Estimation of Serum C Reactive Protein in Oral Submucous Fibrosis

***Sahil Kohli[1], Harshkant Gharote[2], and Christopher Vinay Shinde[3]**

*Corresponding Author E-mail: **sahilkohlisk11@gmail.com**

Contributors:

[1]Senior lecturer, RKDF Dental College and Research Centre, Bhopal, India , [2]Professor,Batterjee Medical College, Jeddah, Saudi Arabia, [3]Senior lecturer, Peoples Dental Academy, Bhopal,India.

Abstract

Oral submucous fibrosis (OSMF) is a slowly progressive chronic fibrotic disease of the oral cavity and oropharynx, characterized by fibroelastic change and inflammation of the mucosa. C - reactive protein (CRP) is an acute phase plasma protein that can be used as marker for activation of the immune system. CRP is termed acute phase because the time course of the rise above normal levels is rapid within 6 hours, peaking at about 48 hours. It represents a good marker for disease activity, and to some degree ,severity.The aim of the study was to estimate serum C reactive protein and compare it in OSMF patients,habit group and healthy controls and to calculate body mass index in OSMF patients,habit group and healthy controls.Also,our objective was to correlate serum C reactive protein levels with body mass index, in OSMF patients,habit group and healthy controls.There was no statistically significant variation in serum C reactive protein levels in OSMF patients and other study groups but a positive correlation between serum C reactive protein level and body mass index was found.

Keywords: Oral Submucous Fibrosis, C - reactive protein, Oral Cavity, Inflammation.

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, progressive scarring disease that mostly affects the people of South East Asian origin..[1] There is substantial evidence that lends support to a critical role of areca nut in etiology behind OSMF. Arecoline has the capacity to modulate matrix metalloproteinases and collagenases, all influencing metabolism of collagen, which causes increased fibrosis.[2] the initial symptom of this disease is inflammation, followed by hypovascularity and fibrosis visible as blanching of the oral mucosa[3]. C - reactive protein (CRP) is an acute phase plasma protein that can be used as marker for activation of the immune system.[4] CRP is named for its tendency to precipitate the somatic C polysaccharide of Streptococcus pneumonia and is a sensitive systemic marker of inflammation and tissue damage. Plasma CRP is synthesized only by hepatocytes, predominantly under transcriptional control by the cytokine IL-6, although other sites of CRP production have also been suggested.[5] .CRP can trigger immune cascade reactions leading to neurodegeneration[6]

MATERIAL AND METHOD

This prospective randomized single outcome based study was conducted in the Department of Oral Medicine and Radiology, People's College of Dental Sciences and Research Centre, Bhopal, India from July 2014 till July 2016. Prior to starting the study an ethical clearance was obtained from the Institutional Ethical Committee. The study included 64 individuals of either sex and was divided in three groups as OSMF group (number of patients=24), individuals with habit but without OSMF (number of individuals=20) and healthy control group (number of individuals=20). Diagnosis of OSMF was made on the basis of characteristic clinical features of the disease. A written informed consent was obtained from all the patients before inclusion in the study.

INCLUSION CRITERIA:

1. Patient with clinically diagnosed OSMF.
2. Healthy individuals without underlying illness.
3. The habit group comprised of individuals with areca habit but without OSMF and any systemic disease.

EXCLUSION CRITERIA:

1. Patients having other oral lesions besides OSMF.
2. Individuals having systemic disorders and chronic inflammatory disorders.
3. Previously treated OSMF patients.

