

Serum C-reactive protein in oral submucous fibrosis and oral squamous cell carcinoma: A cross-sectional study

Suchitra Rajesh Gosavi, Amruta Appasaheb Torkadi

Department of Oral Pathology and Microbiology, Government Dental College and Hospital, Nagpur, Maharashtra, India

Abstract

Context: Early detection of oral squamous cell carcinoma (OSCC) and oral submucous fibrosis (OSMF) transforming into malignancy can drastically improve the treatment outcomes and prognosis. Carcinoma development is an intricate complex mechanism and the multifactorial causation makes it more difficult to find specific prognostic and therapeutic biomarkers. Thus, the development of newer diagnostic and predictive approaches that are less invasive, economical and amenable to repeated sampling is imperative. Serum C-reactive protein (CRP) serves a useful marker for prognosis and monitoring of oral potentially malignant disorders (OPMDs) including OSMF as well as OSCC. Secreted by hepatocyte as an acute inflammatory marker, various studies have shown association between serum CRP and presence of OPMDs, as well as with presence and prognosis of OSCC.

Aims: The aim of this study was to measure and compare serum CRP levels in patients with OSMF and OSCC as compared to healthy individuals and to establish baseline data for serum CRP levels in patients with OSMF and OSCC.

Settings and Design: Cross-sectional observational prospective study.

Materials and Methodology: The study includes 150 individuals, with 50 healthy, 50 OSMF and 50 OSCC individuals. Spinreact CRP-turbilatex Kit (SPINREACT) and Prietest-EXP semi-automatic biochemical analyzer were used for quantitative determination of serum CRP.

Statistical Analysis Used: Data were analyzed by SPSS 16[®] (Statistical Package for the Social Sciences) software.

Results: The mean value of serum CRP in Group I was 2.20 mg/L with standard deviation of 1.74; in Group II, it was 5.40 mg/L with standard deviation of 4.79 mg/L and in Group III, it was 12.17 mg/L with standard deviation of 11.38.

Conclusions: Serum CRP levels in patients with OSMF were raised, but not statistically significant as compared to the control group. Values of serum CRP were significantly higher in patients with OSCC as compared to the control group, and it was statistically significant; these values also showed positive association with primary tumor size.

Keywords: C-reactive protein, oral potentially malignant disorder, oral squamous cell carcinoma, oral submucous fibrosis

Address for correspondence: Dr. Amruta Appasaheb Torkadi, Omkar Nivas, Sharda Nagar, Latur, Maharashtra, India.

E-mail: dramrutatorkadi@gmail.com

Submitted: 06-Nov-2019, **Accepted:** 21-Jan-2020, **Published:** 08-May-2020

Access this article online

Quick Response Code:



Website:

www.jomfp.in

DOI:

10.4103/jomfp.JOMFP_317_19

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Gosavi SR, Torkadi AA. Serum C-reactive protein in oral submucous fibrosis and oral squamous cell carcinoma: A cross-sectional study. *J Oral Maxillofac Pathol* 2020;24:46-51.

INTRODUCTION

In India, oral squamous cell carcinoma (OSCC) ranks number one in terms of incidence among men and third among women.^[1] India has always been cited as a country with the highest incidence of oral cancer along with potentially malignant disorders (OPMD), with registration of over 100,000 new cases of oral cancer every year.^[2] It has one of the lowest 5-year survival rates of all cancers, probably because most lesions are not diagnosed in the initial stages. However, if detected early, the probability of survival from oral cancer is remarkably better when compared to most other types of cancers.^[1]

Oral submucous fibrosis (OSMF) is a premalignant condition of the oral cavity. It is characterized by inflammation and progressive generalized submucosal fibrosis, leading to limitation of mouth opening. The possible precancerous nature of submucous fibrosis was first mentioned by Paymaster in 1956, who described the development of slow-growing squamous cell carcinoma (SCC) in one-third of the cases with submucous fibrosis.^[3] Malignant transformation rate of OSMF was found to be different in various studies; in a long-term follow-up study, it was found to be in the range of 7%–13%, and hence it is very important to monitor these patients to identify early transformation into OSCC patients. If identified at initial stages, the incidence of death rates due to OSCC can be reduced considerably.^[4,5]

