

Role of Serum C-reactive Protein Levels in Oral Potentially Malignant Disorders and Oral Cancer: A Cross Sectional Study

Ayushi Pravin Shah¹, Palak H. Shah², Rashmi Venkatesh³, Khimya Tinani⁴

ABSTRACT

Introduction: Inflammation plays an important role in the process of carcinogenesis. It is associated with initiation, progression as well as metastasis of cancer. C-reactive protein (CRP) is a reliable marker for inflammation.

Aim: The present study is designed to determine and compare the serum CRP levels in Oral Submucous Fibrosis (OSMF), Oral Leukoplakia (OL), Oral Cancer (OC) and Healthy Controls (HC) in order to assess its possible role in carcinogenesis.

Materials and Methods: A total of 40 participants were equally divided in four groups i.e. OSMF, OL, OC and HC. From each participant, 5 ml of blood was obtained from antecubital vein. The serum was analysed for CRP levels using nephelometric analysis method.

Results: There were total 29 males and 11 females, who were between 16 to 77 years of age, with a mean age of 44.45 ± 7.45 years. The serum CRP levels were significantly increased in OSMF, OL and OC groups as compared to HC group ($p < 0.05$).

Conclusion: Serum CRP levels can be used as diagnostic and prognostic marker for oral premalignant and malignant lesions.

Keywords: C-reactive protein, Oral Submucous Fibrosis, Oral Leukoplakia, Oral Cancer, Nephelometry

INTRODUCTION

Cancer is one of the most dreadful diseases in today's world. In the cancers of head and neck region, oral cancer is the most common type of cancer which has annual incidence of 3,00,000 cases in the world.¹ Indian subcontinent is cited with the highest incidence of oral precancer and Oral Cancer (OC) with over 1,00,000 new cases registering every year.² Oral cancer is documented with the lowest 5 year survival rates among all the cancerous lesions.³

Oral cancer is often preceded by prodromal signs and symptoms known as Oral Potentially Malignant Disorders (OPMD's). Among OPMD's oral leukoplakia (OL) and oral submucous fibrosis (OSMF) are common precancerous conditions involving oral cavity with significant malignant potential.⁴ It has been noted that 90% of oral cancers are squamous cell carcinomas and they have their origin from OPMD's.⁵ Thus, early detection and treatment of these lesions play vital role in decreasing their malignant transformation and thereby decreasing overall incidence of oral cancer.

It is well established fact the inflammation plays an important role in the process of carcinogenesis. Chronic inflammation is associated with initiation, promotion as well as metastasis of cancer.⁶ The complex association of inflammation and carcinogenesis makes it interesting and yet challenging to explore the cause-effect relationship between them. Inflammation reflects its presence by changes in concentration of certain molecules in serum which are collectively termed as

¹K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara, Gujarat, India; ²Department of Oral Medicine and Radiology, Manubhai Patel Dental College and Hospital, Vadodara, Gujarat, India; ³Department of Oral Medicine and Radiology, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara, Gujarat, India; ⁴Department of Statistics, Sardar Patel University, Vallabh Vidyanagar, Anand, Gujarat.

Corresponding author: Palak H. Shah, Department of Oral Medicine and Radiology, Manubhai Patel Dental College and Hospital, Vadodara, Gujarat, India. Email: shahpalak98@gmail.com

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inflammatory markers. Certain markers are at times pathognomonic of certain conditions and show specific changes before and after treatment. Such markers can serve as prognostic marker for that specific condition. C-reactive protein (CRP) is one such inflammatory marker.^{3,7}

CRP is a classical acute phase plasma protein which belongs to pentraxin protein family. In response to tissue injury, it gets synthesized in hepatocytes. Its production is stimulated by inflammatory cytokines such as Interleukin-1, Tumour Necrosis Factor- α etc. Within several hours of infection or inflammation, CRP gets synthesized in liver and gets secreted in the blood stream. This robust and reliable response to inflammation makes CRP an ideal marker for inflammation.^{7,8,9}