Classification of OSMF was followed as per Khanna and Andrade classification (1995).[7]

Patients were made to seat comfortably in the dental chair and extra and intra oral examinations were carried out wearing sterile gloves and mouth mask under artificial illumination. The diagnostic data, general history, OSMF history habit history and clinical examination were recorded in a structured Performa. Mouth opening was measured using a divider and a graduated scale taking incisal edges of maxillary and mandibular central incisors as reference point. Normal value for males=35 to 45 mm and females=30 to 42mm. Presence of intensity of burning sensation was determined on Visual analogue scale ranging from 0-100. Pain was recorded on Visual analogue scale ranging from 0-100. Blanching, depapillation of tongue, presence and extent of fibrous bands and leathery mucosa were determined based on inspection and palpation of the affected parts of the mucosa. The hsCRP (high sensivity C reactive protein) ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the CRP molecule.The use of serum samples is required for this test. Specimen was collected using standard venipuncture techniques. Serum was removed from the coagulated or packed cells within 60 minutes after collection.

MATERIALS PROVIDED WITH THE TEST KITS

1. Antibody-Coated Wells (1 plate, 96 wells) Microtiter wells coated with mouse monoclonal anti-CRP.

2. Reference Standard Set (1.0 ml/vial) contains 0, 0.005, 0.010, 0.025, 0.050 and 0.100 mg/l CRP in phosphate buffer-BSA (bovine serum albumin) solution with preservatives; lyophilized.

3. hsCRP Sample Diluent (50 ml/vial) contains phosphate buffer-BSA solution with preservatives.

4. CRP Enzyme Conjugate Reagent (12 ml/vial) contains goat anti-CRP conjugated to horseradish peroxidase with preservatives.

5. TMB (tetramethylbenzidine) Reagent (11 ml/bottle) contains one-step TMB solution.

6. Stop Solution (1 bottle, 11 ml/bottle) contains diluted hydrochloric acid (1N HCl).

MATERIALS PROCURED FOR TEST

1. Precision pipettes: 5 µl, 10 µl, 50 µl, 100 µl and 1.0 ml

2. Disposable pipette tips

3. Microtiter well reader capable of reading absorbance at 450 nm.

4. Vortex mixer, or equivalent

5. Absorbent paper

6. Graph paper

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement ELISA (Enzyme linked immune sorbent assay) Reader was used and it had 10 slots and six optical filters were available of wavelengths—340 nm, 405 nm, 450 nm, 490 nm, 560 nm, 630 nm. Out of these, the

optical filter of wavelength 490 nm was used in our study.

Patient and control serums were diluted 100 fold prior to use. Desired number of coated wells was secured in the holder. CRP standards, diluted specimens and diluted controls were dispensed into appropriate wells. CRP Enzyme Conjugate Reagent was dispensed into each well. Incubation was done at room temperature for 45 minutes. The incubation mixture was removed by flicking plate contents into a waste container. The microtiter wells were rinsed and flicked 5 times with deionized or distilled water. The wells were stroke sharply onto absorbent paper or paper towels to remove all residual water droplets. 100 µl TMB solution was dispensed into each well and gently mixed for 5 seconds and incubated at room temperature for 20 minutes. Reaction was stopped by adding 100 µl of Stop Solution to each well and gently mixed for 30 seconds. It was made sure that all the blue color changes to yellow color completely. Absorbance was read at 450 nm with a microtiter well reader within 15 minutes.

RESULTS

Comparison of mean CRP levels between all the groups was found to be statistically non-significant. (Table 1)

Table 1: COMPARISON OF SERUM CRP LEVELS BETWEEN GROUPS (t TEST)

PARAMETER	MEAN SERUM CRP(mg/L)	T value	p value	Inference
Healthy group Habit group	3.505±1.4449 3.805±2.9328	-0.410	0.341925	Not significant
Healthy group OSMF group	3.505±1.4449 4.3708±3.9279	-0.9330	0.178056	Not significant
Habit group OSMF group	3.805±2.9328 4.3708±3.9279	-0.5320	0.298759	Not significant

The mean BMI (body mass index) for all the groups were calculated and compared as given in Table 2.Comparison the mean BMI between healthy group and habit group, healthy group and OSMF group were found to be statistically non-significant. Further comparison between habit and OSMF groups was found to be significant statistically.

