ASSESSMENT OF SERUM PARAOXONASE-1 ENZYME ACTIVITY, MALONDIALDEHYDE AND VITAMIN-C IN ORAL PREMALIGNANCIES

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ABSTRACT

Background: Oral premalignancies are a group of disease or syndromes which if left untreated can lead to cancer. It carries a great significance in Indian perspective. The actual figure of oral cancers arising from oral premalignancies is not known and to predict accurately the malignant transformations of them is still not possible. Oxidative stress is a known player behind cancerogenesis. Recently decreased Paraoxonase-1 activity and increased oxidative stress markers ware found to be associated with Oral Squamous Cell Carcinoma. So, there is a strong possibility of a similar finding in Oral Premalignancies too. **Aim**: This study aims to investigate the correlation between serum PON-1 activity and oxidative stress markers (MDA & Vitamin C) in patients with Oral Premalignancies. **Material and Methods**: A total of 62 patients with clinically diagnosed oral premalignant lesions and diseased controls were chosen for the study. Venous blood samples were collected and PON-1, MDA (in serum) & Vitamin c (in plasma) were analysed spectro-photometrically. **Results:** A significant decreased serum PON-1 activity ($P<0.05$) and concomitant significantly increased serum MDA ($P<0.05$) and decreased Vitamin C levels ($P<0.05$) were observed in premalignancies compared to the controls. These finding were more pronounced in Oral Leukoplakia (OL) than in Oral Submucous Fibrosis (OSMF) with a significant difference. Mean levels of the analysed parameters differed accordingly in the clinical grades of oral premalignancies. **Conclusion:** It can be envisaged that serum PON-1 activity and increased oxidative stress might be a contributing factor behind pathogenesis and progression of Oral Premalignant Diseases.

KEYWORDS: Premalignancies; Cancerogenesis; PON-1.

INTRODUCTION

Oral premalignant lesions also known as potentially malignant disorders are a group of disease or syndromes which if left untreated can lead to oral cancer [1]. This way it carries a great significance in Indian perspective, as oral cancer shares about 13-16% of the cancer load of India [2]. It is seen that maximum oral cancers are squamous cell cancers, where some of them can arise from an apparently normal mucosa while others arise from some clinically obvious premalignant lesions [3]. In Indian context, in contrary to that of western countries, these diseases are seen to be maximally coexistent with life style related problems like smoking, chewing tobacco, paan, betel nut, ghutkha, alcohol drinking and so on [4]. These known addiction substances can induce oxidative stress by several modes. Interplay of oxidative stress and chronic inflammation is one of them [5]. Serum PON-1 is a part of body's complex antioxidant system that is known to confer antioxidant and antiatherogenic role to HDL [6]. A greater prevalence of PON-1 192 glutamine allele and reduced PON-1 activity was seen in Oral Squamous Cell Carcinoma Patients (OSCC) and in Turkish Lung cancer patients [7, 8]. These findings in OSCC suggest that PON-1activity may be a contributing factor to the progression of Oral Premalignancies too.

The present study was therefore undertaken to understand the molecular etiopathogenesis of oral premalignancies by assessing the extent of lipid peroxidation and oxidative stress level through estimation of PON-1 activity, MDA and vitamin C levels.

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MATERIALS AND METHODS

Study design: This analytical case control observational study.

Ethics approval: The protocol of this study was approved by the Institutional Human Ethics Committee.

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Study location: The study was carried out in Dept. of Biochemistry in collaboration with Dept. of ENT at MGIMS, Sewagram.

Sample selection: Patients with oral premalignant lesions and diseased controls were recruited after obtaining their written consent in regional language.

Sample size: A total number of 63 samples.

Inclusion criteria: Cases of premalignancies were chosen totally on clinical diagnosis. They were recruited within the age group of 20 to 60years without any sex differentiation. Controls were taken considering the exclusion criteria

Exclusion criteria: with no history of smoking and any past or present history of oral premalignancy were excluded from the study.

