambeau M, Redl A, Respondek F. Proteins from wheat. In: Nadathur S, Wanasundara JPD, Scanlin L. Sustainable Protein Sources 1st ed. Academic press. 2017;67-78. https://doi.org/10.1016/B978-0-12-802778-3.00004-4
25. Schoenlechner R, Siebenhandl S, Berghofer E. Pseudocereals. Gluten-free cereal products and beverages. 2008;149–*VI*. https://doi.org/10.1016/B978-012373739-7.50009-5
26. Orona-Tamayo D, Paredes-López O. Amaranth Part 1—Sustainable Crop for the 21st Century. In: Nadathur S, Wanasundara JPD, Scanlin L. Sustainable Protein Sources 1st ed. Academic press. 2017; 239-56. https://doi.org/10.1016/B978-0-12-802778-3.00015-9
27. Scanlin L, Lewis KA. Quinoa as a Sustainable Protein Source. Nadathur S, Wanasundara JPD, Scanlin L. Sustainable Protein Sources 1st ed. Academic press. 2017;223-38. https://doi.org/10.1016/B978-0-12-802778-3.00014-7
28. Arya SS, Salve AR, Chauhan S. Peanuts as functional food: A review. J Food Sci Technol. 2016; 53(1):31-41. https://doi.org/10.1007/s13197-015-2007-9
29. Marambe PWMLHK, Shand PJ, Wanasundara, JPD. An *in-vitro* investigation of selected biological activities of hydrolysed flaxseed *(Linum usitatissimum* L.) proteins. J Am Oil Chem Soc. 2008;85(12):1155-64. https://doi.org/10.1016/j.plaphy.2014.04.020
30. Orona-Tamayo D, Valverde ME, Paredes-López O. Chia-The New Golden Seed for the 21st Century. In: Nadathur S, Wanasundara JPD, Scanlin L. Sustainable Protein Sources 1st ed. Academic Press. 2017;265-81. https://doi.org/10.1016/B978-0-12-802778-3.00017-2
31. Wanasundara JPD, McIntosh TC, Perera SP, Withana-Gamage TS, Mitra P. Canola/rapeseed protein-functionality and nutrition. OCL. 2016; 23(4):D407. https://doi.org/10.1051/ocl/2016028
32. Bachmann, HP. Cheese Analogues: A review. Int Dairy J. 2001;11:505-15. (Cross Ref). https://doi.org/10.1016/S0958-6946(01)00073-5
33. Söderberg J. Functional properties of legume proteins compared to egg proteins and their potential as egg replacers in vegan food.
34. Sethi S, Tyagi SK, Anurag RK. Plant-based milk alternatives an emerging segment of functional beverages: A review. Journal of Food Science and Technology. 2016 Sep;53(9):3408-23. https://doi.org/10.1007/s13197-016-2328-3
35. Koyande AK, Chew KW, Rambabu K, Tao Y, Chu DT, Show PL. Microalgae: A potential alternative to health supplementation for humans. Food Science and Human Wellness. 2019 Mar 1;8(1):16-24. https://doi.org/10.1016/j.fshw.2019.03.001
36. Zhu Y, Fang Q. Analytical detection techniques for droplet microfluidics. Anal Chim Acta. 2013;787:24-35. https://doi.org/10.1016/j.aca.2013.04.064
37. Hadnadev M, Hadnadev TD, Pojic M, Saric B, Misan A, Jovanov T, Sakac M. Progress in vegetable proteins isolation techniques: A review. Food Res. 2017; 44(1):11-21. https://doi.org/10.5937/FFR1701011H
38. Moure A, Sineiro J, Domínguez H, Parajó JC. Functionality of oilseed protein products: a review. Food Res Int. 2006; 39(9):945-63. https://doi.org/10.1016/j.foodres.2006.07.002
39. Gendag E, Gorguc, A, Yilmaz FM. Recent advances in the recovery techniques in plant based proteins from agro industrial by products. Food Rev Int. 2020;1-22. https://doi.org/10.1080/87559129.2019.1709203
40. Chemat F, Huma Z, Khan, M. Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrason Sonochem. 2011;18(2011):813-35. https://doi.org/10.1016/j.ultsonch.2010.11.023
41. Preece K, Hooshyar N, Krijgsman A, Frye P, Zuidam N. Intensified soy protein extraction by ultrasound. Chem Eng Process. 2016;113:94-101. https://doi.org/10.1016/j.cep.2016.09.003
42. Dickey L, Parris N, Craig JC, Kurantz MJ. Ethanolic extraction of zein from maize. Ind Crops Prod. 2000;13(2001):67-76. https://doi.org/10.1016/S0926-6690(00)00054-6
43. Takiguchi Y, Osada O, Uematsu M. Thermodynamic properties of {xC2H5OH+(1–x)H2O} in the temperature range from 320 K to 420 K at pressures up to 200 MPa. J Chem Thermodyn. 1996; 28(12):1375-85. https://doi.org/10.1006/jcht.1996.0120
44. Shewry P, Casey R. Seed Proteins. 1st ed. Springer publishing House. 1999;1-11. https://doi.org/10.1007/978-94-011-4431-5_1
45. Nessa M, Rahman M, Kabir, Y. Plant-produced monoclonal antibody as immunotherapy for cancer. Biomed Res Int. 2020;3038564. https://doi.org/10.1155/2020/3038564
46. Wadhave A, Jadhav A, Arsul, V. Plant proteins applications: A review. World J Pharm Sci. 2014;3(3):702-12.
47. Hao F, Kumar S, Yadav N, Chandra D. Neem components as potential agents for cancer prevention and treatment. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2014 Aug 1;1846(1):247-57. https://doi.org/10.1016/j.bbcan.2014.07.002
48. Bose A, Baral R. Neem leaf glycoprotein in cancer immunomodulation and immunotherapy. In: New Look to Phytomedicine. Academic Press. 2019 Jan 1;391-408. https://doi.org/10.1016/B978-0-12-814619-4.00016-1
49. Saha A, Nandi P, Dasgupta S, Bhuniya A, Ganguly N, Ghosh T et al. Neem leaf glycoprotein restrains VEGF production by direct modulation of HIF1α-linked upstream and downstream cascades. Frontiers in Oncology. 2020 Mar 6;10:260. https://doi.org/10.3389/fonc.2020.00260
50. Dangaran K, Tomasula PM, Qi P. Structure and function of protein -based edible films and coatings. In: Huber K, Embuscado M. (Editors) Edible Films and Coatings for Food Applications. Springer, New York, NY. 2009. https://doi.org/10.1007/978-0-387-92824-1_2
51. Mariotti F, Gardner C. Dietary protein and amino acids in vegetarian diets-A review. Nutrient. 2019;11(11):2661. https://doi.org/10.3390/nu11112661
52. Vanga SK, Raghavan V. How well do plant based alternatives fare nutritionally compared to cow's milk?. J Food Sci Technol. 2018;55(1):10-20. https://doi.org/10.1007/s13197-017-2915-y
53. Grant CA, Hicks AL. Comparative life cycle assessment of milk and plant-based alternatives. Environ Eng Sci. 2018;35(11):1235-47. https://doi.org/10.1089/ees.2018.0233
54. Paul AA, Kumar S, Kumar V, Sharma R. Milk analog: Plant based alternatives to conventional milk, production, potential and health concerns. Crit Rev Food Sci Nutr. 2019; 1-19. https://doi.org/10.1080/10408398.2019.1674243
55. Joshi VK, Kumar S. Meat analogues: Plant based alternatives to meat products-A review. International Journal of Food and Fermentation Technology. 2015;5(2):107-19. https://doi.org/10.5958/2277-9396.2016.00001.5
56. Dagnelie PC, Mariotti F. Vegetarian diets. Vegetarian and plant-based diets in health and disease prevention. 2017;3-10. https://doi.org/10.1016/B978-0-12-803968-7.00001-0
57. Samaei SP, Ghorbani M, Tagliazucchi D, Martini S, Gotti R, Themelis T et al. Functional, nutritional, antioxidant, sensory properties and comparative peptidomic profile of faba bean (*Vicia faba* L.) seed protein hydrolysates and fortified apple juice. Food Chemistry. 2020 Nov 15;330:127120. https://doi.org/10.1016/j.foodchem.2020.127120

