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Original Research Article

Assessment of serum levels of ascorbic acid and malondialdehyde as oxidative stress marker in patients with oral lichen planus

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ABSTRACT

Background: Oral Lichen Planus (OLP) is a chronic inflammatory mucosal disease with unknown etiology. The cause might because of reactive oxygen species and oxidative damage to the tissues.In recent studies, free radicals have been postulated as the possible etiology of inflammatory and autoimmune disease.
Materials and Methods: A total of 60 participants were studied and they were grouped in to cases and controls. Each group had 30 participants, 30 cases and 30 healthy controls. Serum levels of MDA and Vitamin C were estimated**.** The data collected was analyzed statistically by using descriptive statistics namely mean, standard deviation and any significant difference between the mean values of cases and controls will be tested by using sample Student t-test. A p value ≤ 0.05 has been considered statistically significant.
Objective: The present study was conducted to estimate the levels of Serum Ascorbic acid (vitamin C) and Malondialdehyde (MDA) levels, as biomarkers of oxidative stress in patients with oral lichen planus.
Results: The mean Serum Vitamin C levels in OLP patients and healthy controls were 2.5±0.46 mg/L and 9.28± 1.04 mg/L. The mean serum levels of MDA in OLP patients and healthy controls were 3.99±0.71nmol/mL and 1.39±0.20 nmol/ml.
Conclusion: The current study demonstrates, existence of oxidative stress, compromised anti- oxidant defenses with higher levels of MDA and decreased levels of Vitamin C, in patients with oral lichen planus. Further studies are to be conducted to draw definite conclusion and significant correlation of the levels of the biomarkers in OLP.

1. Introduction

Oral Lichen Planus (OLP) is a chronic mucocutaneous inflammatory oral mucosal disease with unknown etiology. OLP lesions appearance is as white striations, papules, plaques, erythema, erosions or ulcers in the mouth and commonly effects the buccal mucosa, tongue, gingiva.[1] Pain from OLP varies from being minimally bothersome to annoying and unables patients to perform their daily routine. About 2% of people around the world has incidence of OLP, its more common in women aged between 40 to 60 years.[2] The trigger factors for OLP are genetic susceptibility, immunological illness, malnutrition as well as infectious agent.[3] The state of increased Reactive oxygen species (ROS) due to excess production, or decreased function of antioxidants or impaired ROS removal is called as Oxidative Stress. ROS could be injurious to cells by inactivation of enzymes, denaturation of proteins, DNA destruction, Lipid peroxidation and leads to cell membrane damage, increase in reactive aldehydic materials like Malondialdehyde (MDA). MDA is a useful determinant in case of increased Lipid peroxidation, that leads to impaired function of cell.[4] Different types of scavengers

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for free radicals which consist of various minerals, enzymes and vitamins like A and E which inhibits lipid peroxidation of cell membrane. Vitamin C by stabilizing collagen triple helical structure acts as cofactor for enzymes as well as helps to regenerate vitamin E.[5]

The existing literature providing information on the role of oxidative stress in Oral Lichen Planu is very limited. Hence this current study aims to estimate the oxidative stress and compromised antioxidant defenses in OLP with Vitamin C and MDA (end product of lipid peroxidation) as a biomarker.

2. Materials and Methods

2.1. Study participants

The study was initiated after obtaining permission from the institutional ethics committee of V.S. Dental College. The study was conducted by Department of Biochemistry, Kempegowda Institute of Medical Sciences, in collaboration with Department of Oral medicine and Radiology, V. S Dental College, Bangalore, India.

Participants were enrolled in the study after a written informed consent. Subjects of either sex aged between 20-70 years attending the outpatient department of Oral medicine and Radiology and consenting to participate in the study were grouped in to two groups. A total of 60 subjects were evaluated, of them the first group consisted of 30 subjects who are clinically and biopsy proven cases of OLP and the second group consisted of apparently healthy individuals.

Subjects with known history of co morbid conditions like Endocrine disorders, autoimmune disorders, Diabetes Mellitus, smoking, alcohol and tobacco products consumption, subjects on therapeutic regimen in the last three months consisting of vitamin supplements, chronic periodontitis subjects and immunosuppressive drugs were excluded from the study. Clinical examination was undertaken to rule out any systemic illness in patients. The study was carried out for a period of 6 months from January to June 2016.

