

A comparative study of biochemical markers in precancerous and cancerous subjects with normal healthy individuals in Gujarat Population

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Abstract

Introduction: Globally oral cancer is the sixth most common cause of the death with India accounts for 86% of the world's oral cancer cases.⁽¹⁾ Chronic use of tobacco, areca nuts and betel quid consumption results in progressive premalignant condition called oral sub mucous fibrosis.⁽²⁾ Free radicals are released during the metabolism of tobacco and areca nut, that's associated cell damage, leads to an imbalance between the pro-oxidant and antioxidant states. This imbalance plays an important causative role in carcinogenesis.⁽³⁾ This malignant transformation rate is around 7.6%.⁽⁴⁾

Materials and Method: Total 150 patients are selected for this study, out of which 50 patients are diagnosed oral submucous fibrosis, 50 patients are oral cancer and 50 patients are healthy non tobacco consuming control were included. In this study oxidative stress, Ascorbic acid, α -tocopherol, copper, iron and acute phase protein were measured by using standard kit method.

Result: Serum Melondialdehyde, C-reactive protein and copper concentration was found to be significantly higher ($p < 0.05$) as compared to healthy control due to the generation of free radicals by lipid peroxidation. Serum iron, Ascorbic acid and α -tocopherol were found to be significantly reduced ($p < 0.001$) than healthy control due to damage the epithelium and disturbance in collagen metabolism.

Conclusion: Our observation suggested that serum Melondialdehyde, vitamin C, vitamin E can be used as marker of OSMF. Decreased Vitamin C & E is closely associated with increased Melondialdehyde, as the marker of oxidative stress is significant. Among the all trace elements, Serum copper and iron are highly significant markers for the early diagnosis of oral submucous fibrosis. The CRP Concentration is associated with development of oral cancer and also serves as a potential prognostic biomarker.

Keywords: Oxidative stress, Antioxidant status, Trace elements, C-reactive protein

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Introduction

Oral submucous fibrosis (OSMF) is a precancerous condition caused by use of the areca nut. Oral submucous fibrosis (OSMF) is a chronic, progressive, and debilitating disease that was first reported from India in 1953.⁽⁵⁾ Initially the disease was found only amongst people living in the Indian subcontinent or in Indian migrants to other countries. Later, it was reported from many South-east Asian populations.⁽⁶⁾ In this disease the oral mucosa loses its elasticity and fibrous bands develop. The epithelium is atrophic and there is a marked intolerance to spicy food. The opening of the mouth progressively reduces and in extreme cases it may be difficult for even a straw to pass into the mouth.⁽⁵⁾

The etiology of oral submucous fibrosis is multifactorial. The etiological factors include local irritants such as chili consumption, areca nut chewing, tobacco chewing and smoking. Systemic factors include anemia (iron deficiency), vitamin deficiencies (B-complex and folate) together with the malnourished state (protein deficiency), genetic predisposition to the disease and autoimmunity.⁽⁷⁾

Oral Cancer is one of the most common causes of mortality and morbidity today with more than 10

million new case and more than 6 million deaths each year worldwide. Oral cancer is the sixth most common cause of cancer related death. Oral cancer accounts for approximately 30-40% of all cancers in India.⁽⁸⁾ Despite the recent advance in tumor surgery and multimodal treatment regimes, the prognosis of oral squamous cell carcinoma is still relatively poor. This may be because symptoms that indicate the presence of the carcinoma often appear when the tumor is in an advanced stage.⁽⁹⁾

As is the norm with other disease afflictions, wide ranges of investigations have been carried out in the condition to identify the causation and pathogenesis. Biochemical investigations of blood, serum, and tissues have been the earliest form of interventions. Such investigations have largely helped to localize parameters that predispose to the development of the condition, modify its behavior and prognosticate on its malignant transformation potential.⁽⁸⁾

The present review attempts to collate the biochemical investigation carried out in OSMF and relates the importance of the parameters evaluated.^(8,9)

Materials and Method

This study was conducted in Department of Biochemistry, Pramukhswami Medical College,

Karamsad affiliated to Sardar Patel University, Vallabh Vidyanagar with the collaboration of oral diagnostic department in College of dental Sciences & research center and Gujarat cancer Institute of medical Research. Total 150 patient serum samples were collected in three different groups, in which 50 diagnosed oral submucous fibrosis Patients, 50 diagnosed oral cancer patients and 50 normal healthy controls. Five to seven ml blood was collected in fasting stage under aseptic condition with the patient consent. All the parameters were determined by using semi auto analyzer and fully auto analyzer (Erbospital and sinta cam 50 & Mura logo tech respectively) with ready to used kits.