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Angiogenic Potential and Its Modifying Interventions in Dental Pulp Stem Cells: a Systematic Review

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Abstract

Background

Dental pulp stem cells (DPSCs) have been reported to have a pro-angiogenic effect indicative of the inherent property. Angiogenesis is a phenomenon crucial for wound healing, tissue regeneration, and vascularization of engineered scaffolds. Therefore, it is crucial to explore the angiogenic potential and the contributing factors in order to exploit this potential in stem cell research and tissue engineering.

Aim

To systematically review the literature on the angiogenic potential of DPSCs as well as to explore the effect of external environmental interventions on the angiogenic potential.

Methods

Three databases, PubMed, MEDLINE, and Cochrane, were systematically reviewed for literature with a suitable inclusion criterion. The data was screened for quantitative data for use in a meta-analysis.

Result

Overall, the studies indicated that DPSCs possess an inherent angiogenic potential that can be enhanced through external stimulation. The experimental methodology lacked consistency, and thus no quantitative data could be extracted for meta-analysis.

Conclusion

DPSCs are pro-angiogenic, which can be enhanced through external manipulation, making them a feasible option for use in tissue engineering and regenerative medicine. Consistency in experimental methodology is required in order to quantitatively analyze the angiogenic potential of DPSCs.

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Isolation of Stenotrophomonas pavanii DSM 25135(T) from Textile Effluent and Bioremediation of Carcinogenic Dye Basic Fuchsin in Free Cell vs Immobilized Cell System

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Full Text

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Abstract

The study focuses on the ability of bacterial strain *Stenotrophomonas pavanii* DSM 25135(T) isolated from textile effluent to decolourize one of the commonly used textile colourants, Basic Fuchsin, which is a carcinogen. The isolated bacterial strain was screened for its biodegrading capability at high dye concentrations (0.05% and 0.1% w/v) and at different time intervals (24 hr, 48 hr, 72 hr, 96 hr and 120 hr). The experimental results showed that *Stenotrophomonas pavanii* has a high capability for decolourizing this triphenylmethane dye at a dye concentration of 0.1% w/v. The capability of this bacterial strain to degrade the dye was tested in the free cell system as well as in the immobilized cell system. The isolate showed enhanced degradation of the dye (90.4%) in the immobilized state within 120 hr. The isolated microbe can, therefore, be utilized as a pre-treatment tool in the decolourization step adopted by various textile industries.

Keywords

Bioremediation, Biodegradation, Textile effluent, Water pollution, Decolourization, Basic Fuchsin, Immobilization, Calcium-alginate beads

References

1. Nigam, P., *et al.* 1996. Microbial process for the decolourization of textile effluent containing azo, diazo and reactive dyes. *Process Biochem.*, 31(5): 435-442.

2. Shrivastava, J.N., N. Raghav and A. Singh 2012. Laboratory scale bioremediation of the Yamuna water with effective microbes (EM) technology and nanotechno-logy. *J. Bioremed. Biodegrad.*, 3(8).
3. Gita, S., A. Hussan and T.G. Choudhury. 2017. Impact of textile dyes waste on aquatic environments and its treatment. *Env. Ecol.*, 35(3C): 2349-2353.
4. Křížová, H. 2015. Natural dyes: Their past, present, future and sustainability. In Recent development in fibrous material science. Prague: Kosmas Publishing.
5. Yang, X., *et al.* 2015. Highly effective removal of Basic Fuchsin from aqueous solutions by anionic polyacrylamide/graphene oxide aerogels. *J. Colloid Interface Sci.*, 453: 107-114.
6. Ren, S., *et al.* 2006. Decolourization of triphenylmethane, azo and anthraquinone dyes by a newly isolated aeromonas hydrophila strain. *Appl. Microbiol. Biotech.*, 72(6): 1316-1321.
7. El Haddad, M. 2016. Removal of Basic Fuchsin dye from water using mussel shell biomass waste as an adsorbent: Equilibrium, kinetics and thermody-namics. *J. Taibah University Sci.*, 10(5): 664-674.
8. Anonymous. Nutrient broth: Technical data. HiMedia Laboratories. Available at http://himedia labs.com/TD/M002.pdf.
9. Kumar, N.M. and D. Saravanan. 2015. Isolation of dye degrading bacteria from textile effluent. *J. Chem. Pharm. Res.*, 7(3):2214-2218.
10. Sharma, D.K., *et al.* 2004. Isolation and characterization of microorganisms capable of decolouri-zing various triphenylmethane dyes. *J. Basic Microbiol. Int. J. Biochem. Physiol. Genetics Morphol. Ecol. Microorganisms.*44(1): 59-65.
11. Keweloh, H., *et al.* 1989. Protection of bacteria against toxicity of phenol by immobilization in calcium alginate. *Appl. Microbiol. Biotech.*, 31(4): 383-389.
12. Gao, H., *et al.* 2016. Immobilization of multi-biocatalysts in alginate beads for cofactor regeneration and improved reusability. *J. Visualized Experiments.* 110: 53944.
13. Beshay, U. 2003. Production of alkaline protease by *Teredinobacter turnirae* cells immobilized in ca-alginate beads. *African J. Biotech.*, 2(3): 60-65.
14. Ren, S., *et al.* 2006. Decolourization of triphenylmethane, azo and anthraquinone dyes by a newly isolated *Aeromonas hydrophila* strain. *Appl. Microbiol. Biotech.*,72(6): 1316-1321.
15. An, S.Y., *et al.* 2002. Decolourization of triphenylmethane and azo dyes by Citrobacter sp. *Biotech. Letters.*24(12): 1037-1040.
16. Hemraj, V., S. Diksha and G. Avneet. 2013. A review on commonly used biochemical test for bacteria. *Innovare. J. Life Sci.*, 1(1): 1-7.
17. Brink, B. 2010. Urease test protocol. American Society for Microbiology, Washington, DC.
18. Shields, P. and L. Cathcart. 2010. Oxidase test protocol. American Society for Microbiology, Washington, DC.
19. Kim, M. and J. Chun. 2014. 16S rRNA gene-based identification of bacteria and archaea using the EzTaxon server. *Methods Microbiol.*, 41: 61-74.
20. Koyani, R.D., *et al.* 2014. Biodegradation of synthetic textile dyes by Mn dependent peroxidase produced by *Phanerochaete chrysosporium. Int. J. Env. Sci.*, 5(3): 652-663.

