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SERUM AND SALIVARY LEVELS OF NITRIC OXIDE AND C - REACTIVE PROTEIN IN PATIENTS WITH ORAL LICHEN PLANUS

Oral Pathology

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ABSTRACT

Background: The etiology and pathogenesis of oral lichen planus (OLP) has been the focus of research since a long time and various etiological factors like autoimmunity, heredity, drugs, dental materials, psychological factors, especially stress and anxiety have been reported to be associated with this disease. **Aim And Objectives:** To determine serum and salivary level of nitric oxide (NOx) and C - reactive protein (CRP) as oxidative stress and inflammation status in patients with oral lichen planus 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 two groups. Group A included 15 cases of normal oral mucosa and Group B comprised 15 cases of oral lichen planus confirmed by histopathological examination. Salivary and serum levels of NOx and CRP were determined. **Results:** In this study, serum and salivary levels of NOx and CRP increased significantly from normal oral mucosa patients to oral lichen planus. A significant correlation was found between NOx and CRP values in serum and saliva. **Conclusion:** Oxidative stress cause damage to various organs in the human body. The results of our study revealed that NOx and CRP play an important role in the etiopathogenesis of oral lichen planus. Salivary and serum CRP and NOx may be used as a non-invasive and prognostic marker for OLP.

KEYWORDS

Oral lichen planus, oral potentially malignant disorders, C-reactive protein, Nitric oxide

INTRODUCTION

Lichen Planus is a term derived from the Greek word leikhēn, means "a tree moss" and the Latin word planus, means "flat".[1] Lichen planus was first explained by British physician Erasmus Wilson in 1869 that generally affects 0.5-1 percent of the world's population.[2] Oral lichen planus is the mucosal counterpart of cutaneous lichen planus and occurs most commonly in the fourth decade of life. It demonstrates a preponderance in females with an occurrence ratio of 1.4:1.[3-5] Clinically, the lesion may exhibit reticular, papular, plaque-like, erosive, atrophic or bullous pattern. The buccal mucosa, tongue and gingiva are commonly involved in oral lichen planus and the lesions may appear weeks or months before the onset of cutaneous lesions. The skin lesions appear as violaceous, flat-topped papules in ankles, wrist and genitalia though typically, the facial skin is spared. [6, 7] More commonly, the oral lesion appears as a radiating white, gray, velvety, thread-like papules in a linear, annular and retiform pattern forming typical lacy, reticular patches, rings and streaks in the oral cavity. A tiny white elevated dot is present at the intersection of white lines known as Wickham striae as compared to striae of Wickham seen in the skin.[2,8,9]

Both specific and non-specific mechanisms may play an important role in the etiopathogenesis of oral lichen planus. In specific mechanisms, antigen presentation by basement layer keratinocytes as well as cytotoxic T lymphocyte lead to death of antigen-specific keratinocytes, whereas non- specific mechanisms suggests that degranulation of mast cell and activation of matrix metalloproteinase are responsible. Both mechanisms acting concurrently cause accumulation of T lymphocytes in the lamina propria beneath the epithelium, intraepithelial migration of T lymphocytes, breakdown of basement membrane and apoptosis of keratinocyte, all of which are distinctive features of oral lichen planus.[3] Dendritic cells (DC) play a major role in these immunopathological features with regard to antigens presented to T-cell. [10]