Grouping: Subjects were mainly divided into two group:

Group 1: Control.

Group 2: Test group, clinically according to the severity each of them were again divided into different subgroups. OSMF cases were divided into clinical Grades of I, II & III and OL group was divided in Homogenous and Non-Homogenous clinically.

Methodology: Clinical and demographic data was collected regarding their smoking habits and other risk behaviors by proforma method.

About 10 ml venous blood was collected from participants in plain (for serum) and EDTA (for plasma) vials. Serum was used for PON-1, MDA estimation and plasma for Vitamin C.

Estimation of PON- 1 Activity: Serum Paraoxonase 1 activity was measured spectrophotometrically (ELICO) according to Gan et al. (1991) [9] with some modifications. Using Paraoxon (Paraoxon Ethyl of Sigma-Eldrich) as a substrate, concentration of the product p-nitrophenol was measured at 420 nm using molar extinction coefficient of 18,290 M-1 cm-1 at pH 10.

Estimation of Malondialdehyde (MDA): Serum MDA level was measured according to Buege & Aust SD (1978) [10]. MDA in serumreacts with thiobarbituric acid (TBA) (Sigma-Eldrich) to form a colored product, absorbance of which was measured spectrophotometrically (ELICO) at 535 nm. The malondialdehyde concentration of the sample was calculated using the extinction coefficient of 1.56 x 105 M-1 cm-1.

Estimation of Vitamin C: Plasma Vitamin C was measured according to Aye Kyaw, (1978) [11]. The acid phototungstate used in this method serves as plasma protein precipitant as well as ascorbic acid extractant and color developing agent, as it gets reduced to tungstate blue by ascorbic acid. The blue color was measured in spectrophotometer (ELICO) at 700 nm. Absorbancewas read is read against blank, constituted with distilled water (instead of plasma), and subjected to all treatments simultaneously as test samples. For every set, a standard (Sigma-Eldrich) and blank are run through the procedure.

Statistical analysis: For comparison in the groups we used unpaired t test, ANOVA, TUKEY HSD and for correlation analysis we used Pearson's Correlation Test. A 'p-value' less than 0.05 was considered significant.

RESULTS

OSMF cases were divided into clinical Grades of I, II & III (after Anil K Ghom) [12] and OL group was divided in Homogenous and Non-Homogenous clinically (after S Warnakulasuriya et al) [13].

There were 5, 7 & 8 subjects in the subgroups of Grades of I, Grade II & Grade III OSMF accordingly. Likewise, in Oral Leukoplakia there were 12 subjects in Non-homogenous subgroup and 10 subjects in Homogenous leukoplakia subgroups. Tobacco related addiction like Paan, Kharra, tobacco chew, smoking were the common addiction habits in both the premalignant groups with a mean age addicting of 6 years.

Mean serum PON-1 activity was significantly lower in Leucoplakia then OSMF (*P<0.05*), OSMF than controls (*P<0.05*) and Leucoplakia than controls (*P<0.05*) Likewise, Vitamin C level was also significantly lower in Leucoplakia than OSMF (*P<0.05*), OSMF than controls (p<0.05) and Leucoplakia than controls (*P<0.05*). But in contrast the serum MDA level were significantly higher in the same respective groups that is in Leucoplakia than OSMF (*P<0.05*), OSMF than control (*P<0.05*) and Leucoplakia than control (*P<0.05*).

DISCUSSION

The most commonly found PMD are OSMF, OL and erythroplakia [14]. In our study we got two groups of premalignancies i.e. OSMF and Oral Leukoplakia. Clinically, on inspection of oral mucosa, one can find a chronic fibrotic change in OSMF and white patches that cannot be characterized otherwise in Oral Leukoplakia. The malignant transformation rate for OSMF is 7.6% in17 years of follow up and for OL it is about 13.6% globally [15, 16]. In India, according to

various studies, there is a strikingly varied prevalence of both OSMF (0.03 to 3.2%) and OL (0.2% to 4.9%) [17,18].

