DOI: 10.7860/JCDR/2017/20815.9254

Original Article

Dentistry Section

Estimation of Serum Protein in Oral Potentially Malignant Disorders and Oral Malignancy – A Cross-Sectional Study

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ABSTRACT

Introduction: In carcinogenesis, increased oxidative stress and weakened antioxidant defense produces damage to the macromolecules like proteins. Thus, protein can act as potential biomarker in oral premalignant and malignant lesions.

Aim: To determine and compare the levels of serum proteins in Oral Submucuous Fibrosis (OSMF), Oral Leukoplakia (OL), Nicotina Stomatitis (NS), Oral Malignancy (OM) and Healthy Controls (HC).

Materials and Methods: A total of 250 participants, were equally divided in five groups i.e., OSMF, OL, NS, OM and HC. Five ml of blood was collected from antecubital vein from each participant. The serum was analyzed for total protein, albumin and globulin levels using EBRA EM 200 semi-quantitive analyzer with the help of diagnostic kits.

Results: There were total 193 males and 57 females, who were between 18 to 82 years of age, with a mean of 46.32±13.89 years. The serum protein and globulin levels were significantly decreased in OSMF, OL and NS and increased in OM as compared to HC ($p<0.001$). No statistically significant difference was found in serum albumin levels between the study groups ($p>0.05$).

Conclusion: Serum proteins can be used as diagnostic and prognostic marker for oral premalignant and malignant lesions.

Keywords: Albumin, Globulin, Nicotina stomatitis, Oral Leukoplakia, Oral malignancy, Oral submucous fibrosis, Serum protein

INTRODUCTION

Like an evil with many faces, tobacco and areca nut habits are practiced in different forms in India since centuries. India is world's second largest consumer of tobacco, where about one-third of the adults consume some or the other form of tobacco [1]. The carcinogens of these substances may cause oral precancerous and cancerous lesions, which are associated with significant morbidity and mortality. Daily the number of new cases and deaths is increasing worldwide, and more than half of all cancer cases occur in developing countries [2].

The oral cancerous lesions are always preceded by Oral Potentially Malignant Disorder (OPMD's) like OL, OSMF, NS etc., [3]. Although, the assessment of probable behaviour of OPMD's is mainly based on histological examination, various biochemical alterations occur at every step of oncogenic process, during which, various substances change quantitatively in the serum and are collectively termed as tumour markers or biochemical serum markers, which may act as reliable indicator. Studies involving relationship of enzymes, proteins and glycoproteins are reported by many workers and had observed significant alterations in serum levels of OPMD's and OM [4-6].

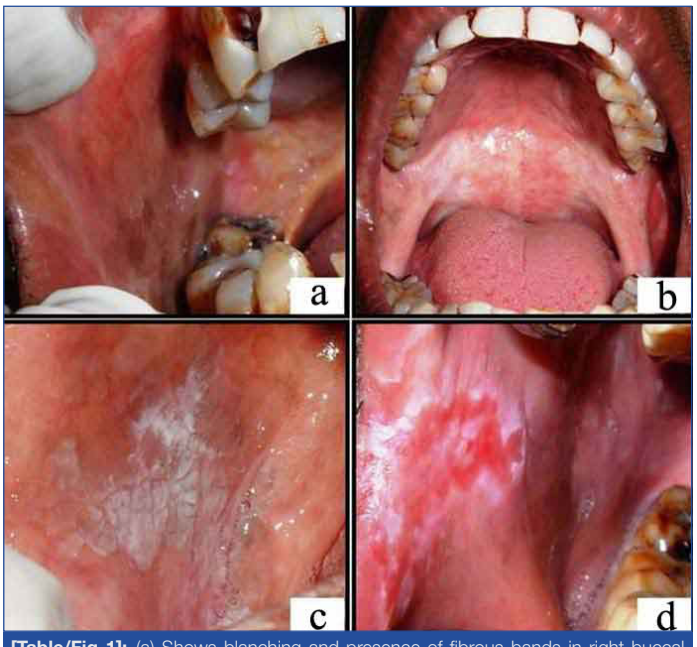
Free radicals attack the healthy cells of the body leading to loss of structure and function [7]. Excessive production of reactive oxygen species within the tissue can damage DNA, proteins, lipids and carbohydrates. The oxidation of proteins plays an important role in pathogenesis of oral cancer [8,9]. Hypoproteinemia is commonly observed in oral malignancy and it is expressed as cachexia [10]. Thus, serum protein may serve as an important diagnostic and prognostic marker for OPMD's and OM. Hence, the present study was planned to assess and correlate the serum levels of proteins in OPMD's and OM.