Development of OSCC *de novo* and transformation of OSMF into OSCC involves contribution of both external and internal factors. There are not many studies on proteins and early biomarkers that could help clinicians select a suitable treatment strategy for patients with OPMD and oral cancer and particularly for monitoring the course for OPMD.^[5]

The search for a suitable biomarker which indicates immune system responses in cancer patients has been long and arduous; in the last few years, a widely known biomarker has emerged as a potential candidate for this purpose. C-reactive protein (CRP) is an acute-phase plasma protein that can be used as a marker for activation of the immune system. The short plasma half-life and relatively robust and reliable response to inflammation makes CRP an ideal candidate marker for inflammation. Usually, static sampling of CRP has been used for clinical studies and these can predict disease presence or recurrence, notably for a number of cancers. It has been identified that CRP increases during both acute and chronic inflammatory conditions.^[6]

As it is a well-known fact that inflammation and cancer are linked, the role of this protein has to be identified in many OPMDs including OSMF and OSCC.^[5] Still, in literature, very few studies have tried to establish a cause–effect relationship between serum CRP and oral potential malignant disorders such as OSMF and OSCC. Our study is an attempt to establish a baseline relationship between serum levels of CRP in healthy individuals and to compare them with these levels in cases of OSMF and OSCC.

Aim and objectives

To measure and compare serum CRP levels in patients with OSMF and OSCC as compared to healthy individuals.

MATERIALS AND METHODOLOGY

The study was carried out at the Department of Oral Pathology and Microbiology in our Dental College and Hospital. The study was approved by the Institutional Ethics Committee, Dental College and Hospital.

Study design

This was a prospective, cross-sectional study.

Sample size

One hundred and fifty individuals were equally divided into three groups. Group I consisted of 50 apparently healthy people who visited our institute for routine dental checkup or oral prophylaxis irrespective of age and sex. This group served as “control” in our study. Group II consisted of 50 clinically diagnosed cases of OSMF with different amount of interincisal opening (IIO) and different stages of progression of disease. Group III consisted of 50 clinically diagnosed cases of OSCC with different tumor size and grade at different location in oral cavity. These cases with OSCC were grouped according to primary tumor size for which TNMS classification of clinical grading was used.^[7] Only T, i.e., size of primary tumor, was considered for the study purpose.

All these cases were assessed clinically and their detailed case history was recorded; informed written consent was obtained and 5 ml of intravenous blood was collected using standard aseptic conditions. These samples were centrifuged and serum was seen separated as the top transparent layer. This separated serum was then used immediately for quantitative determination of serum CRP by Spinreact CRP-turbilatex Kit (SPINREACT) and Prietest-EXP semi-automatic biochemical analyzer. CRP-Turbilatex is a quantitative turbidimetric test for the measurement of CRP in human serum or plasma. Serum CRP levels 0–5 mg/L were considered to be normal.^[8]

The data were collected, tabulated and analyzed by SPSS 16[®] (Statistical Package for the Social Sciences) software IBM Corporation, Chicago IL, USA. Appropriate test of significance was applied and $P < 0.05$ was considered statistically significant.

OBSERVATION AND RESULTS

Out of 50 individuals in Group I, 24 (48%) were male and 26 (52%) were female. In Group II, out of 50 cases, 40 (80%) were male and 10 (20%) were female. Occurrence of OSMF was observed to be more in male cases. In Group III, out of 50 cases, 41 (82%) were male and 9 (18%) were female.

The value of serum CRP in Group I ranged from 0 to 6.24 mg/L with a mean value of 2.20 mg/L and standard deviation of 1.74. This was within normal limits. The value of serum CRP in Group II ranged from 0 mg/L to 21.62 mg/L with a mean value of 5.40 mg/L and standard deviation of 4.79 mg/L. The value of mean serum CRP in Group II was compared with that of Group I, and the results are shown in Table 1.