CRP is an inflammatory marker for diseases such as atherosclerosis, myocardial infarction and bone fracture.¹⁰ Association of elevated CRP levels with an increased risk of malignancy has also been demonstrated in past researches.^{6,11,12} Prognostic role of CRP has been demonstrated in various systemic cancers such as Lung cancer, Breast cancer, Colon and Rectal cancer, Esophageal cancers, Liver cancer, Pancreatic cancer, Kidney and Urinary tract cancer, Blood cancer and Sarcomas.^{6,9}

Role of CRP in oral cancer has also been studied and it has shown promising results.^{12,13,14} But, there are very few research studies^{1,3,15} in past which has demonstrated role of CRP in OPMD's. This justifies the need for further researches in this field. Hence, the present study has been designed to determine the CRP levels in oral leukoplakia, oral submucous fibrosis, oral cancer and compare it with healthy individuals.

MATERIALS AND METHODS

A permission to conduct the present study was obtained from Institutional Ethics Committee. (IEC).

A total of 40 participants, equally divided into four study groups - Oral Submucous Fibrosis (OSMF), Oral Leukoplakia (OL), Oral Cancer (OC) and Healthy Controls (HC) - formed the study population. The lesions were clinically diagnosed as per the predetermined diagnostic and staging criteria (More et al classification for OMSF16, WHO classification for OL17 and TNM staging for OC18) by two Oral Medicine and Radiology specialists. Participants who had undergone treatment for the lesions and having systemic disease were excluded from the study.

Sample Collection

Written consent was obtained from each and every study participant. Total 5 ml of blood was aspirated from right / left

Table 1 : Distribution of participants according to age and sex

Group	Age (Yrs.)		Sex	
	Min-Max Age range (Yrs.)	Mean Age (Yrs.)	Male	Female
OSMF	16-53	36.7±10.33	06	04
OL	35-68	47.9±12.73	10	00
OC	40-77	52.6±10.31	07	03
HC	29-73	40.6±13.85	06	04
Overall	16-77	44.45 ± 7.45	29	11

(OSMF – Oral Submucous Fibrosis, OL – Oral Leukoplakia, OC – Oral Cancer, HC – Healthy Control, Yrs. – Years)

antecubital vein taking necessary aseptic precautions. Serum from the blood was extracted by centrifugation method. The serum was analysed for CRP levels by nephelometric analysis method using Mispa-i2 semi-automated specific protein analyser.

Statistical Analysis

The collected data was subjected to statistical analysis by using a statistical software, IBM SPSS v. 22.0. The tests applied were descriptive statistics, Kruskal Wallis test and Mann Whitney U test. The level of significance was set at p<0.05.

RESULTS

The age of the study participants was ranging from 16 to 77 years with a mean age of 44.45 ± 7.45 years. In sex distribution, total 29 (72.5%) male participants and 11 (27.5%) female participants were included in the study. (Table 1)

Serum CRP levels were estimated in all the study groups and the statistical difference among the groups was analysed by using Kruskal-Wallis test. The mean serum CRP levels were maximum in OC group (22.26 ± 53.84 mg/l) and minimum in HC group (1.36 ± 1.08 mg/l). The difference between the groups was found to be statistically significant. (p<0.05). (Table 2)

The co-relation of serum CRP levels in HC group with other study groups was performed by Mann Whitney U test. The difference was statistically significant with OC group and OSMF group (p<0.05) and statistically not significant with OL group (p>0.05). (Table 3)

Table 2: Serum CRP levels in different study groups (Kruskal – Wallis Test)

Study Groups	Serum Concentration of CRP (mg/l)			P Value
	Min	Max	Mean	
OMSF	1.58	12.1	5.17 ± 3.70	0.012 Significant (<0.05)
OL	0.5	8.02	2.45 ± 2.52	
OC	0.5	174.76	22.26 ± 53.84	
HC	0.5	3.8	1.36 ± 1.08	

(OSMF - Oral Submucous Fibrosis, OC - Oral Cancer, OL – Oral Leukoplakia, HC = Healthy Control, CRP- C-Reactive Protein, mg/l – milligram/litre, min - Minimum, Max - Maximum)

Table 3: Intergroup Co-relation of SERUM concentration of CRP in HC group with other study groups (Mann – Whitney U Test)

Study Groups	p-value	Significance
OSMF	0.002	Significant (p<0.05)
OC	0.029	Significant (p<0.05)
OL	0.396	Not Significant (p>0.05)