MATERIALS AND METHODS

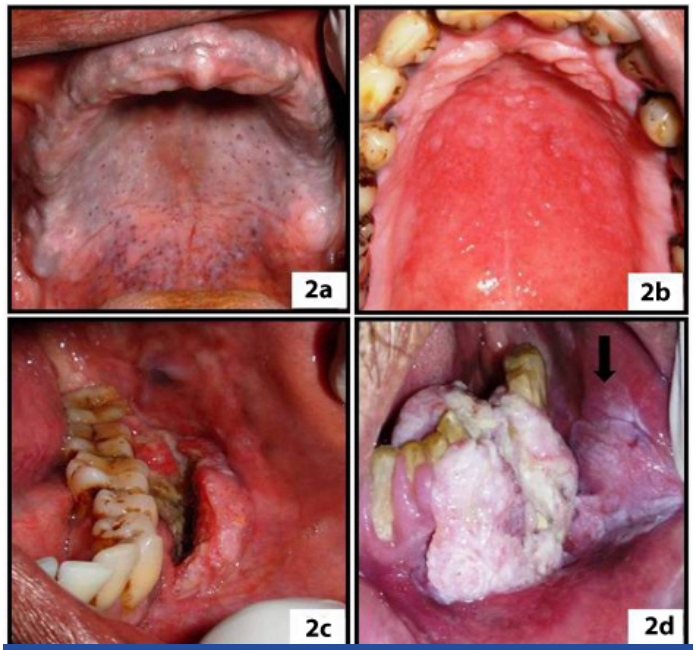
The present prospective study was conducted in the Department of Oral Medicine and Radiology, after obtaining the approval from the Institutional Ethics Committee (IEC) of Sumandeep Vidyapeeth, bearing number SVIEC/ON/DENT/RP/1520 dtd. 04/12/2014 and SVIEC/ON/DENT/RP/15034 dtd. 29/06/2015.

The total of 250 participants, equally divided into five study groups – OSMF, OL, NS, OM and age and sex matched HC, which were diagnosed clinically and histopathologically and were classified accordingly [3,11,12] depending on the severity; formed the part of the study [Table/Fig-1,2]. Participants who had undergone treatment for the lesions and having systemic disease (like scleroderma, anemia etc.,) were excluded from the study.

After obtaining informed consent, 5 ml of blood was aspirated from right/left antecubital vein, which was centrifuged at 2000 rpm for 10 minutes to separate the serum. The serum was then analyzed for total proteins and albumin levels using diagnostic kit-Liquixx Total Protein Erba Mannheim and Liquixx Albumin Erba Mannheim respectively, and EBRA EM 200 fully automatic analyzer. The serum globulin levels were obtained by subtracting the values of total proteins and albumin.



[Table/Fig-1]: (a) Shows blanching and presence of fibrous bands in right buccal mucosa; (b) Shows blanching of soft palate; (c) Shows homogenous oral leukoplakia on right buccal mucosa; (d) Shows non-homogenous oral leukoplakia on right commissure and buccal mucosa.