The value of mean serum CRP in cases with OSMF was slightly higher as compared to normal controls. However, when independent samples *t*-test was applied, *P* value was found to be 0.07, i.e., statistically not significant.

Group II cases were also subgrouped according to IIO as shown in following Table 2. For this purpose, Khanna and Andrade classification was used.^[9] One-way ANOVA test was applied and $P = 0.16$, i.e., statistically not significant [Table 2].

The mean value of serum CRP in cases with OSCC was higher as compared to the control group, i.e., 12.17 mg/L, with standard deviation of 11.38, as shown in Table 3. Independent samples *t*-test was applied to compare the mean CRP levels in Group III and Group I, $P < 0.001$, i.e., statistically significant. Hence, we can conclude that

the mean CRP value in cases with OSCC is significantly higher as compared to the control group.

One-way ANOVA test was applied and $P = 0.004$ (statistically significant). This implies that there was a significant difference in the value of mean serum CRP in cases with OSCC when they are categorized based on primary tumor size.

Further *post hoc* test was applied for intergroup comparison, and it was found that the mean CRP level in cases with tumor size T3 was significantly higher than that found in cases with tumor size T1 and T2 [Table 4].

One-way ANOVA test was applied, $P < 0.001$, i.e., statistically significant. This implies that a statistically significant difference was noted between the mean serum CRP values of Group I, Group II and Group III.

When *post hoc* test was applied for further intergroup comparison, the mean serum CRP level was significantly high in cases with OSCC (Group III) as compared to both remaining groups, i.e., control group (Group I) and cases with OSMF (Group II) [Table 5].

On summarizing all the above observations, it can be stated that serum CRP levels in normal individuals were within normal range, and in cases with OSMF, they were raised, but no statistical significant difference was observed.

In cases with OSCC, the values of serum CRP were significantly higher as compared to normal individuals and these values showed positive association with primary tumor size; however, no significant difference in values of serum CRP was observed in these cases based on the site of occurrence and histopathological difference.

DISCUSSION

In India alone, over 100,000 new cases of OSCC are registered every year. In a survey done over a decade ago, there were more than 250,000 OSMF cases recorded, a figure that must have increased sharply till date.^[2] Pathogenesis of OSMF is not well established. Since its first description, various etiopathological concepts have been put forward such as spices (chillies), nutritional deficiencies, genetic susceptibility, lysyl oxidase (Lox) and autoimmunity,

Table 1: Comparison of mean serum CRP values in Group I and Group II subjects

Group	Number	Mean CRP	Std. deviation	<i>P</i>
Group I	50	2.20	1.74	0.07
Group II	50	5.40	4.79	

Table 2: Comparison of mean serum CRP values in Group II subjects based on interincisal opening (IIO)

Group	Number	Mean CRP	Std. deviation	<i>P</i>	<i>Post hoc test</i>
Group IIA (IIO >35 mm)	2	1.35	1.52	0.16	Group IIA=Group
Group IIB IIO=26 to 35 mm	16	7.34	6.51		IIB=Group
Group IIC IIO= 16 to 25 mm	22	4.37	3.71		IIC=Group IID
Group IID IIO <15 mm, without any other premalignant lesion	10	5.36	2.97		
Group IIE IIO <15 mm , with presence of premalignant lesion	0	0	0		

Table 3: Comparison of mean serum CRP values in Group I and Group III subjects

Group	Number	Mean CRP	Std. deviation	P
Group I	50	2.20	1.74	<0.001
Group III	50	12.17	11.38	

Table 4: Comparison of mean serum CRP values in Group III subjects based on primary tumor size (T)

Size	Mean CRP	Std. deviation	P	Post hoc Test
T1 (2 cm or less, n=12)	6.03	4.51	0.004	(T1=T2)
T2 (2 cm-4 cm, n=15)	8.42	2.09		< T3
T3 (size>4 cm, n=23)	17.70	14.68		

Table 5: Comparison of mean serum CRP values in Group I, Group II and Group III subjects