21. Partovinia, A. and B. Rasekh. 2018. Review of the immobilized microbial cell systems for bioremediation of petroleum hydrocarbons polluted environments. *Critical Reviews Env. Sci. Tech.,* 48(1): 1-38.
22. Iþýk, M. and D.T. Sponza. 2004. Decolourization of azo dyes under batch anaerobic and sequential anaerobic/aerobic conditions. *J. Env. Sci. Health Part A.* 39(4): 1107-1127.
23. Sneha, U., R. Poornima and S. Sridhar. 2013. Decolourization of synthetic textile dyes using *Pseudomonas putida. J. Chem. Pharm. Res.,* 5(5): 219-225.
24. Pratiwi, D., *et al.* 2017. Decolourization and degradation of Batik dye effluent using *Ganoderma lucidum. IOP Conf. Series Earth. Env. Sci.,*101(1): 012034.
25. Asad, S., *et al.* 2007. Decolourization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. *Bioresour. Tech.,*98(11): 2082-2088.
26. Chen, K.C., *et al.* 1999. Microbial decolourization of azo dyes by *Proteus mirabilis. J. Ind. Microbiol. Biotech.,* 23(1): 686-690.
27. Chen, C.C., *et al.* 2007. Biodegradation of crystal violet by *Pseudomonas putida. Biotech. Letters.*29(3): 391-396.
28. Jang, M.S., *et al.* 2005. Triphenylmethane reductase from Citrobacter sp. strain KCTC 18061P: Purification, characterization, gene cloning and overexpression of a functional protein in *Escherichia coli. Appl. Env. Microbiol.,* 71(12): 7955-7960.
29. Jing, W.U., *et al.* 2009. Isolation and characterization of *Pseudomonas otitidis* WL-13 and its capacity to decolourize triphenylmethane dyes. *J. Env. Sci.,*21(7): 960-964.
30. Hayase, N., K. Kouno and K. Ushio. 2000. Isolation and characterization of Aeromonas sp. B-5 capable of decolourizing various dyes. *J. Biosci. Bioeng.,* 90(5): 570-573.
31. Chen, K.C., *et al.* 2003. Decolourization of the textile dyes by newly isolated bacterial strains. *J. Biotech.,* 101(1): 57-68.

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Research article

Progins progesterone receptor gene polymorphism as a risk factor for preterm delivery

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ABSTRACT

The study's aim and the objective were to evaluate the association between progesterone receptor PROGINS and preterm delivery by correlating with clinical data. The study had begun with 150 preterm delivery cases (PTD) among which it has classified into 3 cohorts as extreme preterm i.e. 24 - 28 gestation weeks, n= 37, very preterm i.e. 28 to <32 gestation weeks, n =36; and late preterm i.e. 32 to <37 gestation weeks, n =77. 150 cases of term delivery were evaluated for progesterone receptor PROGINS gene polymorphism. Software R version 3.6.3 was used for the statistical analysis. PTD cases were having higher PR mutation. The mutant allele of T2 frequencies were .23 in preterm delivery women and .01 in controls with odds ratio 18.0 (CI, 4.25- 76.1). Homozygous for T2 allele was present in 2.6% of preterm delivery women. PROGINS polymorphisms had showed association in preterm labor and are evidently enhancing risky genetic factors in terms of the susceptibility of PTD.

Keywords: Preterm delivery, mutation, genotype, homozygous, heterozygous.

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INTRODUCTION

World health organization (WHO) has defined that if birth occurs before 37 weeks of gestation is preterm delivery. [1,2] PTD plays a major role in contributing to increased global neonatal morbidity and mortality [3], with prolonged negative effect for health and poor intellectual development as well as financial support for health. [4-6]

The gestational period acts as the most important clinical marker for determining the health and survival of an infant. Statistically on an average 80-85% number of preterm babies born between 32 to < 37 weeks of gestation (moderate/late preterm); on the other hand, 10% of premature babies are born in between 28 to 32 weeks and 5% of babies born prematurely in < 28 gestation weeks. It has also resulted in fetal mortality cases, which are found in alarming numbers in countries with low income. Additionally, the global prevalence rate shows the highest number of preterm birth cases where India occupies the first position. [7]

Genetic findings have proved to be an associated factor of preterm birth since a couple of decades. Due to the complexity of the disorder, the causes of the disorder are still unclear. Moreover, it is involved with several other factors such as pathological, genetic and environmental factors. [4,8] There are a few pathways which assume a crucial part in the support of a pregnancy which is fruitful. Progesterone pathway and folate pathway are the two most fundamental pathways related with fruitful pregnancy are. So any disturbance in these fundamental pathways prompts the intricacy in pregnancy. [4,8,9]

Progesterone hormones has an important role in pregnancy maintenance particularly in later gestation.[10] Progesterone is a composite hormone of Carbon 21 a steroid hormone it is produced in brain, ovaries, placenta and the adrenal glands which help in menstrual cycle in females, embryogenesis and of pregnancy that is found in several mammalian groups. [11] Progesterone receptors are found in many pregnancy-associated tissues including fetal amniotic and chorionic membranes.[12] In progesterone receptor (PR) gene (PROGINS) polymorphisms has been identified, with the insertion of 306 base pair PV/HS-1 (an Alu subfamily in intron G).[13]

In this paper, we assessed the role of genetic variation in progesterone receptor qualities to distinguish ladies who might be at sequential danger of preterm conveyance contrasted and everybody hazard. Uncovering hereditary variations in the progesterone receptor that are related to a high danger of preterm birth could prompt the designated utilization of progesterone interventional treatments later on. Also, one of the genuine worries that the western locale of India is a zone with an exceptionally large number of instances of preterm conveyance cases. Shockingly, no logical examinations have been embraced to date, to recognize the basic sub-atomic mechanism(s) or

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hereditary inclination related to preterm conveyance and related inconveniences from this space. [9,14]

MATERIALS AND METHODS

Study Group and Design

Mothers who had undergone labor (n=300) were selected for the study. Pregnant women of the suburban population who had preterm labor (n = 150) had selected on the bases of the gestation period (>37 weeks of gestation). Three cohort's gestation age were considered under the preterm delivery cases as: extremely preterm i.e. 24 to 28 weeks, very preterm i.e. 28 to <32 weeks, and moderately or late pre-term i.e. 32 to <37. Additionally, term delivery cases (n = 150) were selected as a control group, with all the clinical findings for both pre as well as term deliveries from MGM Medical College and Hospital, Kalamboli, Navi Mumbai following the guidance of a practitioner who is registered for medical practice with the informed consent and necessary clinically important data along with in this mothers with Tuberculosis, HIV, Jaundice, Infection of HPV, HBV, UTI or urinary tract infection, eclampsia, pre-eclampsia, miscarriage, pregnant female's twin fetus, a fetus with congenital anomalies and IUGR had been excluded. The Institutional Ethics Committee of MGMIHS, Navi Mumbai had approved the study.

