



INFLAMMATORY ASSAY IN ORAL SUBMUCOUS FIBROSIS

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ABSTRACT

Introduction: Oral submucous fibrosis (OSMF) is a chronic precancerous condition associated with inflammatory and fibrotic changes in the deeper layers of connective tissue. Even though the etiology of OSMF is multifactorial; it is particularly associated with chronic and frequent chewing of areca nut. Micro trauma due to repeated chewing habit along with the carcinogenic effects of arecanut products in OSMF; induce inflammatory changes. C-reactive protein (CRP) is an acute phase protein; indicator of inflammatory response for any stimulus. Thus acute phase proteins may have significant prognostic value in early cancer diagnostics.

Materials and Methods: The study consists of 30 clinically and histologically diagnosed cases of OSMF taken as study group with an age range between 20 to 45 years and 30 control cases of same age range without any detectable oral lesions. All the cases were taken from the outpatient in the Department of Oral Medicine and Radiology, A.J. Institute of Dental Sciences, Mangalore.

The blood which was withdrawn from the subjects, was allowed to clot, later centrifuged, serum separated and bio chemically evaluated for c-reactive protein (CRP) LEVEL, serum separated and biochemically evaluated for C - reactive protein (CRP) level. Mean serum level of CRP in both the group was statistically evaluated using student's t test and compared in SPSS software.

Results: The study revealed increased serum CRP level in OSMF patients compared to control group.

Conclusion: In the present study CRP was used as one of the prognostic marker to show the influence of inflammation on OSMF to evaluate the mechanisms by which inflammation influences the risk of cancer.

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INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, premalignant condition of the oral mucosa. Pindborg in 1966 defined OSMF as "an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by with vesicle formation, it is always associated with juxta-epithelial inflammatory reaction followed by fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat."¹ It is particularly associated with areca nut chewing. The alkaloids and Flavonoids of areca nut act as irritant and causes progressive fibrosis of the oral mucosa. Furthermore, the micro trauma due to chewing habit results in juxta-epithelial inflammatory cell infiltration.^{1,2}

Paymaster in 1956 was first to describe the malignant transformation of OSMF, when he observed slow growing Squamous Cell Carcinoma (SCC) in many of the patients with OSMF. Epithelial dysplasia in OSMF tissues appeared to vary from 7 to 26% depending on the study population. Malignant transformation rate of OSMF was found to be in the range of 7-13%.

The hypothesis for malignant transformation stated that metabolites released from areca nut, betel nut and tobacco due to quid placement in vestibular sulcus for chronic duration helps in the interaction of carcinogens with oral mucosa, resulting in dense fibrosis, less vascularity of the corium along with altered cytokine activity. This creates a unique environment for carcinogens from both tobacco and arecanut to act on the epithelium. The process may also be influenced by increased secretion of inflammatory cytokines and growth factors. Nutritional deficiencies, HLA and genetic predisposition could have synergistic role³.

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C-reactive protein, a member of the pentaxin protein family was named because of its ability to bind and precipitate the C-polysaccharide of pneumococcus. It is an alpha globulin with a molecular weight of 110,000 to 140,000 Daltons and is composed of five identical subunits which are non covalently assembled as a cyclic pentamer. It is an acute phase protein which is increased in inflammation.⁴ Acute phase proteins are the class of proteins whose concentration is altered at least 25% in response to inflammation due to release of cytokines from the inflammatory cells. The liver responds to such cytokines by producing a large number of acute-phase proteins. The major acute phase proteins include C-reactive protein, serum amyloid A and fibrinogen whose concentration increases with inflammation.

Micro trauma due to repeated chewing habit along with the carcinogenic effects of arecanut products in OSMF; induce inflammatory changes. (CRP) C-reactive protein is an indicator of inflammatory response for any stimulus Thus acute phase proteins may have significant prognostic value in early cancer diagnostics.^{4,5}

Thus in the present study CRP was used as one of the prognostic marker to show the influence of inflammation on OSMF to evaluate the mechanisms by which inflammation influences the risk of cancer.