Role of oxidative stress in pathophysiologic changes of basal cells of epidermis in lichen planus has also been studied.[11] Nitric oxide is a short living product of nitrogen metabolism which is formed by various cells in the organism with very significant physiological function. Endothelial cells and neural cells produce NOx, macrophages and other inflammatory cells can provoke its synthesis and release. Bacterial products are the most important inductors of NOx synthesis. The biological function of NOx can be illustrated in two ways. First, it acts as an endothelial-derived vascular smooth muscle relaxant, an inhibitor of platelet aggregation and adhesion, and a neuronal messenger. Second, the NOx synthesized in large amounts by activated macrophage is a cytotoxic molecule which influences the ability of cells to kill bacteria, viruses and protozoa along with tumour cells. However, NOx secreted by macrophages has destructive effects against cellular proteins, DNA and a lipid leading to periodontitis.[12-14] C-reactive protein (CRP) is an acute phase protein that acts as a systemic marker of chronic inflammation. The levels of C-reactive protein may vary on daily basis and also demonstrates an increases with aging, increased blood pressure, smoking, coffee and alcohol consumption. The decreased level of CRP occurs during physical activity, raised levels of triglycerides, insulin resistance and diabetes, high-protein diet, chronic tiredness and suffering from sleep disturbances, and depression.[15] CRP pertains to pentraxins protein family and pentraxins are the ancient proteins in which cyclic pentameric arrangement of five non-covalently bound identical subunits [16] placed in a symmetric cyclic design around a central pore, determining a pentameric, discoid, and flattened configuration. [17] CRP is also considered as a prognostic factor for various malignancies like OSCC, multiple myeloma etc and the risk of cancer is increased when pre-diagnostic CRP levels are high. The aim of the present study was to determine serum and salivary level of nitric oxide (NOx) and C - reactive protein(CRP) as oxidative stress and inflammation status in patients with oral lichen planus as well as to evaluate their significant role as a prognostic marker.

MATERIALS AND METHOD

A cross-sectional study was conducted on 30 subjects in the IDST, Modinagar and the study group was divided into two groups:-

Group A- 15 subjects with normal oral mucosa

Group B- 15 subjects with oral lichen planus

Histopathological confirmation was done on all cases of oral lichen planus 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 receiving therapy for or suffering from any systemic condition like hepatic or renal disorders; patients who were pregnant or taking antibiotics or NSAIDS within a month of sample collection, patients with underlying systemic diseases, oral cancer or previously treated oral lichen planus, chronic alcoholics were excluded from the study to rule out altered liver function which could contribute to alterations of CRP and NOx levels.

Sample collection was done in the morning from the study patients to prevent confounding arising from 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 was collected by centrifuging the blood at 3,000rpm for 5 to 10 minutes in 10cc sterile tubes and it was stored at -20^0C till the time of analysis.

From each subject, 10 ml of unstimulated whole saliva was collected

into a sterile centrifuge tube. After centrifugation, the separated clear salivary fluid was stored in disposable storage vials at -80 ^{0}C until the test day.

Serum and salivary NOx determination was based on the Griess reaction in which a chromophore with a strong absorbance at 545nm is formed by the reaction of nitrite with a mixture of N-naphthylethylenediamine and sulfanilamide. For NOx detection, fractions of the sample were mixed with cadmium in glycine buffer at pH 9.7 to covert nitrate to nitrite, which was then mixed with fresh reagent. The absorbance was measured by spectrophotometer. Salivary and serum levels of CRP were obtained 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 was 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 was measured at 546 nm. The increased turbidity corresponded to increased concentration of CRP in the test specimen. All the data was analyzed using statistical software (SPSS version 21.0). Mean and standard deviation were calculated for each individual group. A probability value (p) of ≤0.05 was considered as statistically significant.

RESULTS:

In the present study, 60.00% and 40.00% of the subjects in Group A were males and females respectively. In Group B, 46.67% and 53.33% were males and females respectively. (Table 1). The mean age of the patients in Group A and Group B were 25.75±7.08 years and 33.50±6.32 years respectively (Table 2).

The mean serum level of CRP in Group A and Group B were 1.82±1.08 and 4.26±2.86 mg/l respectively. The mean serum level of CRP was significantly higher in Group B. The mean serum level of NOx in Group A and Group B again demonstrated the same statistically significant pattern with NO_x values of 37.65±7.85 and 59.75±19.45µmol/l respectively. (Table 3).

The mean salivary CRP levels in Group A and Group B were 09.32±2.8 and 13.40±6.2µg/l respectively with greater levels observed in those with oral lichen planus. The p-value was found to be statistically significant. The mean salivary level of NOx in Group A and Group B were 64.67±22.41and 85.04±35.62µmol/l respectively. The p-value was found to be statistically significant (Table 4).