[Table/Fig-2]: (a) shows grayish black pigmentation on palatal mucosa with inflamed openings of minor salivary glands; (b) Shows erythematous palatal mucosa; (c) Shows deep burrowing ulceration in lower left gingival; (d) Shows proliferative lesion in lower canine premolar region. The black arrow denotes the extent of the lesion towards the buccal mucosa.

The statistical analysis was performed using SPSS version 19.0 and tests applied were one-way ANOVA and Tukey's Post-Hoc analysis for multiple comparisons.

RESULTS

The participant's age ranged from 18 to 82 years, with a mean age of 46.32±13.89. There were total 193 males and 57 females with the M:F ratio of 3.4:1 [Table/Fig-3].

The serum protein levels were estimated in all the groups and the statistical difference was analyzed by using one-way ANOVA test. The mean value of total protein was minimum (5.012±1.493) g/dl in NS group and was maximum (6.212±1.618) in OM group; which

Group	Age			Sex	
	Minimum Age (Yrs)	Maximum Age (Yrs)	Mean Age (Yrs)	Male	Female
OSMF	18	79	43.66	45	05
OL	24	75	46.32	47	03
NS	20	80	51.74	50	00
OM	30	82	50.20	29	21
HC	21	65	39.68	22	28
Overall	18	82	46.32±13.89	193	57

[Table/Fig-3]: Distribution of participants according to age and sex.
(OSMF – Oral Submucous Fibrosis, OL – Oral Leukoplakia, NS – Nicotina Stomatitis, OM – Oral Malignancy, HC – Healthy Control, Yrs. – Years)

Study Group	Serum Protein levels (g/dl) Mean±SD	p-value	Serum Albumin levels (g/dl) Mean±SD	p-value	Serum Globulin levels (g/dl) Mean±SD	p-value
OSMF	5.442±1.214	0.000 Highly Significant (p<0.001)	3.762±0.716	0.102 Not Significant (p>0.05)	1.680±0.830	0.000 Highly Significant (p<0.001)
OL	5.410±1.192		3.417±0.870		1.994±0.790	
NS	5.012±1.493		3.389±0.911		1.623±0.844	
OM	6.212±1.618		3.548±0.799		2.675±1.145	
Healthy	6.154±1.579		3.644±0.619		2.509±1.219	

[Table/Fig-4]: Serum levels of proteins (one-way ANOVA).
(OSMF – Oral Submucous Fibrosis, OL – Oral Leukoplakia, NS – Nicotina Stomatitis, OM – Oral Malignancy, HC – Healthy Control, g/dl – gram/deciliter, p- probability)

Study Groups		Mean Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
OSMF	OL	0.03220	0.28610	1.000	-0.7541	0.8185
	NS	0.43060	0.28610	0.560	-0.3557	1.2169
	OM	-0.77000	0.28610	0.058	-1.5563	0.0163
	HC	-0.71120	0.28610	0.097	-1.4975	0.0751
OL	OSMF	-0.03220	0.28610	1.000	-0.8185	0.7541
	NS	0.39840	0.28610	0.633	-0.3879	1.1847
	OM	-0.80220	0.28610	0.043(S)	-1.5885	-0.0159
	HC	-0.74340	0.28610	0.074	-1.5297	0.0429
NS	OSMF	-0.43060	0.28610	0.560	-1.2169	0.3557
	OL	-0.39840	0.28610	0.633	-1.1847	0.3879
	OM	-1.20060	0.28610	0.000(HS)	-1.9869	-0.4143
	HC	-1.14180	0.28610	0.001(S)	-1.9281	-0.3555
OM	OSMF	0.77000	0.28610	0.058	-0.0163	1.5563
	OL	0.80220	0.28610	0.043(S)	0.0159	1.5885
	NS	1.20060	0.28610	0.000(HS)	0.4143	1.9869
	HC	0.05880	0.28610	1.000	-0.7275	0.8451
HC	OSMF	0.71120	0.28610	0.097	-0.0751	1.4975
	OL	0.74340	0.28610	0.074	-0.0429	1.5297
	NS	1.14180	0.28610	0.001(S)	0.3555	1.9281
	OM	-0.05880	0.28610	1.000	-0.8451	0.7275