PCR genotypic investigation of PR (PROGIN) Gene mutation: 3 ml of Peripheral blood was collected from MGM Hospitals in EDTA vacutainer. The collected blood was additionally prepared for extraction of DNA utilizing NucleoSpin Dx Blood DNA, RNA and protein refinement Kit (Macherey-Nagel, Germany). The DNA was put away at 4°C until investigated. For the polymerase chain reaction (PCR), we utilized the accompanying oligonucleotide introductions for in vitro intensification of explicit progesterone receptor fragment of gene: 5′ GGC AGA AAG CAA AAT AAA AAG A 3′ (forward) and 5′ AAA GTA TTT TCT TGC TAA ATG TC 3′ (reverse).[15] The wild sort of the PR was characterized as the insertion shortfall. At the point when this PCR technique was utilized, the wild-type created a 149–base pair section and the freak produced a base pair of 455. PCR items were settled by using 2% concentration of agarose gel. Alleles were recorded on the basis of the individual band molecular weight.

Statistical analysis

The frequencies of Allele were compared with genotype in the control groups and patient group were compared and analyzed with the Fisher exact test. One-way ANOVA and Post hoc test were used for the demographic tests. The strength of the association was measured using the odds ratio (OR) between the frequencies of allele, genotype, and preterm. Statistical analysis was done using the software R version 3.6.3. All p-values were two-tailed, and 95% CIs were calculated. p-value less than .05 was considered statistically significant.

RESULT

Clinical Demographic patient's profile: In the current study, we had enrolled women who had underwent labor (n=300). The details of which are represented in the table:1

Table 1: Demographic profile

Cases Term	Maternal Age (in range)	Parity					Birth Status		Birth Weight
		1	2	3	4	5	Live	Death	
Extremely Preterm (24-28 weeks)	28.01±6.04	56	64	24	5	1	137	13	2.75±0.58
Very Preterm (28-<32 weeks)	28.02±6.92	10	16	10	0	1	4	28	1.16±0.66
Late Preterm (32-<37 weeks)	28.36±6.98	14	16	3	2	1	22	12	2.04±0.57
	28.14±6.88	24	40	9	3	1	80	4	1.80±0.71

The subjects were enlisted with complete details which were clinically important of the baby and the mother along with the weight at birth of the baby (table 1). In our investigation we had discovered that childbirth weight was fundamentally lower in all the preterm cases of the delivery as compared with the term cases of the delivery (p-value 0.0001); just as in extremely preterm delivery cases contrasted with moderately preterm cases of the delivery. The birth weight of the baby was also significantly lower to very preterm delivery cases compared to the moderately preterm delivery cases. Hence it signifies that gestational age is proportional to birth weight of the infant. Extremely premature infant and extremely low birth weight infant (ELBW) are at high risk for disability and death with mortality rate 30–50% and 20–50% at least risk of morbidity in survivors.[16]

In our findings, we had found that there is a difference of 1.83-1.51 kg of weight in extremely newborn and term infants, and therefore there is the highest number of deaths seen in extremely premature infants n=28 as compared to term infants n=13. Therefore, survival rate among the term babies is higher compared to preterm babies (Table 1). Similar patterns were expressed in other parts of the world that in the USA the Preterm birth complicates 12.5% of all deliveries, prominently describing itself as the main cause of perinatal mortality and morbidity, accounting for as many as 75% of perinatal deaths.[17-20] In recent years, preterm delivery incidence has increased leading to different clinical risk factors and epidemiological factors. But it is not just epidemiological or clinical factors which can cause preterm labor, but also genetic aspect is vital and remains relatively unexplored. [21]

The wild type allele (T1) is of 149bp and polymorph allele (T2) band is of 455bp. Lane (L) 1 represents 50 base pair (bp) ladder; L 2,4,6,8-10 represents

PROGINS polymorphism which is associated with the Alu insertion that decreases the progesterone activity during pregnancy [28]. In current study we had found association between PROGINS and preterm delivery. It was observed that intron G insertion had shown significant in preterm delivery cases (22.6%) and in term it was observed (1.3%) of genotype distribution. The association of PROGINS gene polymorphism had also shown significant (OR=20.06[95% CI: 4.71-85.45]; p= 0.0001) and has shown 20 folds and in the variant genotype of PROGINS polymorph had resulted as (OR=18 [95% CI: 4.25-76.1]; p=0.0001) which had also shown as statistically significant with 18 folds. Later, the analysis was done between the term groups and sub-cohorts of preterm our data had given a pleasant results, when term was compared with extremely preterm (OR=14.32, [95% CI: 2.67-74.31], p=0.0015) that resulted statistically significant, next term was compared with very preterm (OR=21.14, [95% CI: 4.26-104.85], p=0.0002) which also significant, and lastly term was compared to late preterm (OR=22.57 [95% CI: 5.07- 100.34], p=0.0001) that too resulted statistically significant. Our data had shown correlating results with a study which was conducted in north-eastern part of India (OR=2.652[95% CI: 0.830-8.474] p=0.115) [9]. Our study also resembled a study which was conducted by Langmia et.al., 2015 in Malaysia had found association between progesterone receptor gene polymorph and preterm delivery (OR 2.3, [95 % CI: 1.2–4.5] P = 0.011) where they had found it significant different [29]. On the other hand, some studies did not match our analysis and these are the studies which had proved that progesterone receptor does not play a key role in any manifestation. A study which was held to detect the polymorphism of progesterone receptor and its association with recurrent spontaneous abortion (RSA) in pooled (OD: 1.82 [95% CI: 0.98-3.37] p=0.056) but the data did not show any significant difference [30]. Progesterone plays a vital role in sustaining pregnancy and has been successfully used to prevent preterm labor reoccurrence. By analyzing both maternal and fetal effects we examined the role of genetic mutations in the progesterone receptor (PGR) gene in altering risks to preterm labor. With enormous availability of data and studies there is still vast gap between the associations of PROGINS with preterm or related complications. Although our data has given a descriptive path where the research could be continued but the actual results will be only state if large sample size is been presented.

CONCLUSION

PROGINS polymorphisms had showed association in preterm labor and are evidently enhancing risky genetic factors in terms of the susceptibility of PTD.

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FUNDING

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CONFLICT OF INTEREST

Authors contributes no conflicts of interests.