Statistical Analysis: All data were expressed as mean and standard deviation; SPSS 17 program was used for the analysis. The statistical significance of the mean in the subject and control groups was assessed using the paired Student t test. Correlations were computed based on Pearson's correlation analysis. A significant level of

95% was chosen for all tests $p < 0.05$. For student t test, the significance was represented as $p < 0.001$, where in it was considered as highly significant.

Results

These results were obtained following the study undertaken on OSMF and Oral cancer in Gujarat population. Result of c-reactive protein and MDA have been highlighted, in the light of mouth cancer. The total number of subjects in the study is 50 OSMF patients and 50 oral cancer patients with 50 healthy controls; the mean age of subjects is 20-65 years. The Mean and standard deviation of biochemical measurements of control and subjects are considered in distribution which pertains to tobacco and betel nut chewing patients. The results show the significance as form of 'P' value. $P < 0.05$ was the level of statistical significance.

Table 1: Status of copper and iron in precancerous and cancerous stage

Variables	Study Group	N	Mean	Std. Dv	'p' Value
Copper	Control	50	114.07	7.79	<0.002**
	OSMF	50	153.57	7.68	
	Oral Cancer	50	164.43	15.75	
Iron	Control	50	141.03	5.56	<0.001**
	OSMF	50	77.62	15.27	
	Oral Cancer	50	50.04	19.97	

*indicates a significant value ($P < 0.05$), ** indicate a highly significant ($p < 0.001$)

The compare to mean and SD between the both study group its indicate that the copper levels are elevated in cancer patients compare to OSMF patients due to excessive tobacco, gutkka, pan masala consume and Iron levels are decreased in cancer patients compare to OSMF patients due to the defect in DNA synthesis and oxidative metabolism.

Table 2: Comparison of Mean &SD in all different study group

Study Group	Study parameters	
	Vitamin C	Vitamin E
	Mean±SD	Mean±SD
Healthy individuals	1.09±0.17	1.39±0.85
Precancerous patients	0.55±0.10	1.18±0.16
Cancerous Patients	0.44±0.09	0.8±0.12
'p' value	< 0.001**	< 0.0001**

*indicates a significant value ($P < 0.05$), ** indicate a highly significant ($p < 0.001$)

Vitamin C- Ascorbic acid **Vitamin E-** α - Tocopherol

The comparison of mean and standard deviation values between the oral sub-mucosal fibrosis patients and oral cancer patients has low vitamin c values than oral submucous fibrosis due to the defect in collagen metabolism and vitamin E is antioxidant that's goes down in cancer patients than OSMF patients due to increased free radicals.

Table 3: Evaluation of oxidative stress and Acute phase protein in pre cancerous and cancerous stage

Parameter	Group-1 (normal individuals)	Group-2 (pre-Cancerous Patients)	Group-3 (cancerous Patients)	'P' Value
	Mean±SD	Mean±SD	Mean±SD	
CRP	0.7±0.6	1.21±0.78	6.31±13.16	<0.05*
MDA	2.4±0.5	2.77±0.56	5.86±0.99	<0001**

*indicates a significant value ($P < 0.05$), ** indicate a highly significant ($p < 0.001$)

CRP- C-reactive Protein, MDA- Melondialdehyde

The comparison of mean and standard deviation values between the oral sub-mucosal fibrosis patients and oral cancer patients CRP values are increased in oral cancer individuals than the OSMF patients same as MDA levels are also high in cancer patients than OSMF patients due to increased levels of reactive oxygen species leads to increase the oxidative stress than the normal individual.