Table 2 COMPARISON OF MEAN BMI BETWEEN GROUPS (t TEST)

PARAMETER	MEAN BMI(kg/m^2)	T value	p value	Inference
Healthy group Habit group	22.745±3.5894 25.145±5.3139	-1.67377	0.051195	Not significant
Healthy group OSMF group	22.745±3.5894 22.7542±3.8723	-0.00808	0.496796	Not significant
Habit group OSMF group	25.145±5.3139 22.7542±3.8723	1.72379	0 .04605	significant

Table 3 CORRELATION OF BMI WITH SERUM CRP LEVELS IN HEALTHY GROUP

parameter	Healthy group	Correlation coefficient	p value	inference
Mean BMI Mean serum CRP	22.745±3.5894 3.505±1.4449	-0.09746	0.34	Not significant

Table 4 CORRELATION OF BMI WITH SERUM CRP LEVELS IN OSMF GROUP

	OSMF group	Correlation coefficient	p value	inference
Mean BMI Meanserum CRP	22.7542±3.8723 4.3708±3.9279	0.441266	0.015	significant

Table 5 CORRELATION OF BMI WITH SERUM CRP LEVELS IN HABIT GROUP

Parameter	Habit group	Correlation coefficient	p value	inference
Mean BMI Meanserum CRP	25.145±5.3139 3.805±2.9328	0.452923	0.022	significant

In healthy control group the correlation between BMI and serum CRP level was statistically non-significant (Table 3). Correlation of BMI with serum CRP level was significant in OSMF and habit groups (Tables 4 and 5).

DISCUSSION

Oral submucous fibrosis is a gradual but harmful, chronic disease affecting any part of the oral cavity and sometimes,also, the pharynx. The subepithelial and submucosal myofibrosis causes stiffness of the oral mucosa and deeper tissues with progressive restriction in opening of the mouth and protusion of the tongue, thus causing difficulty in eating, swallowing and speech.[8] many studies suggested that areca nut is the main causative factor for this disease. Other aetiological factors suggested are lime, tobacco, chillies, immunological disorders, nutritional deficiencies and collagen disorders.[9] the betel nut has psychotropic and antihelminthic property because of the presence of areca alkaloids. Four alkaloids have been conclusively identified in biochemical studies and these are arecoline, arecaidine, guvacine and guvacoline.[20] Patients complain of burning sensation while eating spicy food. The fibrosis leads to difficulty in mastication, speech, pain in throat and ears, and relative loss of auditory sharpness because of abnormal narrowing of the opening of Eustachian tube. In advanced cases, there may be severe trismus and chronic ulceration.[10] Body mass index (BMI) is considered as a predictor of overall health and nutritional status of an individual. The exact pathophysiology behind the association between OSMF and BMI is not clear, but lower BMI can be considered as a risk factor and can trigger premalignant conditions of the oral cavity.[17] Oral squamous cell carcinomas comprise of 90% of malignancies in the oral cavity which may develop from premalignant oral lesions such as OSMF, leukoplakia, erythroplakia and oral lichen planus.[18] CRP is one of the common test parameters used in clinical practice to assess, diagnose and prognoses inflammation.[13] William Smith Tillet was an American internist and microbiologist who discovered CRP in 1930, in sera, from patients with Streptococcus pneumonia infection. It is so named because of interaction with phosphocholine residues of pneumococcal C-polysachcharide, which is a constituent of teichoic acid in the pneumococcal cell wall.[19] CRP is an important member of the pentraxin family, identified by proteins of a cyclic pentameric structure and calcium dependent ligand binding.[14] It is an acute phase reactant produced mainly by the liver. Acute phase proteins can be explained as proteins whose concentration is changed by at least 25% in response to inflammation.[11] Serum CRP levels are elevated in response to acute infections, inflammatory conditions and trauma. In these clinical situations, the serum CRP levels rise rapidly generally beyond 10 mg/l with accompanying rise of erythrocyte sedimentation rates.CRP has a stable pentraxin structure and that is why it has a relatively long half-life of 18 to 20 hours.[15] Conventionally CRP values are reported as mg/dL, to differentiate the results from high sensitivity CRP results that are reported in mg/L.[16] Two hypotheses could be suggested with increased CRP levels as a marker of chronic inflammation. First the induction hypothesis, chronic inflammation causes uncontrolled cell proliferation and triggers a cascade of cellular actions, causing induction of irreversible DNA damage. Second, the response hypothesis, the immune response of the host as a result of tumour growth itself could be the cause for rise in CRP levels.[21] the analysis of blood is done in a medical laboratory. The blood is often collected in a serum separating tube and various analytical methods are available for CRP determination, for example ELISA as it can conduct other forms of ligand binding assays instead of strictly immunoassays, though the name carried the original “immuno” because of the common use and history of development of this method.[12] Mean serum CRP levels in OSMF group, habit group and healthy group were