ETHICS APPROVAL

The study is approved by institutional ethical committee

AUTHORS CONTRIBUTIONS

MT and SS conceived and planned the experiments. SP carried out the experiments. MT and SP, SJ planned and carried out the simulations. SP and SS contributed to sample preparation. SP, and MT contributed to the interpretation of the results. MT, SP and SJ took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

REFERENCES

1. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, Rubens C, Menon R, Van Look PF, 2010. The worldwide incidence of preterm birth a systematic review of maternal mortality and morbidity, Bull World Health Organ, 88:31-8.
2. Challis JR, Mechanism of parturition and preterm labor, 2000. Obstetrical & gynecological survey 1; 55:650-60.
3. Norwitz E R, Robinson J N, 2001. A systematic approach to the management of preterm labor, Semin Perinatol, 25:223–235.
4. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, Rubens C, Menon R, Van Look PF, 2010. The worldwide incidence of preterm birth a systematic review of maternal mortality and morbidity, Bull World Health Organ, 88:31-8.
5. Gissler M, Järvelin MR, Louhiala P, Rahkonen O, Hemminki E, 1999. Can children's health be predicted by perinatal health? Int journal of epidemol, 1;28:276-80.
6. Wong HS, Edwards P, 2013. Nature or nurture a systematic review of the effect of socio-economic status on the developmental and cognitive outcomes of children born preterm, Matern child health, 17:1689-700.
7. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, Adler A, Garcia CV, Rohde S, Say L, Lawn JE, 2012. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries a systematic analysis and implications, the lancet, 379:2162-72.
8. Goldenberg RL, Culhane JF, Iams JD, Romero R, 2008. Epidemiology and causes of preterm birth, The lancet, 371:75-84.
9. Tiwari D, Bose PD, Das S, Das CR, Datta R, Bose S, 2015. MTHFR (C677T) polymorphism and PR (PROGINS) mutation as genetic factors for preterm delivery, fetal death and low birth weight, A Northeast Indian population-based study, Meta Gene, 1;3:31-42.
10. Challis JR, Matthews SG, Gibb W, Lye SJ, 2000. Endocrine and paracrine regulation of birth at term and preterm, 21: 514-50.
11. Aruna M, Nagaraja T, Andal S, Tarakeswari S, Sirisha PV, Reddy AG, 2010. Thangaraj K, Singh L, Reddy BM, Role of progesterone receptor polymorphisms in the recurrent spontaneous abortions Indian case, PLOS one, 5: e8712.

heterozygous polymorph; L 3 represents wild type; L 5 & 7 represents homozygous polymorph Distribution of *PR* mutation and its association with preterm delivery

Figure 1: Representation of 2% Agaroe gel showing 306bp intron G insertion of Progesterone Receptor Gene PROGINS polymorphisms

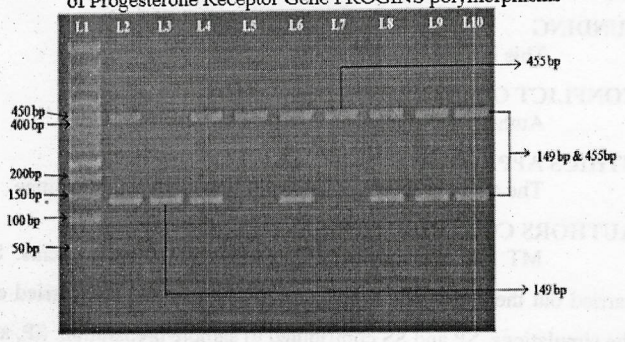

Table 2: Allele and Genotype frequencies among mothers with preterm and term deliveries

	Preterm (n=150)	Term (n=150)	ODDS ratio (95% CI)	*P value*
Genotypes				
T1/T1	118(78.66%)	148(98.67%)	20.06 (4.71 – 85.45)	0.0001[a] [b*]
T1/T2	28(18.67%)	2(1.33)		
T2/T2	4 (2.66)	0		
T1/T2 & T2/T2	32(22.6%)	2 (1.3%)		
Alleles				
T1	150(100%)	150(100%)	18 (4.25-76.1)	0.0001
T2	36 (24%)	2 (1.33)		

Data stated otherwise are number of patients in percentage
[a] T1/T2 and T2/T2 Vs T1/T1
[b] Fisher's Test

Genotype Analysis

Genotype analysis was conducted in all enrolled cases for the detection of wild type T1 and mutant T2 alleles shown in Figure 1. Women underwent preterm deliveries and term resulted significantly difference, in the distribution of genotype frequency for allele T2 (T1/T2 and T2/T2 Vs T1/T1 or 20.06 {95% CI 4.71-85.45}; p- 0.0001) and in the frequency of the mutant T2 allele (0.23-0.01; or 18 {95% CI: 4.25 – 76.1; p- 0.0001}). Four women from the study group (preterm) and none in term (control) group were found homozygous mutant for allele T2 (Table 2).

Preterm and its incidence by parity

Albeit, numerous interventions have been assessed, few have a moderate-to-high proof for diminishing preterm birth: smoking end and progesterone treatment in ladies with a high danger of preterm birth in low to average-pay nations and cervical cerclage for those in big-time salary nations. Risk partum and post-pregnancy mediations (eg, antepartum maternal steroid organization, or kangaroo mother care) to improve preterm neonatal endurance after birth have been exhibited to be compelling yet have not been broadly executed. [22]

In our study cohort, heterozygous variation was seen in 2 patients from the control group and 28 patients from preterm group (study group). In the control group both the babies were born alive. But from the preterm group it was seen that only 5 (17.85%) neonates survived and 23 (82.14%) of them were either still born or died immediately after birth. Additionally, the birth weight of 23 neonates who had died and 5 neonates who had survived from the study group were found in the range of 0-1 kg and 1-2 kg respectively. Similarly, in preterm cases the homozygous variation was 4, among which 3 neonates were still born and 1 was alive. Neonates who survived weighted 1.2kg and the still born weighted 0.80kg or less. This shows how in both variations (heterozygous and homozygous) the neonates who were still born were comparatively lower in weight as compared to the neonates who survived.

As it is known, the age of the mother during pregnancy plays another vital aspect for the outcome of the pregnancy and is strongly linked with genetic variations we also found that 100% of our study group mothers had access to full ANC and PNC care which is very vital for proper monitoring and management of the pregnancy outcomes. Therefore, it shows that the cause of preterm birth and stillborn neonates seen in mentioned 23 preterm cases with heterozygous variation and 3 stillborn neonates seen in preterm cases with homozygous variation occurred due to progesterone receptor PROGINS polymorphisms.