Oral Premalignancies are found to share common etiological insults and common geographical distribution like Oral Cancers in India [19]. Thus, in these backgrounds mentioned and moreover, the absence of an established etiopathogenic mode and inability to predict a malignant transformation, makes oral

Table 1. The mean serum PON -1 activity, MDA concentration and plasma Vitamin C level in the test groups.

Parameters	Controls (Mean ± SD)	OSMF (Mean ± SD)	Oral Leukoplakia (Mean ± SD)
PON-1 Activity (unit/ ml)	313.02 ± 87.93	265.00 ± 26.64*	170.39 ± 33.42*£
MDA (µmol/L)	0.7645 ± 0.178	2.34 ± 0.67*	2.94 ± 0.64*£
Vitamin C (mg/dl)	3.14 ± 0.395	2.09 ± 0.55*	1.51 ± 0.47*£

Statistically Significant (*P<0.05*) in comparison to Control (*), OSMF (£)

Table 2. Mean value in different disease subgroups

Mean Value in different stages				
Disease	**Subgroups**	**PON-1 activity(unit/ml)**	**MDA (µmol/L)**	**Vitamin C (mg/dl)**
OSMF	**Grade I**	278.99 ± 27.24	1.113 ± 0.417	2.625 ± 0.25
	Grade II	269.69 ± 27.19	1.7181 ± 0.219	2.2 ± 0.434
	Grade III	251.63 ± 22.94	2.4844 ± 0.235	1.6571 ± 0.475
OL	**Homogenous**	184.24 ± 33.02	2.58 ± 0.482	1.9 ± 0.326
	Non- homoge-nous	159.73 ± 30.77	3.3192 ± 0.522	1.2061 ± 0.305
Control		313.02 ± 87.93	0.7645 ± 0.178	3.14 ± 0.395

Table 3: Inter-comparison of PON-1, MDA & Vitamin C Level in Different clinical grades of OSMF & Oral Leukoplakia by TUKEY HSD

	OSMF		**P value**	**Oral Leukoplakia**	**P value**
Pon1	Grade I	Grade II	0.645	Homogenous vs Non-homogenous	0.030
		Grade III	0.119		
	Grade II	Grade III	0.372		
MDA	Grade I	Grade II	<0.05	Homogenous vs Non-homogenous	0.004
		Grade III	<0.05		
	Grade II	Grade III	<0.05		
Vitamin C	Grade I	Grade II	0.308	Homogenous vs Non-homogenous	0.001
		Grade III	<0.05		
	Grade II	Grade III	<0.05		

premalignancy an important candidate to be investigated in line of oral cancer, at least in Indian perspective.