4.3708±3.9279, 3.805±2.9328 and 3.505±1.4449 mg/dl respectively. Comparison of serum CRP levels between all the groups were found to be statistically non-significant. (Tables 1)**.** In a study by Kaja et al [22], Mean CRP level in OSMF was 0.58±0.83 where as in controls it was 0.26±0.05 however the difference was not significant statistically. These findings are in accordance with the present study. However, in a study by Anand Kumar et al (2011)**,** mean serum CRP levels in OSMF patients were 0.68±0.10 mg/dl and statistically significant as compared to controls (p=0.00) and concluded that mean serum CRP levels can be a useful prognostic marker in oral precancer. [23]

In present study, we compared the mean BMI between OSMF group and habit group that was found to be statistically significant (p = 0.046). However comparison between OSMF group and healthy group, and habit group and healthy group was found to be statistically non-significant. (Table 2). Similar findings were also observed by Singh P et al in a study on OSMF where comparison of mean BMI between OSMF (20.5 ± 3.2 kg/m^2) and controls (21.5 ± 2.3 kg/m^2) was statistically not significant.[20] A study by Hashibe et al (2002) investigated the association between BMI, smoking, drinking, and the risk of OSMF. They observed an inverse dose-response relationship between BMI and the risk of OSMF. They correlated that alcohol drinking may possibly be associated with the risk of OSMF and further establish that BMI was inversely associated with the risk of OSMF for both genders when potential confounding factors were adjusted[24]. However present study did not show inverse correlation with BMI.

In the present study, Correlation of BMI with serum CRP levels for OSMF group (Group I) (p value=0.015) (table 4), and habit group (Group II)(table 5) and (p=0.022), was statistically significant however in control group, inference was found to be not significant (p = 0.34) (Table 3). This finding may suggest that habit of areca and inflammatory markers of OSMF including CRP could play some role in regulation of body mass. However there is no literature available for the support of this finding.

CONCLUSION

The present study was an attempt to evaluate serum C reactive protein as potential marker in oral sub mucous fibrosis. However there was no statistically significant variation in its level between OSMF and other study groups. Nevertheless a positive correlation between Serum c reactive protein level and body mass index was found which may suggest some role of areca and progression of OSMF in regulation of weight of an individual. Further studies on larger size of population need to be carried out to get more accurate results.

REFERENCES:

1. SharmaR,RajSS,MiahraG,Reddy,YG,Shenava S,Narang P.Prevalence of Oral Submucous Fibrosis in patients visiting dental college in rural area of Jaipur,Rajasthan.J Indian Aca Oral Med Radiol 2012;24(1):1-4
2. Greenberg MS,Glick M,Ship JA.Burket's Oral Medicine.11th edn.India:CBS Publishers & Distributors Pvt Ltd;2012.p.88
3. Wollina U, Verma SB, Ali FM, Patil K. Oral submucousfibrosis:anupdate.Clin.Cosmet.Investig.Dermatol.2015;8:193-204
4. CoventryBJ,AshdownML,QuinnMA,Markovic SN,Clarke SL,Robinson AP. CRP identifies homeostatic immune oscillations in cancer patients: a potential treatment targeting tool?. J. Transl. Med. 2009; 7:102.
5. PepysMB,HirschfieldGM.C-reactive protein:a critical update.J.Clin.Invest.2003;111:1805-1812.
6. Gorska-CiebiadaM,Saryusz-Wolska M,Borkowska A,Ciebiada M,Loba J.C-reactive protein,advanced glycation end products,and their receptor in type 2 diabetic,elderly patients with mild cognitive impairment.Front.Aging Neurosci.2015;7:1-9