2.2. Sample collection and estimation

5ml plain venous blood was obtained by venipuncture under aseptic conditions, followed by centrifugation and the serum was stored at -20°c till the investigations were carried out. Serum levels of MDA were estimated by Thiobarbituric acid method (TBA) and Vitamin C by 2, 4 Dinitrophenyl hydrazine method manually by colorimetric analysis.

2.2.1. Measurement of MDA levels

MDA is used to measure lipid peroxidation by Thiobarbituric acid (TBA) reactive substances method.TBA method is commonly and widely used as an indicator of Peroxidative free radical damage.[6] MDA reacts with two molecules of Thiobarbituric acid with elimination of two water molecules forming a pink crystalline pigment with the absorption maximum at 535nm measured colorimetrically. The results are expressed in nmol/ml

2.3. Measurement of Vitamin C levels

Vitamin C a water soluble antioxidant vitamin acts as a defense against oxidative stress. Vitamin C is measured by 2,4 Dinitrophenyl hydrazine method. Ascorbic acid is oxidized to form dehydroascorbic acid and diketogulonic acid, in the presence of 2,4 Dinitrophenyl hydrazine and sulphuric acid. They form an orange coloured product with absorption maximum at 520 nm. The results are expressed in mg/L.

The data collected was analyzed statistically by using descriptive statistics namely mean, standard deviation, and any significant difference between the mean values of the study groups and the control group was tested using sample Student t-test. A P value ≤ 0.05 was considered statistically significant in the study.

3. Result

The mean age of the OLP patients and controls individuals were 43.6 and 35.1 years old, respectively.(Figure 1) The mean Serum Vitamin C levels in OLP patients and healthy controls were 2.29±0.48 mg/L and 9.85± 2.99 mg/L(Figure 2). The mean serum levels of MDA in OLP patients and healthy controls were 4.12 ± 0.71nmol/ml and 1.96 ± 0.35 nmol/ml respectively. (Figure 3) The difference between mean values of Vitamin C and MDA among OLP and cases and healthy controls was found to be statistically significant (p value < 0.001) (Table 1). There is a negative correlation (-0.008), though not very significant (P value 0.98) between MDA and Vitamin C, that is as MDA levels rise there is a fall in Vitamin C levels.