Women who had received early prenatal care appears to have a lower incidence of preterm birth than women who receive late care or no prenatal care, and some researcher believes that this decreased incidence results directly from some beneficial influence of prenatal care.[23] The continuing elucidation of the mechanisms that regulate preterm labor, combined with rigorous clinical assessment, offer hope for future solutions.[20] To conclude, in this study the variation in genetic factors, plays a significant role in preterm delivery. However, this study resembles the studies where genetics associations are found between SNP and clinically associated factors. The major drawbacks in these studies are lack of evaluations of SNP, small sample size, patient's demographical data etc. However, our finding puts a light on the SNP, whose research needed to be taken into consideration to avoid the adverse pregnancy outcome in future. With this current study, it has been analyzed that genetic factors are involved with different clinical classification of Preterm deliveries occurred in Navi Mumbai Population. Moreover, including the cohorts from various regions of Navi Mumbai population is important to correlate the findings from other regional populations that contribute expanded knowledge for the cause of preterm delivery

DISCUSSION

Progesterone receptor gene altogether has seven introns and eight exons (A-G). One of the progesterone receptor variant polymorph consists of PV/HS-1 of 320/306bp Alu gets insert at intron G and their [15, 24-27].PR involves in the physiological effects of the progesterone and PR gene polymorphism, mostly in

12. Merlino A, Welsh T, Erdonmez T, Madsen G, Zakar T, Smith R, Mercer B, Mesiano S, 2009. Nuclear progesterone receptor expression in the human fetal membranes and decidua at term before and after labor, Reprod Sci, 16: 357-63.
13. Rowe SM, Coughlan SJ, McKenna NJ, Garrett E, Kieback DG, Carney DN, Headon DR, 1995. Ovarian carcinoma-associated TaqI restriction fragment length polymorphism in intron G of the progesterone receptor gene is due to an Alu sequence insertion, Cancer Res, 55:2743-5.
14. Ahankari A, Bapat S, Myles P, Fogarty A, Tata L, 2017. Factors associated with preterm delivery and low birth weight a study from rural Maharashtra, India, F1000 Res, 6.
15. Wieser F, Schneeberger C, Tong D, Tempfer C, Huber JC, Wenzl R, 2002. PROGINS receptor gene polymorphism is associated with endometriosis, Fertil Steril, 77: 309-12.
16. Glass HC, Costarino AT, Stayer SA, Brett C, Cladis F, Davis PJ, 2015. Outcomes for extremely premature infants, Anesth Analg, 120:1337.
17. Ananth CV, Ananth CV, Vintzileos AM, 2006. Epidemiology of preterm birth and its clinical subtypes, J Matern Fetal Neonatal Med, 19: 773-82.
18. Cammu H, Dony N, Martens G, Colman R, 2014. Common determinants of breech presentation at birth in singletons a population-based study, Eur J Obstet, Gynecol Reprod Biol, 177:106-9.
19. Fruscalzo A, Londero AP, Salvador S, Bertozzi S, Biasioli A, Della Martina M, Driul L, Marchesoni D, 2014. New and old predictive factors for breech presentation our experience in 14 433 singleton pregnancies and a literature review, J Matern Fetal Neonatal Med, 27: 167-72.
20. Bin YS, Roberts CL, Nicholl MC, Nassar N, Ford JB, 2016. Contribution of changing risk factors to the trend in breech presentation at term, Aust N Z J Obstet Gynaecol, 56:564-70.
21. Slattery MM, Morrison JJ, 2002. Preterm delivery, The Lancet, 360: 1489-97.
22. Simmons LE, Rubens CE, Darmstadt GL, Gravett MG, 2010. Preventing preterm birth and neonatal mortality, exploring the epidemiology, causes, and interventions, In Seminars in perinatology, Vol 34, No 6, pp, 408-415, WB Saunders.
23. Shwartz S, Vinyard Jr JH, 1965. Prenatal care and prematurity, Public Health Rep, 80:237.
24. Romano A, Delvoux B, Fischer DC, Groothuis P, 2007. The progins polymorphism of the human progesterone receptor diminishes the response to progesterone, Journal of molecular endocrinology, 1; 38 (2): 331-50.
25. Agoulnik IU, Tong XW, Fischer DC, Körner K, Atkinson NE, Edwards DP, Headon DR, Weigel NL, Kieback DG, 2004. A germline variation in the progesterone receptor gene increases transcriptional activity and may modify ovarian cancer risk, The Journal of clinical endocrinology & Metabolism, 1;89 (12): 6340-7.
26. Fabjani G, Tong D, Czerwenka K, Schuster E, Speiser P, Leodolter S, Zeillinger R, 2002. Human progesterone receptor gene polymorphism PROGINS and risk for breast cancer in Austrian women, Breast cancer research and treatment, 72 (2): 131-7.
27. Rowe SM, Coughlan SJ, McKenna NJ, Garrett E, Kieback DG, Carney DN, Headon DR, 1995. Ovarian carcinoma-associated TaqI restriction fragment length polymorphism in intron G of the progesterone receptor gene is due to an Alu sequence insertion, Cancer Research, 1;55 (13): 2743-5.
28. Gomes MT, Castro RD, Villanova FE, Silva ID, Baracat EC, Lima GR, Girão MJ, 2006. Relação entre polimorfismo do gene do receptor de progesterona, raça, paridade e ocorrência de leiomioma uteri no, Revista Brasileira de Ginecologia e Obstetrícia, 28 (5): 278-84.
29. Langmia IM, Apalasamy YD, Omar SZ, Mohamed Z, 2015. Progesterone Receptor (PGR) gene polymorphism is associated with susceptibility to preterm birth, BMC medical genetics, 16 (1): 1-5.
30. Aruna M, Nagaraja T, Andal S, Tarakeswari S, Sirisha PV, Reddy AG, Thangaraj K, Singh L, Reddy BM, 2010. Role of progesterone receptor polymorphisms in the recurrent spontaneous abortions Indian case, PLOS one, 14;5 (1): e8712.

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Original Article

Community mapping of COVID-19 cases admitted from April to June 2020 at a tertiary health care hospital in Raigad district in Maharashtra, India

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Abstract

Introduction: At end of 2019, a novel coronavirus caused severe acute respiratory syndrome, which emerged in Wuhan, Hubei province of China. Health professionals have always used conventional mapping (in recent times geographic information systems [GIS] mapping) as a useful tool for better tracking which further facilitated better management of deadly contagions such as SARS-CoV 2. This study aimed to map geographically the positive patients admitted in a dedicated COVID-19 hospital which is a tertiary care hospital from April to June 2020 to gain insight into the local viral transmission and pattern of geographical spread because of ongoing cluster transmission.

Objectives: The aims of this study were (1) to locate geographically the COVID-19 cases admitted from April to June 2020 at a tertiary health-care facility, (2) to study trends and patterns of geographical spread, and (3) to identify geographical clustering of cases, if any.

Materials and Methods: This was an observational, cross-sectional, secondary data-based study. The study was conducted at MGM Medical College Hospital, Kamothe. The data were collected from existing surveillance and lab data records. The data were analyzed in Excel and Epi info. Specialized GIS software was used for mapping to Taluka level based on patients' addresses using standard ".shp" files for the local area.

Results: There were a total of 968 cases. The majority of which were from Raigad district (839, 87%). The Panvel taluka in Raigad District having Panvel as a major city and the thickly populated urban area has shown clustering of cases extending to neighboring Uran taluka.