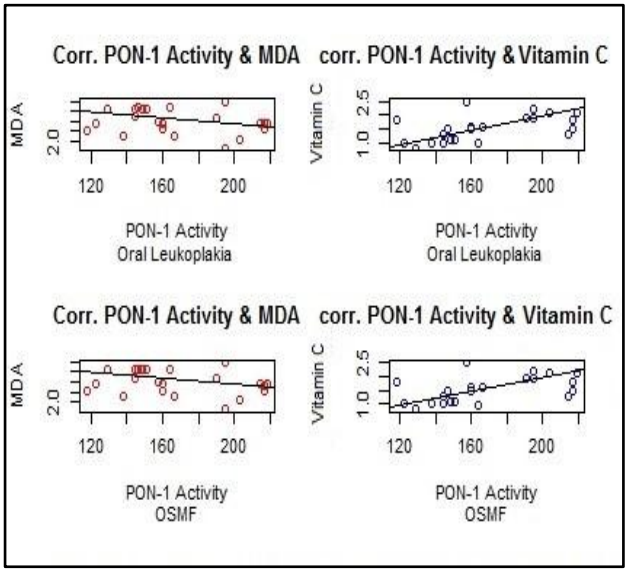
Talking about molecular mechanisms behind, oxidative stress is a known culprit behind cancerogenesis and amongst the various mechanisms that our body has developed to fight back, Paraoxonase -1 enzyme is one of them. PON -1, a hydrolytic enzyme with a wide range of substrate, gathered a significant interest as a protein that is mainly responsible for most of the antioxidant properties of High Density Lipoprotein (HDL). Its beneficial role against atherosclerosis is already well documented. PON-1 gene is mainly expressed in liver and it is transported in the circulation in association with lipoproteins and get delivered to the multiple other sites who doesn't synthesise PON-1 by themselves. PON-1 by interacting with Apo-A binds to HDL and gets circulated along the blood circulation. An increased accumulation of PON-1 in the media and intima of the aorta in atherosclerotic arties is attributed to macrophages which also connects the link of PON-1 to lipid peroxidation. Thus, it is seen that PON-1 has a capacity to protect our cells from lipid peroxidation [20]. By virtue of antioxidant property PON-1 enzyme achieved a heavily suspected role in pathogenesis of OSCC, which makes it an important candidate to be investigated in Oral Premalignancies too. In our study, wefound a significant decrease in serum PON-1 activity in both the premalignancies than the control group (Table1). Even in oral leucoplakia group mean serum PON-1 activity was significantly lower (*P<0.05*) than OSMF (Table1). A gradual decrease in the mean serum PON-

1 activity in clinical grades of OSMF from Grade I to Grade III was observed, though the difference was not statistically significant (Table2, Table 3). Probably this is due to small sample size of these subgroups. However, interestingly, there was a statistically significant decrease (*P<0.05*) in PON-1 activity from Homogenous to Non- Homogenous Leukoplakia subgroups (*P<0.05*) (Table2, Table 3).Smokers are known to have a significant decrease in PON-1 activity along with higher propensity to have oxidized Low-Density Lipoprotein (LDL) and HDL. But the molecular mechanism is not clear till now [21].

Table 4. Correlation between serum PON-1 activity with MDA and Vitamin C in the study groups

Correlation in Pon-1 and MDA		Correlation in Pon-1 and Vitamin C	
Group	Pearson Correlation	Group	Pearson Correlation
OSMF	-0.421	OSMF	0.025
leukoplakia	-0.156	leukoplakia	0.522
Control	0.002	Control	-0.073

Fig 1: Scatter Plot showing Correlation between serum PON-1 activity with MDA and Vitamin C



The addiction habits of our study population substantiate our findings of decreased serum PON-1 activity, andpositively indicates that the decrease in the protective antioxidant role of PON-1 is playing some role in their progression.

Reactive Oxygen species degrades polyunsaturated lipids forming malondialdehyde. This reactive aldehyde by its electrophilic properties produces advanced lipoxidation end products (ALE) by causing toxic stress in cells. This ALE is widely used as a biomarker to measure lipid peroxidation related oxidative stress in organisms.In this study, we found a gradual increase in the serum MDA level in both the Oral PMDs groups as compared to controls. This increase in MDA level was also significantly increased (*P<0.05*) in Oral Leukoplakia than OSMF (Table 1). Our findings were consistent with that of RH Chole et al [22]. On further analysis there were statistically significant increase (*P<0.05*) in the MDA level within all the clinical grades OSMF according to severityand from Homogenous to Non-Homogenous Leukoplakia (Table2, Table3). Our study correlates nicely with S Gupta et al [23] for OSMF cases and Metgud R & Bajaj S [24] for Oral Leukoplakia cases. A finding like this indicates the strong role of lipid peroxidation behind the progression of OSMF and Oral leukoplakia from normal mucosal epithelium.

