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Original Research Article

Serum and Salivary C - reactive protein in Patients with Oral Leukoplakia and Squamous Cell Carcinoma

Dr Sonia Gupta[1], Dr. Nutan Tyagi[2], Dr. Afreen Nadaf[3]

[1]Tutor, Department of Oral Pathology and Microbiology, Govt. Dental College and Hospital Srinagar.
[2]Reader, Department of Oral Pathology and Microbiology, Institute of Dental Studies and Technologies, Kadrabad, Modinagar, (U.P.)
[3]Lecture, Department of Oral Pathology and Microbiology, Govt. Dental College and Hospital Srinagar.

Corresponding Author: Dr Sonia Gupta

ABSTRACT

Aims and objective: To determine the levels of serum and salivary C-reactive protein in patients with oral leukoplakia and squamous cell carcinoma as well as to evaluate their significant role as a prognostic marker.
Materials and method: The study sample consisted of 30 patients and the subjects were divided into three groups. Group A included 10cases of normal oral mucosa, Group B comprised 10 cases of oral leukoplakia and Group C included 10 cases of OSCC confirmed by histopathological examination. Salivary and serum levels of CRP were determined using immunoturbidimetry based on agglutination.
Results: The mean serum and salivary level of CRP in OSCC was more as compared to oral leukoplakia and normal oral mucosa. The p-value was found to be statistically significant
Conclusion: CRP is a biomarker which can be used for the evaluation of the severity of the disease.

Key words: Biomarkers, Leukoplakia, Oral Cancer, C-reactive protein

INTRODUCTION

The term "Leukoplakia" was first proposed in the year 1877 by the Hungarian dermatologist ErnoSchwimmer. [1] Literally; the word leukoplakia means a 'white patch' which was derived from Greek word leukos- white, plakia- patch. In 1978, World Health Organization (WHO) group defined oral leukoplakia as "A white patch or plaque that cannot be characterized, clinically or pathologically as any other disease". [2] Warnakulasuriya et al in 2007 defined leukoplakia as "A plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer". [3] A new definition of leukoplakia was proposed by Shanbhag in 2017 [4] as "a predominantly white, irreversible, non-scrapable lesion of the oral mucosa that cannot be characterized clinically or histopathologically as any other lesion/disease and has increased risk of cancer occurrence than its normal counterpart and is usually associated with consumption of tobacco, betel quid, and alcohol, but otherwise can be of idiopathic in nature.

Oral squamous cell carcinoma (OSCC) is a malignant neoplasm derived from the stratified squamous epithelium of the oral mucosa. [5] The pathogenesis of OSCC is multifactorial, associated with cigarette smoke, alcohol [6] and snuff, as well as the papilloma virus etc. [7] In general, cancers, including OSCC, develop from the accumulation of genetic changes and

epigenetic abnormalities in the signaling pathways that are associated with cancer, resulting in phenotypes that facilitate OSCC development. This process was concised by Hanahan and Weinberg in 'Hallmarks of Cancer'. [8] OSCC is the sixth most common cancer worldwide. [9] More than 90% of all oral cancers are squamous cell carcinoma (SCC). [10,11] A number of conditions have been associated with an elevated risk of developing OSCC including Li-Fraumeni syndrome, Plummer-Vinson syndrome, Fanconi anemia, chemotherapy induced immunosuppression of organ transplantation, dyskeratosis congenita, Xeroderma-pigmentosum and discoid lupus erythematosus. [12]

C-reactive protein (CRP), a systemic marker of chronic inflammation, is an acute phase protein, the levels of which vary on daily basis, rises with aging, increased blood pressure, smoking, coffee and alcohol consumption, decreased physical activity, raised levels of triglycerides, insulin resistance and diabetes, high-protein diet, chronic tiredness and suffering from sleep disturbances, and depression. [13] CRP belongs to pentraxins protein family; pentraxins are the ancient proteins having cyclic pentameric arrangement of five non-covalently bound identical subunits [14] placed in a symmetric cyclic design around a central pore, determining a pentameric, discoid, and flattened configuration. [15] CRP is considered as a prognostic factor for various malignancies and the risk of cancer is increased when pre-diagnostic CRP levels are high. The aim of the present study was to determine the levels of serum and salivary C-reactive protein in patients with oral leukoplakia and squamous cell carcinoma as well as to evaluate their significant role as a prognostic marker.

MATERIALS AND METHODS

A cross-sectional study was conducted on 30 subjects who were divided into three groups:

Group A- 10 subjects of normal oral mucosa

Group B- 10 subjects of oral leukoplakia

Group C- 10 subjects of OSCC

The histopathological confirmation was done on all cases of oral leukoplakia, OSCC and normal oral mucosa. The study was approved by the ethical committee of the institution and an informed consent was obtained from all study subjects. The patients who was receiving therapy or suffering from any systemic condition, such as hepatic or renal disorders, patients who were pregnant or taking antibiotics or NSAIDS within a month of sample collection, patients with underlying systemic diseases, cancers other than oral cancer or previously treated oral cancer, chronic alcoholics were excluded from the study to rule out altered liver function that could contribute to alterations of CRP serum levels.

Sample collection was done in the morning from the study subjects so as to prevent diurnal variation. Blood samples were taken from cubital vein and left to clot at 4^0C in a sterile, clean, dry tube and kept inside the refrigerator. Serum were collected by centrifuge the blood at 3,000rpm for 5 to 10 minutes in 10cc sterile tubes and stored at -20^0C until the time of analysis.