Group	Number	Mean CRP	Std. deviation	P	Post hoc test
Group I	50	2.20	1.74	<0.001	Group III
Group II	50	5.40	4.79		> (Group I=Group II)
Group III	50	12.17	11.38		
Total	150	6.59	8.28		

and one of the most accepted etiopathological concepts is areca nut usage.^[4] The epithelial atrophy may predispose to cancer development in the presence of carcinogens. The precancerous nature of OSMF was first described by Paymaster in 1956 when he observed slow-growing SCC in one-third of the patients with the disease.^[10]

OSCC and OSMF both show a complex pattern of inflammation associated with them, so it is very interesting and yet challenging to explore relationship of these and to establish a cause-effect relationship if at all possible. Inflammation is fundamentally protective response, the ultimate goal of which is to rid of organism of both the initial cause of cell injury (e.g., microbes and toxins) and consequences of such injury (e.g., necrotic cells and tissues). It is mechanism by which body tries to restore its physiological environment.^[11]

Inflammation reflects its presence in serum in the form of various chemokines and various other molecules. These substances at times are either pathognomonic or may be nonspecific; at times after treatment, specific changes are seen in the level of these substances. Hence, these molecules can be used for diagnosis, for monitoring or to predict prognosis in many diseases. Many such inflammatory markers are observed in OSMF and OSCC; those can serve as an excellent opportunity for pathologists for early diagnosis, apt monitoring and/or predicting prognosis. CRP is one such nonspecific inflammatory marker. In humans, the CRP level is low (0.1–0.5 µg/ml), i.e., 0.1–5 mg/L under normal conditions, but increases up to approximately

1000-fold during inflammation, making CRP probably one of the most useful molecules for monitoring inflammation present in many diseases and conditions. Hence, in the present study, serum CRP levels in OSMF and OSCC were estimated as compared to apparently healthy individuals.

Serum CRP level in OSMF cases was slightly higher as compared to the control group; however, this difference was statistically not significant; similar results were observed in a study done by Kaja *et al.*^[5] Our findings are not in accordance with the findings by Kumar and Bhateja. In their study of 25 control and 25 oral precancerous patients (15 – leukoplakia, 7 – OSMF and 3 – oral lichen planus), the mean CRP levels in OSMF patients were 0.68 ± 0.10 .^[12] Our findings are also not in accordance with a recent study done by Metgud and Bajaj, where it was found that the mean serum levels of CRP increased from 2.7 ± 0.89 mg/L in controls to 5.91 ± 0.93 mg/L in oral premalignant patients (10 – OSMF and 10 – leukoplakia) and the difference was statistically significant ($P < 0.001$).^[13]

In both the above studies, however, the study group was a heterogeneous mix of OSMF along with leukoplakia and oral lichen planus. The actual number of OSMF cases participated in these studies was small (7 and 10, respectively). Hence, drawing a definitive conclusion to comment about mean CRP levels in OSMF in their study would be inappropriate.

Inflammation is well associated with chronic irritation to oral mucosa due to chemical irritants such as arecoline and arecadine and various other chemical agents present in areca nut, which is considered as a chief etiological agent for OSMF. In OSMF, there is always presence of juxtaepithelial inflammation in the form of lymphocytes and plasma cells seen in OSMF as initial response and that can be appreciated over a long period of time.^[14,15] Over a period of time, due to persistent habit, chronic inflammation sets in at the site.

Initial irritation leads to further atrophy and ulceration of the mucosa. It can thus be considered that induction of oral mucosal inflammation by betel quid ingredients is a critical event in the pathogenesis of OSMF.^[16] Cytokines such as interleukin (IL)-6, tumor necrosis factor (TNF) and interferon- γ and growth factors such as transforming growth factor- β (TGF- β) are synthesized at the site of inflammation. TGF- β 1 is a key regulator of extracellular matrix assembly and remodeling. TGF- β increases the collagen production and decreases the collagen degradation. IL-6 is a key mediator for synthesis of CRP

by hepatocytes. Hence, increased level of serum CRP is anticipated in OSMF. However, to find these elevated levels statistically significant or to comment to malignant transformation potential in OSMF cases with relation to serum CRP levels, long-term follow-up studies are required.