7. Priyadharshni B.Classification system for oral submucous grading-a review.IJSR[Internet] 2014 Mar;3(3):740-744..Available from : www.ijsr.net
8. Rajendran R. Oral submucous fibrosis:etiology, pathpgenesis and future research. Bull. World Health Organ. 1994;72(6):985-996
9. Khan S, Sinha A, Kumar S, Iqbal H. Oral submucous fibrosis: Current concepts on aetiology and management – A Review. J Indian Acad Oral Med Radiol 2018; 30:407-11.
10. Sabharwal R, Gupta S, Kapoor K, Puri A, Rajpal K. Oral Submucous Fibrosis- A Review. J Adv Med Dent Scie Res 2013;1(1):29-37.
11. Vankadara S, K Padmaja,Balmuri PK, G Naresh,G Vikas Reddy.Evaluation of serum C – reactive protein levels in oral premalignancies and malignancies:a comparative study. J Dent(Tehran) 2018;15(6):358-364
12. Rao M,Gopal S.C-reactive protein-a criticalreview.Int.J.Curr.Microbiol.App. Sci. 2015;4(12):55-61
13. Chandrashekara S. C-reactive protein: An inflammatory marker with specific role in physiology, pathology, and diagnosis. IJRCI. 2014; 2(S1):SR3.
14. Wang CS,Sun CF.C reactive protein and malignancy:clinicopathological association and therapeutic implication.Chang Gung Med J 2009;32(5):471-482
15. Kamath D,Xavier D,Sigamani A,Pais P.High sensitivity C-reactive protein(hsCRP) and cardiovascular disease:an Indian perspective.Indian J Med Res 2015;142:261-268
16. Singh G. C-reactive protein and erythrocyte sedimentation rate: Continuing role for erythrocyte sedimentation rate. ABC 2014;4:5-9
17. Borase AP, Kashid AL, Mohatta A. Association of Body Mass Index and the Risk of Oral Submucous Fibrosis in Maharashtra, India. J Adv Med Dent Scie Res 2017; 5(11):118-121.
18. Chang P, Kuo Y, Wu T, Liao C, Sun Y, Yen T et al. Association and prognostic value of serum inflammation markers in patients with leukoplakia and oral cavity cancer. Clin Chem Lab Med 2013; 51(6): 1291–1300
19. Boncler M, Watala C. Regulation of cell function by isoforms of C-reactive protein:a comparative analysis. Acta.Biochim.Pol.2009 56(1):17-31
20. Singh P, Gharote H, Nair P, Hegde K,Saawarn N, Guruprasad R. Evaluation of Cachexia in Oral Submucous Fibrosis. J Indian Aca Oral Med Radiol 2012; 24(2):130 -132.
21. Kruse AL, Luebbers HT, Gratz KW. C-reactive protein levels: a prognostic marker for patients with head and neck cancer? Head Neck Oncol 2010; 2(21): 1-5
22. Kaja et al. Quantitative analysis of C-reactive protein in potentially malignant disorders: A pilot study. J Orofac Sci 2015; 7(1):3-6
23. Kumar A,Bhateja S. Altered C-Reactive Protein Levels in Serum of Oral Precancer Patients in Comparison With Healthy Controls. J. Oral Maxillofac. Pathol 2011;2(4):16-19
24. Hashibe M, Sankaranarayanan R, Thomas G, Kuruvilla B, Mathew B, Somanathan T et al. . Body mass index, tobacco chewing, alcohol drinking and the risk of oral submucous fibrosis in Kerala, India. Cancer Causes Control 2002;13(1):55-64