From each subject, 10 ml of unstimulated whole saliva were collected into a sterile centrifuge tube. After centrifugation, the separated clear salivary fluid was stored in disposable storage vials at -80^0C until the test day.Salivary and serum levels of CRP were determined using immunoturbidimetry based on agglutination. CRP causes agglutination of the latex particles coated with anti-human CRP. The agglutination of the latex particles is proportional to the CRP concentration and is measured by turbidimetry. The test specimen is mixed with activation buffer and latex reagent which then allowed to react. CRP in the test specimen causes the formation of an insoluble complex that produces turbidity, which is measured at 546 nm. The increased turbidity corresponds to increased concentration of CRP in the test specimen.

The data was analysed by using statistical software (SPSS version 19.0). Mean and standard deviation were calculated for each individual group. A probability value (p) of ≤0.05 was considered to be statistically significance.

RESULTS

In the present study, 40% and 60% of the subjects in Group A were males and females respectively. In Group B, 70% and 30% were males and females respectively. In Group C, 80% were males and 20% were females (Table 1).

In the present study, the mean age of the patients in Group A, Group B and Group C were 24.75±5.09, 32.50±8.33 and 48.52± 9.22 respectively (Table 2).

In the present study, the mean serum level of CRP in Group A, Group B and Group C were 2.2±0.85 mg/l, 6.8±0.98 mg/l and 12.22±1.8 mg/l respectively. The mean serum level of CRP increased from normal oral mucosa to OSCC with a statistically significant difference (Table 3).

In this study, the mean saliva level of CRP in Group A, Group B and Group C were 0.15±0.11 mg/l, 0.39±0.08 mg/l and 0.58±0.05 mg/l respectively. The mean saliva level of CRP in OSCC was more as compared to oral leukoplakia and normal oral mucosa. The p-value was found to be statistically significant (Table 4).

Table 1: Gender wise distribution in normal oral mucosa, oral leukoplakia and oral squamous cell carcinoma

Group	No. of cases	Gender	
		Male	Female
A	10	4(40%)	6 (60%)
B	10	7 (70%)	3 (30%)
C	10	8(80%)	2 (20%)

Group A- Normal oral mucosa, Group B-Oral leukoplakia, Group C- Oral Squamous cell carcinoma

Table 2: Age wise distribution in normal oral mucosa, oral leukoplakia and oral squamous cell carcinoma

Group	No. of cases	Mean ± Standard deviation
A	10	24.75±5.09
B	10	32.50±8.33
C	10	48.52±9.22

Group A- Normal oral mucosa, Group B-Oral leukoplakia, Group C- Oral Squamous cell carcinoma

Table 3: Serum C- reactive protein (mg/l) in normal oral mucosa, oral leukoplakia and oral squamous cell carcinoma

Parameter	Group	No. of cases	Mean ± Standard deviation	P-value
Serum C- reactive protein (mg/l)	A	10	2.2±0.85	
	B	10	6.8±0.98	
	C	10	12.22±1.8	0.001

Group A- Normal oral mucosa, Group B-Oral leukoplakia, Group C- Oral Squamous cell carcinoma

Table 4: Salivary C- reactive protein (mg/l) in normal oral mucosa, oral leukoplakia and oral squamous cell carcinoma

Parameter	Group	No. of cases	Mean ± Standard deviation	P-value
Salivary C- reactive protein (mg/l)	A	10	0.15±0.11	
	B	10	0.39±0.08	
	C	10	0.58±0.05	<0.001

Group A- Normal oral mucosa, Group B-Oral leukoplakia, Group C- Oral Squamous cell carcinoma

DISCUSSION

CRP is a prognostic factor which can be used to determine treatment efficacy in several diseases including connective tissue diseases, cardiovascular diseases, infections, inflammatory bowel disease, lupus erythematosus, pneumococcal pneumonia, rheumatoid arthritis, rheumatic fever, tuberculosis and cancers. [16] In the present study, serum and salivary CRP levels were determined in patients with oral leukoplakia and OSCC to evaluate whether CRP could be a biomarker for oral leukoplakia and OSCC. In this study, serum and salivary CRP levels increased significantly from normal oral mucosa patients to oral leukoplakia patients to patients with OSCC. The results were in accordance with the study done by Acharya et al, Vankadara et al and Metgudet al. [17-19]

An elevated level of CRP signifies that tumor development and progression can induce chronic inflammation. Tumor growth could stimulate an immune response, which results in increased production of inflammatory cytokines. These cytokines could promote tumor growth further by changing tumor cell biology and activating stromal cells in the tumor microenvironment. Therefore, a bidirectional link between chronic inflammation and cancer contributes to

elevated levels of CRP. [20,21] Immune responses produced by premalignant or malignant cells are both anti-neoplastic and pro-neoplastic. In the anti-neoplastic response, the immune system identifies the transformed cells as malignant and thus causes tumor cell destruction. In the case of a pro-neoplastic response, chronic inflammation in the microenvironment of tumors enhances tumor growth, promotes angiogenesis and favors metastasis. Therefore, elevated CRP levels in oral leukoplakia and OSCC could be contributed to either anti- or pro-neoplastic immune response depending on which mechanism overcomes. Besides, increased CRP levels determine an increased predisposition to cancer due to chronic inflammation. [22]

CONCLUSION

The present study determined elevated levels of serum and salivary CRP in patients with OSCC as compared to patients with oral leukoplakia and normal oral mucosa. Also, serum and salivary CRP levels were higher in patients with oral leukoplakia than normal oral mucosa signifying that CRP is a biomarker which can be used for the evaluation of the severity of the disease.

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