MATERIALS AND METHODS

Case selection

The study consists of 30 clinically and histologically diagnosed cases of OSMF taken as study group with an age range between 20 to 45 years and 30 control cases of same age range without any detectable oral lesions. All the cases were taken from the outpatient in the Department of Oral Medicine and Radiology, A.J. Institute of Dental Sciences, Mangalore. History, clinical examination and histopathological assessment were done as per preformed procedure. Institutional Ethical Committee Board reviewed each steps of the study and approved to continue the same. Study group as well as Control group consent in written format was taken after explaining about the objective of study to each individual.

Oral submucous fibrosis in association with any other chronic inflammatory diseases, systemic diseases such as renal, cardiac problems, oral or oropharyngeal malignancies, pre-cancerous lesions; as well as patients under the treatment of OSMF were not involved in the study.

Sample collection and storage

5 ml of intravenous blood from antecubital vein was collected in to red topped tubes from each individual of both study and control groups. Blood was allowed to clot at room temperature for about two hours. Later clotted blood was centrifuged at 3000 rpm for 10min and resulting supernatant serum was separated into a sterile polypropylene tube using Pasteur pipette. Serum was stored by refrigerating and maintaining temperature at -20°C. Obtained serum was then biochemically evaluated for levels of C-reactive protein.

CRP estimation was done by immunoturbidometry method

Synthetic particles coated with antibody to C - reactive protein, aggregate in the presence of C - reactive protein in the sample. The increase in turbidity which accompanies aggregation is

proportional to the C - reactive protein concentration that is assessed photometrically.

Statistics

Mean serum levels of CRP levels were obtained and statistically analyzed in SPSS software using Student't' test, P value<0.05 was considered for statistical significance. Control group consists of 30 healthy individuals and Study group consists of 30 OSMF patients, Serum of these subjects were biochemically evaluated for CRP.

Groups	N	Group Statistics		
		Mean	Std. Deviation	Std. Error Mean
Control Group	30	0.1183	.07666	.01400
Study Group	30	0.7530	.10376	.01894

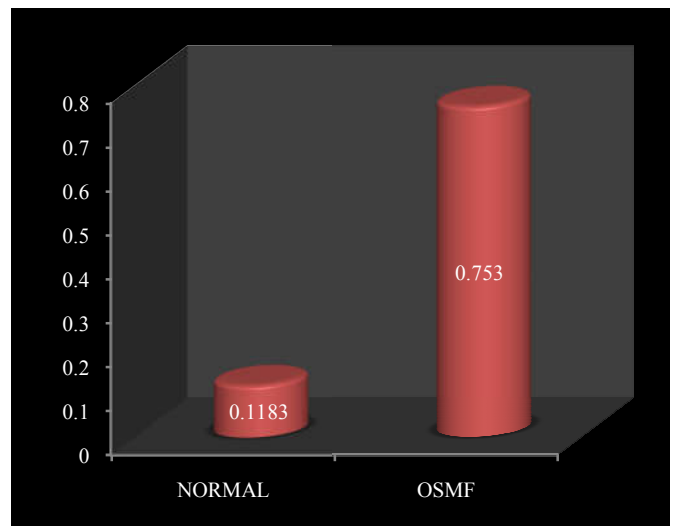
Mean values for the study group is 0.7530 which were higher than the control group whose mean value is 0.1183 with a standard deviation of .07666 in control group and .10376 in study group respectfully. These obtained CRP values are statistically analyzed using 'Student t Test' and was found to be statistically significant.

	Student t test	
	F	Sig.
Equal variances assumed	5.79	0.019
Equal variances not assumed		

The mean CRP level was significantly higher ($P= 0.00$) in OSMF patients as compared to control group.

RESULTS

On comparing mean CRP levels between control group & OSMF patients highly significant difference value ($P = 0.00$) was obtained. Elevated CRP level suggests the immune defense. Results of our study were presented below graphically.