The serum CRP level in OSCC cases was significantly higher as compared to serum CRP levels of control groups, $P < 0.001$. The above observation is similar with the findings by Tariq *et al.*,^[17] Krasteva *et al.*,^[18] Chen *et al.*,^[19] Chang *et al.*,^[20] Chen *et al.*^[21] and Metgud and Bajaj.^[13] CRP elevation indicates a host immune response to tumor growth, with elevated inflammatory cytokines. In the tumor microenvironment, pro-inflammatory cytokines, such as IL-6, IL-8 and TNF, lead to inflammation and angiogenesis, which subsequently upregulate the acute-phase reactant CRP. IL-6 also indirectly helps CRP bind to tumor cells, which may lead to tumor cell lysis. Thus, CRP is not only a response to the tumor microenvironment but also a reflection of tumor cell killing and local tissue damage. This explains the elevated serum CRP in cases with OSCC. Current knowledge suggests a reciprocal induction between chronic inflammation and cancer.^[22] Cancer growth could cause inflammatory response around the cancer, thereby increasing CRP levels. Alternatively, chronic inflammation could lead to the development of cancer. Unfortunately, a direct role of CRP in carcinogenesis has not been experimentally confirmed.

When comparison of mean serum CRP levels was done based on tumor size, we got statistically significant difference ($P = 0.004$), with mean serum CRP value being highest in T3 cases followed by T2 cases and least in T1 cases. Therefore, a positive relationship between tumor size and serum CRP was found. Tariq *et al.*,^[17] Chen *et al.*,^[19] Khandavilli *et al.*,^[23] Chang *et al.*^[20] and Chen *et al.*^[24] also found positive correlation between serum CRP and primary tumor size.

Another hypothesis that is put forward to explain this is, as there is local chronic inflammatory response in tumor microenvironment, the level of CRP increases proportionately which may lead to excessive cell proliferation and subsequent accumulation of DNA damage. The host immune system responds to tumor growth via elevated serum CRP levels again.

CONCLUSIONS

Although the value of serum CRP in cases with OSMF was slightly raised when compared to the control group,

no statistically significant difference was seen. Hence, no association between serum CRP levels and OSMF could be established; based on current knowledge, long-term studies are required.

A positive association between serum CRP and OSCC was observed, with serum CRP levels being significantly high in cases with OSCC as compared to the control group. Positive association was also found between primary tumor size and serum CRP levels. Increased serum CRP levels were observed with increased tumor size.

The mean serum CRP levels in the control group was normal, slightly high in cases with OSMF and a sharp rise in serum CRP level was observed in cases with OSCC. After obtaining these results we suggest close monitoring of serum CRP level in cases with OSMF; these levels might be suggestive of transformation of OSMF into OSCC.

When an association between the elevated CRP levels and increased cancer risk is established, it is essential to define what exactly CRP is: a participant in the pathogenesis of cancer or simply a marker of cancer. Although observational study like this can give some evidence regarding association between serum CRP and conditions such as OSMF and OSCC, it is difficult to prove cause-effect relationship or to predict about its prognostic use. In future, long-term studies with large sample size should be conducted to obtain more definitive evidences. Monitoring serum CRP values can be of great clinical utility and patient management.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ray JG, Ganguly M, Rao BS, Mukherjee S, Mahato B, Chaudhuri K. Clinico-epidemiological profile of oral potentially malignant and malignant conditions among areca nut, tobacco and alcohol users in Eastern India: A hospital based study. *J Oral Maxillofac Pathol* 2013;17:45-50.
2. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol* 2009;45:309-16.
3. Pundir S, Saxena S, Aggrawal P. Oral submucous fibrosis a disease with malignant potential -Report of two Cases. *J Clin Exp Dent* 2010;2:215-8.
4. 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;13:340-1.
5. Kaja S, Naga SS, Kumar KK, Dasari N, Kantheti LC, Reddy BR. Quantitative analysis of C-reactive protein in potentially malignant disorders: A pilot study. *J Orofac Sci* 2015;7:3-6.