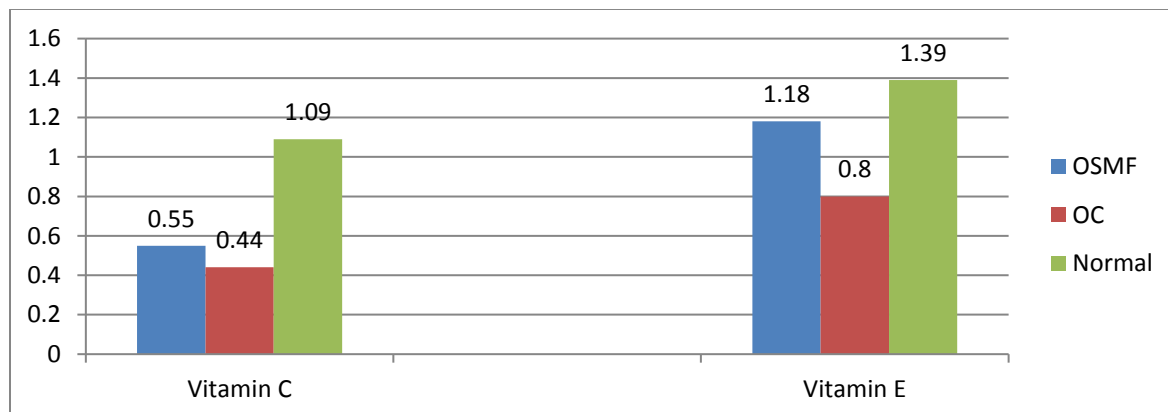


Fig. 1: Comparison of Mean &SD between study groups in Antioxidant status

Fig. 1 is depicts that level of Vit C and Vit E is gradually decreased than healthy control due to the damaged epithelium in oral cavity

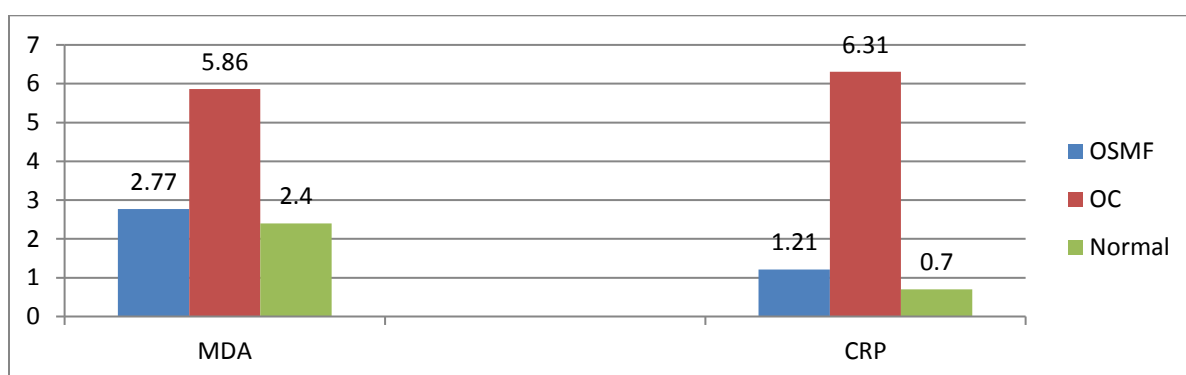


Fig. 2: Compare the mean between study group in oxidative stress and C-reactive protein

Fig. 2 shows that the highly significant status of oxidative stress and inflammatory marker in oral cancer patients than healthy control due to generation of free radicals on lipid peroxidation.