Frie et al showed that plasma ascorbic acid is the only endogenous antioxidant which is able to completely protect the lipids from detectable peroxidative damage induced by aqueous peroxyl radicals [25]. It acts as a co-antioxidant that regenerates α-tocopherol from α- tocopheroxy radical generated during the scavenging of free radicals. In this study we have estimated the antioxidant vitamins in plasma expecting that circulating vitamins are better reflected than their tissue/cellular levels. We found a statistically significant decrease in the mean plasma Ascorbic Acid in Oral PMD from controls (Table 1). The decrease was also significant in OL than OSMF (*P<0.05*) (Table 1). Except for Grade I and Grade II OSMF

Vitamin C level was significantly lower (*P<0.05*) from less severe to more severe clinical subgroup of premalignancies (Table 2, Table 3). Our overall findings are in agreement with those of Dr. Sarita Basu [26]. Vitamin C by its oxidizing properties reacts with superoxide produced from normal cellular metabolism; this in turn inhibits formation of nitrosamines during protein digestion and prevents damage to DNA and other cellular proteins. But very little is documented regarding the ability of Ascorbic Acid to maintain the oral mucosal integrity. In a study conducted by V. Touvinen et al, a significant higher percentage of Oral Leukoplakia than any other Oral Premalignancies were found in Vitamin C deficient cases [27].

Again, in OSMF, it was found that there was an increase in the production of highly cross linked insoluble collagen Type I, increase loss of soluble procollagen type III and collagen type VI. This cross-linking is attributed to the upregulation of lysyl oxidase, playing a crucial role in the development of Grade II OSMF from Grade I [28]. A decrease of such in plasma Vitamin C level probably indicates to its more utilization towards collagen synthesis. This hypothesis is also strengthened by our findings of significant reduction of plasma vitamin C level in oral premalignancies than controls. PON-1 is also found to be negatively correlating with MDA and positively correlating with Vita-

min C in both OSMF and Oral Leukoplakia groups. In OSMF, PON-1 had fair negative correlation with MDA and in Leukoplakia it was rather fair positively correlated with Vitamin C. Despite these minor differences, it can be aptly said that overall finding suggests that decreased PON-1 activity was associated with increased oxidative stress parameters (increased MDA, decreased Vitamin C) in Oral Premalignant Disease patients.

CONCLUSION

From this finding it can be envisaged and concluded that the interplay between decreased serum PON-1 activity and increased in oxidative stress do play some role in the etiopathogenesis and progression of Oral Premalignant Diseases. Further large-scale studies for genetic susceptibility, PON-1 gene polymorphism, PON-1 activity in non -tobacco related oral Premalignancies are needed to substantiate our findings.