[Table/Fig-5]: Intergroup comparison of serum protein levels (Tukey's Post-Hoc Analysis).
(OSMF – Oral Submucous Fibrosis, OL – Oral Leukoplakia, NS – Nicotina Stomatitis, OM – Oral Malignancy, HC – Healthy Control, S – Significant, HS – Highly Significant, p - probability)

Study Groups		Mean Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
OSMF	OL	0.34560	0.15803	0.188	-0.0887	0.7799
	NS	0.37320	0.15803	0.130	-0.0611	0.8075
	OM	0.21460	0.15803	0.655	-0.2197	0.6489
	HC	0.11780	0.15803	0.946	-0.3165	0.5521
OL	OSMF	-0.34560	0.15803	0.188	-0.7799	0.0887
	NS	0.02760	0.15803	1.000	-0.4067	0.4619
	OM	-0.13100	0.15803	0.921	-0.5653	0.3033
	HC	-0.22780	0.15803	0.601	-0.6621	0.2065
NS	OSMF	-0.37320	0.15803	0.130	-0.8075	0.0611
	OL	-0.02760	0.15803	1.000	-0.4619	0.4067
	OM	-0.15860	0.15803	0.854	-0.5929	0.2757
	HC	-0.25540	0.15803	0.489	-0.6897	0.1789
OM	OSMF	-0.21460	0.15803	0.655	-0.6489	0.2197
	OL	0.13100	0.15803	0.921	-0.3033	0.5653
	NS	0.15860	0.15803	0.854	-0.2757	0.5929
	HC	-0.09680	0.15803	0.973	-0.5311	0.3375
HC	OSMF	-0.11780	0.15803	0.946	-0.5521	0.3165
	OL	0.22780	0.15803	0.601	-0.2065	0.6621
	NS	0.25540	0.15803	0.489	-0.1789	0.6897
	OM	0.09680	0.15803	0.973	-0.3375	0.5311

[Table/Fig-6]: Intergroup comparison of serum albumin levels (Tukey's Post-Hoc analysis).
(OSMF – Oral Submucous Fibrosis, OL – Oral Leukoplakia, NS – Nicotina Stomatitis, OM – Oral Malignancy, HC – Healthy Control, p - probability)

was statistically significant (p<0.001). The mean value of albumin was minimum (3.389±0.911) g/dl in NS group and maximum (3.762±0.716) g/dl in OSMF group; which was statistically not significant (p>0.05). Similarly, the mean value of globulin was minimum (1.623±0.844) g/dl in NS group and maximum (2.675±1.145) g/di in OM group; which

Study Groups		Mean Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
OSMF	OL	-0.31340	0.19643	0.502	-0.8532	0.2264
	NS	0.05740	0.19643	0.998	-0.4824	0.5972
	OM	-0.99460	0.19643	0.000(HS)	-1.5344	-0.4548
	HC	-0.82900	0.19643	0.000(HS)	-1.3688	-0.2892
OL	OSMF	0.31340	0.19643	0.502	-0.2264	0.8532
	NS	0.37080	0.19643	0.327	-0.1690	0.9106
	OM	-0.68120	0.19643	0.006(S)	-1.2210	-0.1414
	HC	-0.51560	0.19643	0.069	-1.0554	0.0242
NS	OSMF	-0.05740	0.19643	0.998	-0.5972	0.4824
	OL	-0.37080	0.19643	0.327	-0.9106	0.1690
	OM	-1.05200	0.19643	0.000(HS)	-1.5918	-0.5122
	HC	-0.88640	0.19643	0.000(HS)	-1.4262	-0.3466
OM	OSMF	0.99460	0.19643	0.000(HS)	0.4548	1.5344
	OL	0.68120	0.19643	0.006(S)	0.1414	1.2210
	NS	1.05200	0.19643	0.000(HS)	0.5122	1.5918
	HC	0.16560	0.19643	0.917	-0.3742	0.7054
HC	OSMF	0.82900	0.19643	0.000(HS)	0.2892	1.3688
	OL	0.51560	0.19643	0.069	-0.0242	1.0554
	NS	0.88640	0.19643	0.000(HS)	0.3466	1.4262
	OM	-0.16560	0.19643	0.917	-0.7054	0.3742