6. Coventry BJ, Ashdown ML, Quinn MA, Markovic SN, Yatomi-Clarke SL, Robinson AP. CRP identifies homeostatic immune oscillations in cancer patients: A potential treatment targeting tool? *J Transl Med* 2009;7:102.
7. Rajendaran R. Benign and malignant tumors of oral cavity. In: Rajendaran R, Sivapathsundaram B, editors. *Shafer's Textbook of Oral Pathology*. 7th ed. New Delh: Elsevier Publishers; 2012. p. 81-223.
8. Pepys MB, Hirschfield GM. C-reactive protein: A critical update. *J Clin Invest* 2003;111:1805-12.
9. Khanna JN, Andrade NN. Oral submucous fibrosis: A new concept in surgical management. Report of 100 cases. *Int J Oral Maxillofac Surg* 1995;24:433-9.
10. Pundir S, Saxena S, Aggrawal P. Oral submucous fibrosis a disease with malignant potential – Report of two cases. *J Clin Exp Dent* 2010;2:215-8.
11. Kumar V, Abbas A, Fausto N, editors. *Acute and chronic inflammation*. In: *Robins and Cotran Pathologic Basis of Disease*. 7th ed. New Delh: Elsevier Publishers; 2004. p. 47-87.
12. Kumar AC, Bhateja S. Altered C-reactive protein levels in serum of oral precancer patients in comparison with healthy controls. *Int J Oral Maxillofac Pathol* 2011;2:16-9.
13. Metgud R, Bajaj S. Altered serum and salivary C-reactive protein levels in patients with oral premalignant lesions and oral squamous cell carcinoma. *Biotech Histochem* 2016;91:96-101.
14. Balkwill F, Mantovani A. Inflammation and cancer: Back to Virchow? *Lancet* 2001;357:539-45.
15. Feller L, Altini M, Lemmer J. Inflammation in the context of oral cancer. *Oral Oncol* 2013;49:887-92.
16. Chitra S, Balasubramaniam M, Hazra J. Effect of α -tocopherol on salivary reactive oxygen species and trace elements in oral submucous fibrosis. *Ann Clin Biochem* 2012;49:262-5.
17. Tariq FA, Janjua OS, Khan U. C-Reactive Protein as a prognostic indicator of oral squamous cell carcinoma – A retrospective study. *Pak Oral Dent J* 2011;31:288-91.
18. Krasteva A, Aleksiev E, Ivanova A, Altankova I, Bocheva T, Stanimirov P, *et al*. Salivary components of treated cancer patients and patients with precancerous lesions. *Bulg J IMAB* 2008;14:41-4.
19. Chen HH, Chen IH, Liao CT, Wei FC, Lee LY, Huang SF. Preoperative circulating C-reactive protein levels predict pathological aggressiveness in oral squamous cell carcinoma: A retrospective clinical study. *Clin Otolaryngol* 2011;36:147-53.
20. Chang PY, Kuo YB, Wu TL, Liao CT, Sun YC, Yen TC, *et al*. Association and prognostic value of serum inflammation markers in patients with leukoplakia and oral cavity cancer. *Clin Chem Lab Med* 2013;51:1291-300.
21. Chen HH, Wang HM, Fan KH, Lin CY, Yen TC, Liao CT, *et al*. Pre-treatment levels of C-reactive protein and squamous cell carcinoma antigen for predicting the aggressiveness of pharyngolaryngeal carcinoma. *PLoS One* 2013;8:e55327.
22. Guo YZ, Pan L, Du CJ, Ren DQ, Xie XM. Association between C-reactive protein and risk of cancer: A meta-analysis of prospective cohort studies. *Asian Pac J Cancer Prev* 2013;14:243-8.
23. Khandavilli SD, Ceallaigh PO, Lloyd CJ, Whitaker R. Serum C-reactive protein as a prognostic indicator in patients with oral squamous cell carcinoma. *Oral Oncol* 2009;45:912-4.
24. Chen IH, Liao CT, Wang HM, Huang JJ, Kang CJ, Huang SF. Using SCC antigen and CRP levels as prognostic biomarkers in recurrent oral cavity squamous cell carcinoma. *PLoS One* 2014;9:e103265.