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Conflict of interest: Nil

REFERENCES

1. Carnelio S, Rodrigues GS, Shenoy R, Fernandes D. A Brief Review of Common Oral Premalignant Lesions with Emphasis on Their Management and Cancer Prevention. The Indian Journal of Surgery. 2011;73(4):256-261.
2. Gururaj G, Girish N, Isaac MK. NCMH Background Papers-Burden of disease in India. New Delhi: Ministry of Health & Family Welfare. 2005
3. Markopoulos AK. Current aspects on oral squamous cell carcinoma. The open dentistry journal. 2012; 6:126.
4. Gupta S, Singh R, Gupta OP, Tripathi A. Prevalence of oral cancer and pre-cancerous lesions and the association with numerous risk factors in North India: A hospital based study. National Journal of Maxillofacial Surgery. 2014;5(2):142-148.
5. Katakwar P, Metgud R, Naik S, Mittal R. Oxidative stress marker in oral cancer: a review. Journal of cancer research and therapeutics. 2016;12 (2):438.
6. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol.Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med. 1989;321(17):1196-7.
7. Aksoy-Sagirli P, Cakmakoglu B, Isbir T, Kaytan-Saglam E, Kizir A, Topuz E, Berkkan H. Paraoxonase-1 192/55 polymorphisms and the risk of lung cancer in a Turkish population. Anticancer research. 2011; 31(6):2225-9.
8. Metin ZB, Aydin S, Unur M, Cakmakoglu B, Toptas B, Hafiz G, İsbir T. Oral squamous cell carcinoma and serum paraoxonase 1. The Journal of Laryngology & Otology. 2013; 127(12):1208-13.
9. Gan KN, Smolen AN, Eckerson HW, La Du BN. Purification of human serum paraoxonase/ arylesterase. Evidence for one esterase catalyzing both activities. Drug Metabolism and Disposition. 1991;19(1):100-6
10. Buege JA, Aust SD. The thiobarbituric acid assay Metods Enzymmol 1978; 52:306-7.
11. Kyaw A. A simple colorimetric method for ascorbic acid determination in blood plasma. Clinica chimica acta. 1978 Jun 1; 86(2):153-7
12. Ghom AG, Ghom SA. Textbook of oral medicine. JP Medical Ltd; 2014 Sep 30,Chapter 12,Page 221
13. Warnakulasuriya S, Johnson N, Van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. Journal of oral pathology & medicine. 2007; 36(10):575-80.
14. Yardimci G, Kutlubay Z, Engin B, Tuzun Y. Precancerous lesions of oral mucosa. World Journal of Clinical Cases : WJCC. 2014;2(12):866-872.
15. Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC, Mehta FS. Malignant transformation rate in oral submucous fibrosis over a 17-year period. Community Dent Oral Epidemiol. 1985;13 (6):340-1.
16. Petti S. Pooled estimate of world leukoplakia prevalence: A systemic review. Oral Oncol. 2003;39:770-80
17. Mehta FS, Gupta PC, Daftary DK, Pindborg JJ, Choksi SK. An epidemiological study of oral cancer and precancerous condition among 101,761 villagers in Maharashtra, India. Int J Cancer. 1972;10:134–41.
18. Pindborg JJ, Chawla TN, Mishra RK, Nagpaul RK, Gupta VK. Frequency of oral carcinoma, leukoplakia, leukokeratosis, leukoedema, sub mucous fibrosis and lichen planus in 10,000 Indians in Lucknow, Uttar Pradesh India; Preliminary Report. J Dent Res. 1965;44:61.
19. Saran S, Agrawal GN. Cancer pattern in central U.P. Indian J Cancer. 1984;21(5):133-6.
20. Mackness B, Hunt R, Durrington PN, Mackness MI. Increased immunolocalization of

paraoxonase, clusterin, and apolipoprotein AI in the human artery wall with the progression of atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology. d1997; 17(7):1233-8.

21. Mouhamed DH, Ezzaher A, Mechri A, Neffati F, Omezzine A, Bouslama A, Gaha L, Douki W, Najjar MF. Effect of cigarette smoking on paraoxonase 1 activity according to PON1 L55M and PON1 Q192R gene polymorphisms. Environmental health and preventive medicine. 2012; 17(4):316-21

22. Chole RH, Patil RN, Basak A, Palandurkar K, Bhowate R. Estimation of serum malondialdehyde in oral cancer and precancer and its association with healthy individuals, gender, alcohol, and tobacco abuse. Journal of cancer research and therapeutics. 2010 Oct 1; 6 (4):487.

23. Gupta S, Reddy MV, Harinath BC. Role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. Indian journal of Clinical biochemistry. 2004 Jan 1; 19(1):138.

24. Metgud R, Bajaj S. Evaluation of salivary and serum lipid peroxidation, and glutathione in oral leukoplakia and oral squamous cell carcinoma. Journal of oral science. 2014; 56 (2):135-42.

25. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci U S A. 1989; 86(16):6377-81.

26. Basu S. Antioxidant Changes in Precancerous and Cancerous Lesions of Oral Cavity. International Journal of Life Sciences Research. 2015; 3(1):23-28.

27. Tuovinen V, Väänänen M, Kullaa A, Karinpää A, Markkanen H, Kumpusalo E. Oral mucosal changes related to plasma ascorbic acid levels. Proc Finn Dent Soc.1992;88(3-4):117-22.

28. Rajendran R. Oral submucous fibrosis: etiology, pathogenesis, and future research. Bulletin of the World Health Organization. 1994;72(6):985.

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