DISCUSSION

OSMF is a potentially malignant disorder affecting oral cavity, pharynx and deeper parts of oral mucosa leading to stiffness of the mucosa and restricted mouth opening. It is seen predominantly in people of Asian descent with overall prevalence rate in India being about 0.2% to 0.5 %. Many synonyms have been attributed for this condition; atrophica idiopathica (trophica) mucosae, oris sub mucosal fibrosis of

palate and pillars, diffuse oral submucous fibrosis, idiopathic scleroderma of the mouth, idiopathic palatal fibrosis and sclerosing stomatitis.^{6,7}

Even though the etiology of OSMF is multifactorial, arecanut plays the main role in the pathogenesis of OSMF. Arecanut contains several alkaloids like arecoline, arecaidine, arecolidine, guvacoline and guvacine. Such alkaloid products along with the mico trauma produced by abrasive arecanut products induces chronic inflammation with the activation of macrophages, T cells and an increase in the level of cytokines such as IL6, TNF, IF α and TGF- β .^{8,9} Prostaglandin is one of the main inflammatory mediators and its production is controlled by various enzymes such as cyclo-oxygenase (COX). COX-2 expression is up-regulated during the early phase of OSMF, indicating this to be an early cellular response to arecoline at transcriptional level. This explains the association of OSMF with inflammatory changes and raised levels of inflammatory markers in this precancerous condition¹⁰.

During chronic exposure, the raised oxidative stress and inflammatory markers leads to this preneoplastic condition in the oral cavity.

Pindborg in 1972 put forward five criteria to prove that the disease is precancerous in nature. They include

1. High occurrence of OSMF in oral cancer patients.
2. Higher incidence of SCC in patients with OSMF.
3. Histological diagnosis of cancer without any clinical suspicion in OSMF.
4. High frequency of epithelial dysplasia.
5. Higher prevalence of leukoplakia among OSMF cases.

During tumorigenesis, paracrine and autocrine interaction between epithelium and damaged mesenchyme causes extracellular matrix proteins and growth factors from the mesenchyme to act on the epithelium resulting in; an imbalance in the mesenchymal-epithelial crosslink that render the epithelial cells to acquire multiple genetic mutations.¹¹

Chronic inflammation has been long linked to cancer that occurs as a part of host's immune response. Tobacco and areca nut chewing can also lead to chronic inflammation in the oral mucosa. Acute phase proteins have been used as a promising factor for measuring inflammatory status. The major acute phase proteins include C-reactive protein whose concentration increases with inflammation. Thus C-reactive protein in OSMF patients can be used as a good prognostic marker. Synthesis of CRP in the hepatocytes are regulated by pro-inflammatory cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor-necrosis factor (TNF), whose levels are increased in OSMF as explained earlier and have been linked with malignancies depending on the complex interaction between the tumor and the host's inflammatory response. Patients with cancer of the oral cavity are usually associated with poor nutritional status because of prevailing symptoms like pain, burning sensation, and low immunity. A significant correlation is always present between malnutrition with impaired immunity and elevated serum CRP concentration.

Two hypotheses are made for increased CRP levels in carcinogenesis

1. Induction hypothesis: Cancerous lesions are usually preceded by chronic inflammation, which activate

cascade of events that causes cell death, apoptosis and irreversible DNA damage, which in turn shows increased CRP level.

2. Response hypothesis: Elevation in CRP levels occurs as a response of the immune system as a consequence of tumor growth.^{4,5}

Assessing these marker pose great advantage in the early diagnosis and prognosis of cancer onset because procedure for assessing CRP is simple, less invasive, less time consuming, ease of interpretation, economical.

CONCLUSION

OSMF is a chronic precancerous condition associated with inflammatory changes in the deeper parts of connective tissue. Thus by evaluating inflammatory changes biochemically can help in prevention, better treatment plan as well as better prognosis of precancerous condition. These studies can even help in arrest of cancer risk factors at its earliest stage. Thus in the present study CRP was used as one of the prognostic marker to show the influence of inflammation on OSMF to evaluate the mechanisms by which inflammation influences the risk of cancer.

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