Discussion

The oral cavity is the site of the body where contact with exogenous material, microorganisms and harmful agents like tobacco, alcohol and areca nut are more intense.⁽¹⁰⁾ The oral mucosa functions as a mechanical as well as immunological barrier. Protective mechanisms are noted in the form of increased capacity for epithelial regeneration and increased keratinization. These epithelial changes are reactive and reversible but with progressive loss of normal control mechanisms, it leads to a precancerous state and oral cancer.⁽¹¹⁾ The major risk factor for oral cancer includes tobacco, alcohol and betel quid. Accordingly the geographic distribution of oral cancer is determined by these risk

factors. Tobacco chewing and smoking have been identified as major risk factor for oral cancer in India. On account of a greater consumption of tobacco and betel quid in India, the incidence of oral cancer is high⁽¹²⁾

In our study groups Serum copper levels was gradually increased in OSMF and oral cancer patients than normal healthy control patients it has been suggested that copper regulates the production of fibrosis via enzyme lysyl oxidase, which results in cross linking of collagen and subsequent resistance to degradation by enzyme. It does indicate that there is strong association with this element in the etiology of OSMF. According to Trivedy et al⁽¹³⁾ study copper content in areca nut was found to be more than the contents in the nuts in snacks consumed by the population. When comparing tissue and serum copper levels in patients chewing areca nuts preparations, they found consistently higher levels of copper in the serum. Iron is one of the most abundant and necessary

transition metals in the body, which is an essential component in DNA synthesis and in respiratory and oxidative metabolism⁽¹⁴⁾ In our study, Table 1 suggested that decreased iron levels in OSMF and oral cancer patients compare to normal healthy control due to defect in collagen metabolism by excessive tobacco, gutkha, pan masala consume, related to that defect in DNA synthesis and oxidative metabolism.

Vitamin C is one of the free radical scavenging non enzymatic antioxidants. This interacts with free radicals and terminate chain reaction before vital molecules are damaged. In this study vitamin C levels in both study groups were significantly decreased as compare to control group. The possible mechanism for this decrease could be that in OSMF patients, there is an increase in the production of highly cross linked insoluble collagen, loss of more soluble pro-collagen due to up regulation of lysyl oxidase which is play a crucial role in the development and progression of the condition collagen which is in accordance with study conducted by Shetti A et al.⁽¹⁵⁾ Vitamin E (α -tocopherol) also important antioxidants to prevent the malignancies it could reduce the incidence of primary cancers and improved cancer free survival. Vitamin E supplementation show favorable influence on different prostaglandins made by a body which may prevent cancer. According to our findings its goes down in precancerous and cancerous condition due to gradually increased free radicals its leads to cell damage in oral cavity.⁽¹⁶⁾

According to Prasanna et al. and Silpasree AS et al^(17,18) study Lipid peroxidation is the free radical mediated oxidative degradation of the lipids resulting in cell damage. At the end of lipid peroxidation Melondialdehyde (MDA) is formed which is react with deoxyguanosine and deoxyadenosine in DNA forming adducts which may leads to oxidative stress and cancer. Table 3 shows that the MDA levels was elevated gradually leads to increased oxidative stress in OSMF and oral cancer patients compared to normal healthy control. Vitamin c is one of the free radical scavenging non enzymatic antioxidants this interacts with free radicals and terminate chain reaction before vital molecules are damaged.

Serum C reactive Protein (CRP) is a sensitive marker of inflammation and may have significant prognostic value as early biomarker for cancer diagnostic.⁽¹⁹⁾ This is done by immunoturbidometric assay for detection of acute and chronic inflammatory condition. In this study according to the result of Table 3, CRP levels are increased in oral cancer and OSMF than Control patients due affect in oral malignancies its leads to inflammation and cell damage in oral cavity. C-reactive protein is a receptive marker of inflammation and cancer that is elevated in response to infection and tissue harm in the active phase of disease it's also play a very important role in immunity by acting as a defense against bacterial and viral infection.⁽²⁰⁾

Conclusion

The relative risk of OSMF increased with duration, frequency and style of chewing habits for longer duration and swallowing it without spitting to prevent free radical damage body has a defense system of antioxidants which are involved in the prevention of cellular damage. from our study it was conclude that lipid peroxidation, vitamin C, vitamin E can be used as a marker of OSMF Decreased Vitamin C & E is closely associated with increased Melondialdehyde, as the marker of oxidative stress is significant. Copper Level increased than normal healthy control which is associated over consumption of betel nuts and its additives. Iron levels decreased in OSMF and oral cancer than normal healthy control due to disturb the collagen synthesis and damage the epithelium of oral cavity. The CRP Concentration is associated with development of oral cancer and also serves as a potential prognostic biomarker.

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