(OSMF = Oral Submucous Fibrosis, OC = Oral Cancer, OL- oral leukoplakia)



DISCUSSION

Inflammation is basically a protective response which prevents the body from invading pathogens. It is a complex sequential procedure which acts to neutralize the initial cause of cellular injury, initiate healing and eliminate the necrotic tissue.^{19,20} Inflammation is of two types: Acute and Chronic. In biological systems, while acute inflammation is said to have a protective role, chronic inflammation on the other hand is linked to development of cancer.²¹ Inflammation is identified as a precursor for most of the tumours. The inflammatory molecules and transcription factors are the molecular links between inflammation and cancer. C-reactive protein (CRP) is one such molecule connecting these two processes.^{19,20,21}

The concentration of C-reactive protein increases rapidly in presence of inflammation.⁶ This characteristic makes CRP a reliable marker of inflammation. As inflammation and carcinogenesis are inter-related, CRP can act as a prognostic marker for malignancies. Past researches have demonstrated the prognostic role of CRP in various systemic cancers as well as head and neck cancers.^{9,11,12,13} Raised levels of CRP have also been noticed in oral premalignancies.^{1,3,15} Considering this, the present study is designed with the aim to determine serum CRP levels in Oral Submucous Fibrosis (OSMF), Oral Leukoplakia (OL) and Oral Cancer (OC) and co-relate it with healthy controls.

In our study, mean age of the study participants was 44.45±7.45 years. There were total 72.5% males 27.5% females. The healthy controls included in the study were age and sex matched with the study participants. The findings of our study are consistent with the studies conducted by Shah et al⁴, Metgud et al²² and More et al²³. In all the three disease groups, male predilection was noted. The main reason for this is, in Indian Subcontinent, consumption of various forms of tobacco and areca nut is more common in males as compared to females^{24,25}.

In our study, the serum levels of CRP were determined and compared between all the four study groups. The lowest serum CRP levels were found in HC group. It was observed that, the levels of serum CRP were increased as the severity of the disease increased. It was noticed that the mean serum level CRP was gradually increasing from HC group (1.36 ± 1.08 mg/l) to OL group (2.45 ± 2.52 mg/l) to OSMF group (5.17 ± 3.70 mg/l) to OC group (22.26 ± 53.84 mg/l). These findings confirm our research hypothesis and establish serum CRP levels as a potential tool to understand the role of inflammation in disease progression from normal mucosa to malignancy. When compared statistically by Kruskal-Wallis test, the difference was found to be statistically significant.

When intergroup comparison for serum concentrations of CRP in healthy controls was done with disease groups by Mann Whitney U test, statistically significant difference was obtained for OSMF and OC groups and statistically not significant difference was found for OL group.

Findings of our study were supported by the studies of Vankadara et al¹, Gosavi et al³, Bhattacharjee et al¹⁵, Uppal et al²⁶, Gupta et al²⁷ and Metgud et al²² in which statistically significant differences were found in between the study groups. The findings of our study were not similar to the study conducted by Kaja et al²⁸, Kohli et al²⁹ in which intergroup comparison did not yield statistically significant differences.

The limitations of the present study include:

In our study, histological examination was not performed for participants. Histological examination would have facilitated the co-relation of serum CRP levels with the degree of cellular dysplasia. This could have helped in establishment of possible association between the histological and micro-molecular changes in carcinogenesis, if any.

Also, we could have included larger sample size with the participants equally distributed according to the stages of the disease groups. This would have facilitated intragroup comparison of serum CRP levels and co-relation of disease progression clinically and at micro-molecular level.

Future researches should be designed with larger sample size to overcome these limitations so that the role of CRP becomes clearer in development and progression of malignant process completely in each and every phase of the disease.

CONCLUSION

The findings of present study demonstrate the increase in serum levels of CRP with increasing the severity of disease. Thus, serum CRP levels are strongly associated with progression of the disease process in carcinogenesis. Present study establishes CRP as a reliable prognostic for OPMD's and oral cancer. In addition, this research has provided a baseline data for future researches to co-relate the levels of CRP in all the clinical stages of OPMD's and also with the histological grading of dysplasia. Further researches are also required to know the role of CRP as a potential prognostic marker following the treatment of the disease.

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