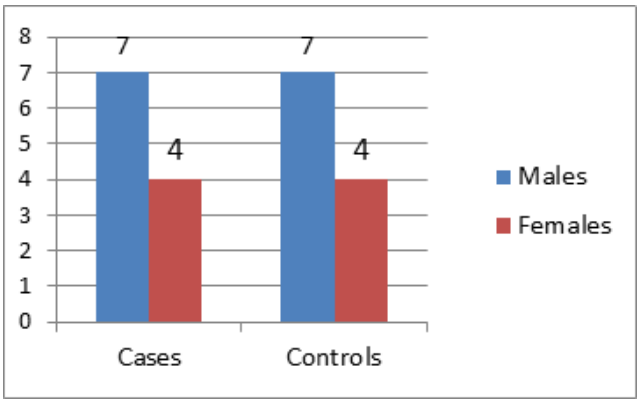

Fig. 1: Distribution of cases and controls according to gender

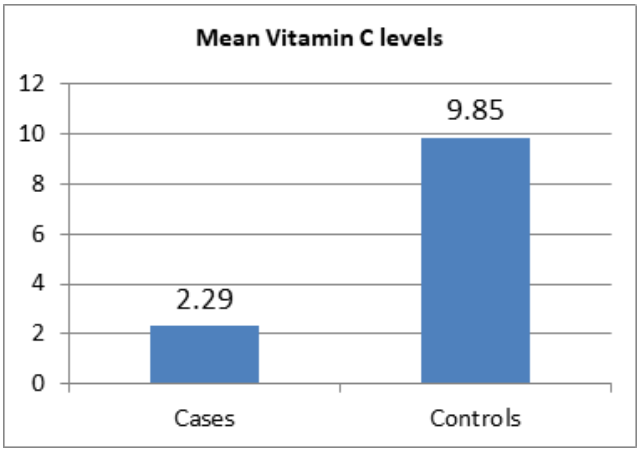

Fig. 2: Mean vitamin C levels among cases and controls

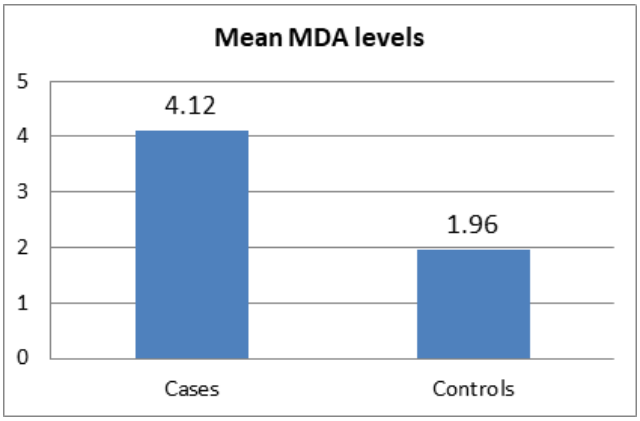

Fig. 3: Mean MDA levels among cases and controls

Table 1: Mean values of Vitamin C and MDA among cases and controls

S.No	Parameter	Mean values		p Value
		Cases	Controls	
1	Vitamin C	2.29 ± 0.48	9.85 ± 2.99	< 0.001
2	MDA	4.12 ± 0.71	1.96 ± 0.35	< 0.001

4. Discussion

The imbalances between ROS and antioxidants, have a role in OLP development.[7,8] Therefore in the current study we aimed at estimating the serum levels of MDA and Vitamin C of patients with OLP. Its the highly complex antioxidant defense systems[4,8] that is effective against ROS. It is important to determine the amount and action of different antioxidants status in vivo. The pathogenesis of OLP has considerable evidence of nutrients particularly anti-oxidant vitamins A,C,E.[8] In the current study we found that the levels of Vitamin C were considerably low when compared to the healthy control group. The antioxidant action in eliminating single oxygen and free radical damage has protective role of Vitamins in reducing the risk of OLP.[9] The fact that antioxidant vitamins are effective against oxidative stress is evident with the significant difference in the levels between the healthy control group and Patients with OLP. Different studies have concluded that the levels of antioxidant vitamin C levels are considerably low compared to the healthy individuals. In quenching the free radicals, antioxidant nutrient is initially consumed followed by lipid soluble antioxidants (a-Tocopherol).[10] In pig coronary artery it has been demonstrated that inhibition of Superoxide production because of alterations in vessel redox status has beneficial effects due to antioxidant vitamins.[11]

The decreased antioxidant vitamin levels in the current study indirectly supports the concept of free radical mediated lipid peroxidation that may be involved in pathophysiologic mechanism of OLP. Antioxidant nutrients can be simply manipulated by dietary and pharmacologic supplementation. The concept of increased utilization of vitamin C in patients raises the possibility of potential protective role for antioxidant nutrients in OLP. Decreased levels of antioxidant nutrients makes the mucosal cells more vulnerable to the cytotoxic effects of reactive oxygen species and it further creates more favorable circumstances for DNA damage and disease progression and also mouth is a critical site for oxidative stress as tissue damage occurs from thermal, chemical and microbial stimuli. Chronic periodontitis is an example of such a damage caused by oxidative stress in the oral cavity. Hence cases with chronic periodontitis were excluded in our study.

Our study also showed MDA levels were significantly higher in OLP patients when compared to healthy control group. Decreased antioxidant defense mechanism led to increased ROS and MDA production. The primary pathogenic event may be increased oxidative stress or the process of inflammation in OLP.[12] Similar results were recorded in other studies namely Hassan et al.[13] Altered oxidative damage of DNA, oxidation of protein, and decreased enzymatic antioxidant defenses increase the level of MDA levels.[7]

Our results indicate that serum levels of antioxidant vitamin C were significantly low and higher levels of MDA in patients with OLP. In conclusion there was an inverse relationship of increased MDA depicting oxidative stress and decreased Vitamin C levels depicting reduced antioxidant defenses in OLP. Further studies are required to demonstrate role of oxidative stress in the pathogenesis and the severity of disease in OLP patients.

5. Conclusion

The current study supports the premise and demonstrates that oxidative stress with higher levels of MDA and compromised anti-oxidant defenses with decreased levels of Vitamin C in patients with oral lichen planus. The major

limitation of our study was that disease severity was not considered. Hence further studies should be investigated by including various forms of oral lichen planus as other forms like erosive and bullous forms of OLP may have high malignant potential compared to the reticular form and also to draw definite conclusion and correlations between various biomarkers in OLP.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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