Conclusion: For better preparedness, we need to keep tracking new outbreaks through GIS and promote further advances in mapping technologies.

Keywords: COVID-19, geographic information systems, mapping outbreak

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INTRODUCTION

In December 2019, a new virus (initially called "Novel Coronavirus 2019-nCoV" and later renamed SARS-CoV-2)

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Table 1: Taluka wise distribution of COVID-19 positive cases admitted at hospital

District Raigad		District Mumbai		District Thane	
Alibag	9	Ward A	1	Ambarnath	3
Karjat	16	Ward E	1	Kalyan	1
Khalapur	17	Ward F/N	5	Navi Mumbai	48
Mahad	11	Ward F/S	5	Thane	28
Mangaon	13	Ward G/N	3	Total	80
Panvel	629	Ward H/E	6		
Pen	7	Ward H/W	1		
Poladpur	1	Ward K/E	1		
Roha	12	Ward L	1		
Shrivardhan	2	Ward M/E	14		
Uran	122	Ward N	4		
Total	839	Ward P/N	1		
		Ward R/C	1		
		Ward R/N	1		
		Ward S	3		
		Ward T	1		
		Total	49		

causing severe acute respiratory syndrome (coronavirus disease-2019 [COVID-19]) emerged in Wuhan, Hubei Province, China,[1] and rapidly spread to other parts of China and other countries around the world, despite China's massive efforts to contain the disease within Hubei. Compared to the 2002/2003 SARS-CoV and the 2012–2014 Middle East Respiratory syndrome-related coronavirus (MERS-CoV), the COVID-19 coronavirus spread strikingly fast. Although MERS took about two and a half years to infect 1000 people and SARS took roughly 4 months, the novel SARS-CoV-2 reached that figure in just 48 days. On January 30, 2020, the World Health Organization (WHO) declared that the new SARS-CoV-2 coronavirus outbreak constitutes a Public Health Emergency of International Concern (PHEIC).[2]

Health professionals have long considered conventional mapping, and more recently geographic information systems (GIS), as critical tools in tracking and combating contagion. The earliest map visualization of the relationship between place and health was in 1694 on plague containment in Italy.[3] The value of maps as a communication tool blossomed over the next 225 years in the service of understanding and tracking infectious diseases, such as yellow fever, cholera, and the 1918 influenza pandemic. From the 1960s, when computerized GIS were born, the possibilities for analyzing, visualizing, and detecting patterns of disease dramatically increased again. A 2014 review of the health GIS literature found that 248 out of 865 included papers (28.7%) focused on infectious disease mapping.[4]

In India, the initial COVID-19 testing strategy included people who had international travel history with symptoms, symptomatic contacts of laboratory-confirmed COVID-19 patients, and symptomatic healthcare workers managing respiratory distress/severe acute respiratory illness (SARI).[5] All over India by May 14, 2020, a total of 51401 active cases, 27919 cured/discharged, and 2649 deaths were reported.[6] Most people infected with the COVID-19 virus have mild disease and recover. Approximately 80% of laboratory-confirmed patients have had mild disease, 15% required hospitalization, and 5% cases were critical requiring ventilator management. But the identification of each case still was a critical part, to avoid those severe 20% cases which add on as a major burden on the healthcare system. Hence, this study was undertaken to identify the pattern of the geographic distribution of cases [Table 1].

Objectives

1. To locate geographically the COVID-19 cases admitted from April to June 2020 at a tertiary health-care facility.
2. To study the trend and pattern of geographical spread in the neighboring area over the time of 3 months.
3. To Identify geographical clustering of cases, if any.

MATERIALS AND METHODS

Study type

This was an observational, cross-sectional study, based on secondary data.

Data collection

The study was conducted at MGM Medical College Hospital, Kamothe, Navi Mumbai, India. The data were collated from lab data records of the college by existing surveillance.

Data sampling

Universal sample. No sampling was done. All patients tested positive for COVID-19 by real-time reverse transcription-polymerase chain reaction (RT-PCR) or Gene Expert technique, admitted at MGM Hospital, Kamothe, Navi Mumbai, India from April 1, 2020 to June 30, 2020.

Inclusion criteria

1. Patients admitted to MGM Hospital, Kamothe, Navi Mumbai, India from April 1, 2020 to June 30, 2020 as COVID-19 cases with positive swab test results on RT-PCR or Gene expert testing.
2. Residing in Mumbai city/Mumbai Suburban/Thane/ Raigad district at the time of onset of symptoms.

Exclusion criteria

None.

Data analysis

Data were collated from surveillance/hospital/lab records and entered in the spreadsheet, Microsoft Excel using the standard coding procedure. The data were analyzed in Excel and Epi info. Specialized GIS software was used for mapping to Taluka level based on patients' addresses using standard ".shp" files for the local area.

Quality control

The process of data collation was supervised closely by all co-investigators using standard cross-checking methods. The Director, MGM School of Biomedical Sciences, Navi Mumbai, India acted as a coordinator for Data Collation. Department of Community Medicine, MGM Medical College, and Hospital, Navi Mumbai, India conducted the study. The principal investigator and all co-investigator acted as guarantors for the integrity of the process of data collection to the publication of results.

RESULTS

There were a total of 968 cases; the majority of which were from the Raigad District (839, 87%) [Table 1]. The Panvel taluka in Raigad District having Panvel as a major city and a thickly populated urban area has shown clustering of cases extending to neighboring Uran taluka [Figure 1].

In comparison, there were few patients from Thane and Mumbai Districts seeking treatment at this particular hospital, however neighboring Navi Mumbai Corporation, being a thickly populated urban area has shown relatively more cases [Figure 2]. Cases from the M/E ward were seen seeking treatment in this hospital, mostly due to road connectivity and possibly being a catchment area [Figure 3].

DISCUSSION

To contain the COVID-19 epidemic, one of the nonpharmacological epidemic control measures is reducing the transmission rate of SARS-COV-2 in the population

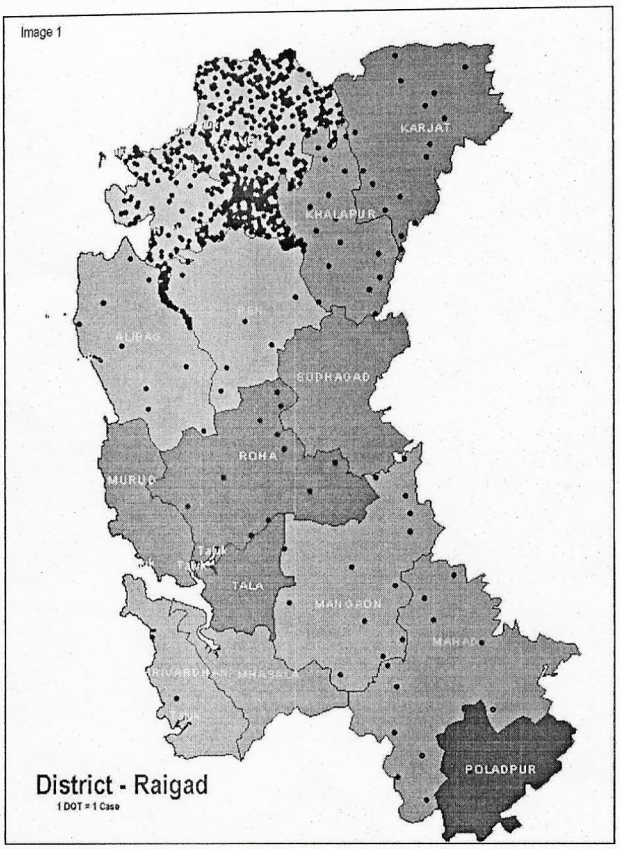

Figure 1: The Panvel taluka in Raigad district having Panvel as a major city and the thickly populated urban area has shown clustering of cases extending to neighboring Uran taluka

through social distancing.[7] To maintain social distancing and breaking the transmission chain, early identification and prompt isolation of individuals is necessary, where mapping proves its benefit as the disease is shown to be spreading rapidly in thick urban populations.