[Table/Fig-7]: Intergroup comparison of serum globulin levels (Tukey's Post-Hoc analysis). (OSMF – Oral Submucous Fibrosis, OL – Oral Leukoplakia, NS – Nicotina Stomatitis, OM – Oral Malignancy, HC – Healthy Control, HS – Highly Significant, S – Significant, p - probability)

was statistically significant (p<0.001) [Table/Fig-4].

The correlation of total protein levels between each study group was performed by Tukey's Post-Hoc analysis. The difference was statistically significant (p<0.05) between OL and OM group, and NS and HC group; whereas statistically highly significant (p<0.001) difference was noted between NS and OM group [Table/Fig-5].

The correlation of albumin level between each study group was performed by Tukey's Post-Hoc analysis and was statistically not significant (p>0.05) [Table/Fig-6].

The correlation of globulin levels between each study group was performed by Tukey's Post-Hoc analysis. The difference was statistically significant between OL and OM groups (p<0.05); whereas statistically highly significant difference was noticed between OSMF and OM, OSMF and HC groups, NS and OM, NS and HC groups (p<0.001) [Table/Fig-7].

DISCUSSION

The term free radical is generally used to describe a molecular fragment containing one or more unpaired electron in its valance shell and is capable of existing independently. Free radicals in high concentrations interact with intracellular macromolecules such as DNA, proteins, carbohydrate and lipid thereby, initiating and promoting inflammation and carcinogenesis [13]. Oxidation of protein plays an important role in pathogenesis of cancer and studies have demonstrated decreased protein levels in cases of OPMD's and oral malignancy [8,9]. In oral cancer, tobacco and areca nut related habit leading to tissue damage and resultant free radicals play a major role as an aetiologic factor. These habits are seen commonly in all the ages and both the sex.

The serum protein levels were decreased in OSMF, OL and NS but increased in OM. This difference was statistically significant (p<0.001). These findings matched with the findings of Patidar KA et al., and Rajendran R et al., in OSMF participants and Dawood RM et al., in OM participants [5,6,14]. But our results did not simulate with the results of Chandran V et al., in OM group in which the plasma protein levels were found to be decreased [8]. The increase in serum protein levels may be explained in terms of inflammatory reaction associated with oral malignancy.

The intergroup comparison of serum albumin was statistically not significant (p> 0.05) and this was not in accordance with the studies of Rajendran R et al., Chandran V et al., Nayyar AS et al., and Singh P et al., [6,8-10]. The intergroup comparison of serum globulin levels was statistically highly significant (p<0.001). The serum globulin levels were decreased in OSMF, OL and NS but increased in OM. This finding was similar to that of Dawood RM et al., [14]. The increase in serum globulin levels may be due to its action as an acute phase reactant.

LIMITATION

The Limitation of the study is that epithelial dysplasia and correlation is not included in the present study.

CONCLUSION

The present study demonstrated decrease in serum protein, albumin and globulin levels in OSMF, OL and NS but increase serum levels of proteins in OM. It may be concluded that serum protein may act as a reliable biomarker for OPMD's and OM.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Apr 19, 2016**
Date of Peer Review: **May 09, 2016**
Date of Acceptance: **Nov 12, 2016**
Date of Publishing: **Feb 01, 2017**