DISCUSSION

Oral Lichen Planus (OLP) is a chronic inflammatory disease which affects skin as well as oral mucosa. OLP is a T-cell mediated autoimmune disease in which the cytotoxic CD8+ T cells elicit basal cells apoptosis of the oral epithelium. The oral lesions appear as white furrows or linear, papules, plaques, erythema, erosions or ulcers in the mouth which mostly affect the buccal mucosa, tongue, and gingiva. Currently, six clinical types of OLP like reticular, erosive, atrophic, papular, plaque-like and bullous have been reported.

C-reactive protein is a marker of chronic inflammation and an acute phase protein that is synthesized in liver and has been determined as an important biomarker in wide spectrum of conditions like systemic infection, rheumatoid arthritis, vasculitis etc. Adipocytes such as IL-6/TNF-alpha are closely related with CRP. [18] In the present study, serum and salivary CRP levels were determined in patients with oral lichen planus to evaluate whether CRP could be a prognostic marker for oral lichen planus. In this study, serum and salivary CRP levels increased significantly from normal oral mucosa patients to oral lichen planus. These results were in accordance with the study done by Shiva et al in 2019.[19]

The findings of the current study suggest potential use of salivary and serum CRP as a parameter for assessment systemic levels of inflammation. The correlation of CRP levels with chronic inflammation is reported that reveals the potentially malignant nature of lesion. The results of the study conducted by Ataset al[20] determined high levels of CRP and ESR with high neutrophil count that might support the systemic inflammatory process in LP patients. Santiago et al [21]showed patients with LP revealed higher lipid peroxidation levels and CRP, hence chronic inflammation may elucidate these findings. Besides, increased CRP levels correspond to an increased tendency to oral lichen planus due to chronic inflammation.

CONCLUSION

The present study determined elevated levels of serum and salivary CRP in patients with oral lichen planus as compared to patients with normal oral mucosa. Salivary and serum CRP may be used as a non-invasive and prognostic marker for OLP and it would be more helpful, if baseline values of CRP could be determined during treatment and follow-up visits of patients, which would further enhance its role in the clinical management of the disease.

Table 1: Gender Wise Distribution In Normal Oral Mucosa And Oral Lichen Planus

Group	No. of cases	Gender	
		Male	Female
Group A	15	09(60.00%)	06 (40.00%)
Group B	15	07(46.67%)	08(53.33%)

Group A- Normal oral mucosa, Group B-Oral lichen plan

Table 2: Age Wise Distribution In Normal Oral Mucosa And Oral Lichen Planus

Group	No. of cases	Mean ± Standard deviation
A	15	25.75±7.08
B	15	33.50±6.32

Group A- Normal oral mucosa, Group B-Oral lichen planus

Table 3: Serum C- Reactive Protein (mg/l) And NOx (µmol/l) In Normal Oral Mucosa And Oral Lichen Planus

Parameter	Group	No. of cases	Mean ± Standard deviation	P-value
Serum C- reactive protein	A	15	1.82±1.08	0.001
	B	15	4.26±2.86	
Serum NOx	A	15	37.65±7.85	0.001
	B	15	59.75±19.45	

Group A- Normal oral mucosa, Group B-Oral lichen planus

Table 4: Salivary C- Reactive Protein (µg/l) And NOx (µmol/l) In Normal Oral Mucosa And Oral Lichen Planus

Parameter	Group	No. of cases	Mean ± Standard deviation	P-value
Salivary C- reactive protein	A	15	09.32±2.8	< 0.001
	B	15	13.40±6.2	
Salivary NOx	A	15	64.67±22.41	0.001
	B	15	85.04±35.62	

Group A- Normal oral mucosa, Group B-Oral lichen planus

REFERENCES:

1. Barnes L, Eveson JW, Reichart P and Sidransky D. World health organization classification of head and neck tumours. Lyon: IARC Press 2004; 140, 177-179.
2. Shafer WG, Hine MK, Levy BM, Rajendran R and Sivapathasundharam B. Text book of oral pathology (7th edition). Elsevier 2012; 94, 808.
3. Sugerman PB, Savage NW, Walsh LJ, Zhao ZZ, Zhou XJ, Khan A, et al. The pathogenesis of oral lichen planus. CritRev Oral Biol Med 2002; 13:350-65.
4. Gorsky M, Epstein JB, Hasson-Kanfi H, Kaufman E. Smoking habits among patients diagnosed with oral lichen planus. Tobacco Induced Diseases 2004; 2(2):103-8.
5. Eisen D. The clinical features, malignant potential and systemic associations of oral lichen planus: a study pf 723 patients. J Am Acad Dermatol 2002; 46(2):207-14.
6. Ismail SB, Kumar SK, Zain RB. Oral lichen planus and Lichenoid reactions; Etiopathogenesis, diagnosis, management and malignant transformation. J Oral Sci 2007; 49:89-106.
7. Canto AM, Muller H, Freitas RR, Santos PS. Oral lichen planus (OLP): Clinical and complementary diagnosis. An Bras Dermatol 2010; 85:669-75.
8. Silverman S, Gorsky M, Lozada-Nur F. A prospective follow-up study of 570 patients with oral lichen planus: persistence, remission and malignant association. Oral Surg Oral Med Oral Pathol 1985; 60: 30-4.
9. Silverman S, Gorsky M, Lozada-Nur F, Giannotti K. A prospective study of findings and management in 214 patients with oral lichen planus. Oral Surg Oral Med Oral Pathol 1991; 72: 665–70.
10. Sloberg K, Jonsson R, Jontell M. Assessment of Langerhans'cells in oral lichen planus using monoclonal antibodies. J Oral Pathol 1984; 13: 516–24.
11. Sander CS, Cooper SM, Ali I, Dean D, Thiele JJ and Wojnarowska F. Decreased antioxidant enzyme expression and increased oxidative damage in erosive lichen planus of the vulva. BJOG 2005; 112: 1572-75.
12. Sezer E, Ozugurlu F, Ozyurt H, Sahin S and Etikan I. Lipid peroxidation and antioxidant status in lichen planus. Clin Exp Dermatol 2007; 32: 430-34.
13. Pali T, Bencsik P, Gorbe A, Ferdinandy P and Csont T. Measurement of NO in biological samples. Br J Pharmacol 2015; 172: 1620-1632.
14. Forstermann U, William C and Sessa A. Nitric oxide synthases: regulation and function. Eur Heart J 2012;33: 829-37.
15. Goldie MP. C-reactive protein, cardiovascular disease, and periodontal disease. What is new in research? Int J Dent Hyg 2004; 2:139-41.
16. Srinivasan N, White HE, Emsley J, Wood SP, Pepys MB, Blundell TL. Comparative analyses of pentraxins: Implications for protomer assembly and ligand binding. Structure 1994; 2:1017-27.

17. Salazar J, Martinez MS, Mervin CC, Victoria N, Roberto A, Yaquelin Tet al. C-reactive protein: An in-depth look into structure, function, and regulation. IntSch Res Notices2014; 2014:1-11.
18. Patil SA, Aditya A and Jangam DK. Evaluation of salivary C-reactive protein and psychological factors in patients with oral lichen planus: A case-control study. JCDR 2021; 15(5): ZC47-ZC51.
19. Shiva A, Arab S, Mousavi SJ, Zamanian A and Maboudi A. Serum and salivary level of nitric oxide (Nox) and CRP in oral lichen planus (OLP) patients. J Dent Shiraz Univ Med Sci 2020; 21(1): 6-11.
20. Ataş H, Cemil BÇ, Kurmuş GI, Gönül M. Assessment of systemic inflammation with neutrophil-lymphocyte ratio in lichen planus. Postepy Dermatol Alergol 2016; 33: 188-192.
21. Arias-Santiago S, Buendía-Eisman A, Aneiros-Fernández J, Girón-Prieto MS, Gutiérrez-Salmerón MT, Mellado VG et al. Cardiovascular risk factors in patients with lichen planus. Am J Med 2011; 124: 543-548.