A similar study done by Zahra Arab-Mazar in March–April 2020 in Iran stated that the highest number of COVID-19 cases were reported in the capital city, using GIS and estimating the incidence/attack rates per province, that one is placed as the seventh, having more cases per population at Qom, Semnan, and Markaz, among other provinces.[8] Similarly in our study with the use of GIS mapping, we were able to conclude that, the maximum number of cases were from Raigad District (Panvel)

The WHO directs and coordinates international health, combating communicable diseases through surveillance, preparedness, and response, and applying GIS technology to this work. On January 26, 2020, the WHO unveiled its ArcGIS Operations Dashboard for COVID-19, which also maps and lists coronavirus cases and the total number of

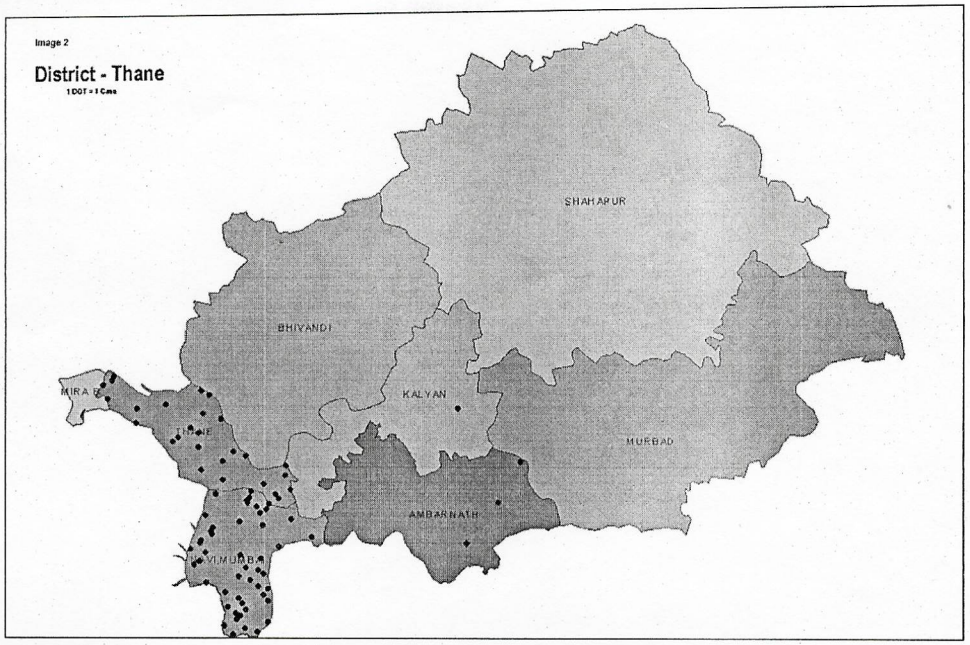

Figure 2: Navi Mumbai Corporation, being a thickly populated urban area has shown relatively more cases

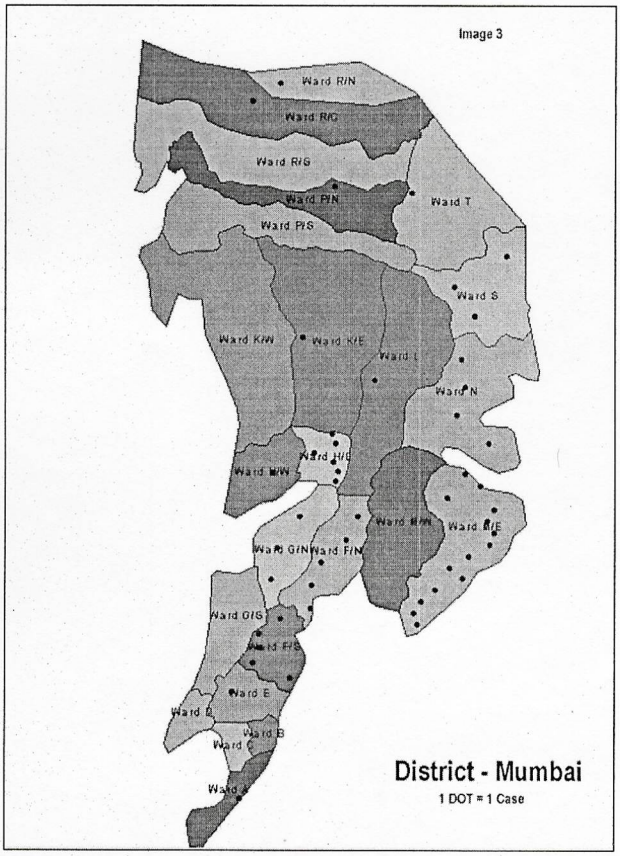

Figure 3: Cases from the M/E (Maharashtra East) ward were seen seeking treatment in the hospital, mostly due to road connectivity and possibly being a catchment area. Ward-ME of Municipal Corporation of Greater Mumbai

deaths by country and Chinese province, with informational panels about the map and its data resources.[9]

John Snow (1813–1858) was able to trace the source of a cholera outbreak in Soho, London, in 1854, thanks to his well-known manual spatial analysis exercise using hand-drawn paper maps of cholera cases and water pumps/water companies supplying them with water. Today, more advanced computerized spatial analyses integrating phyloepidemiological methods are used to identify the likely sources of new outbreaks.[10]

A study by Kamel Boulos and Geraghty[11] in 2020 suggested that dashboards and web maps bring together location and time-sensitive events in relationship to spreading disease and give travelers and officials the potential to reduce exposure by avoiding public gatherings in case of a public event. During the period of our study, it was observed that during the peak phases of the pandemic in the Raigad district, the cases were increasing, and the lockdown rules were intensified, leading to a more strict lockdown.

Understanding of the burden on the health care system can be facilitated by GIS mapping facing treatment facility shortages in Wuhan, government officials commissioned in late January the emergency construction of two new hospitals, which together provide an additional 2600 beds. This increase in requirement for hospital facilities was predicted using GIS mapping.[12]