Assessment of serum and salivary malondialdehyde in patients with oral lichen planus

Aws Waleed Abbas, B.D.S., M.Sc. [1]
Taghreed Fadhil Zaidan, B.D.S., M.Sc., Ph.D. [2]
Abduladheem Y. Abbood Al-Barrak, B.Sc., M.Sc., Ph.D. [3]

ABSTRACT

Background: Free radicals are common consequences of normal aerobic cellular metabolism. Oxidative stress resulting from the increased production of free radicals and reactive oxygen species and/or a decrease in antioxidant defense leads to damage of biological macromolecules and dysregulation of normal metabolism and physiology. Oral lichen planus (OLP) is a chronic inflammatory oral mucosal disease of unknown cause. It has been proven that the imbalances in free radical levels and reactive oxygen species with antioxidants may play a key role in the onset and evolution of several inflammatory oral pathologies. The aim of this study was to assess the role of oxidative stress in the pathogenesis of OLP through the study of serum and saliva malondialdehyde as a marker of oxidative stress.

Methods: The study included (48) patients with OLP (21) with the reticular form and (27) with erosive form and (32) healthy looking volunteers that were age-matched with the patients. Serum and saliva malondialdehyde was measured by reacting with thiobarbituric acid under acidic conditions and heating to a pink color that measured spectrophotometricaly at 532 *nm*.

Results: the mean of serum and saliva malondialdehyde in oral lichen planus patients group was significantly higher than that of control group ($p<0.01$ and $p<0.05$ respectively) and there was no statistically significant differences in serum and saliva malondialdehyde when compared between reticular and erosive forms ($p>0.05$). The study showed that there was no statistically significant correlation between serum and saliva malondialdehyde levels in OLP patients group ($r= 0.053$, $p>0.05$).

Conclusion: Increased serum and salivary malondialdehyde levels refer to the role of oxidative stress in the pathogenesis of OLP.

Keywords: Oral lichen planus, malondialdehyde, oxidative stress, serum, saliva. (J Bagh Coll Dentistry 2014; 26(2): 99-102).

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory disease of unknown etiology [1]. However, the precise cause is unknown, activated cytotoxic T-cells was located close to damaged basal cells which may suggest that they are responsible for the damage and supports the claim that a cell-mediated immune response participates actively in local pathogenetic mechanisms in OLP [2].

Basically there are two classes of oral lesions: reticular and erosive [3]. The majority of cases of lichen planus present as white lesions. An erosive form of this disease presents as chronic multiple oral mucosal ulcers. Erosive lesions of lichen planus occur in the severe form of the disease when extensive degeneration of the basal layer of epithelium causes a separation of the epithelium from the underlying connective tissue [4].

In the healthy human body, there is an approximate balance between production of reactive species and antioxidant defences. Tissue injury in human disease is accompanied by an imbalance in the oxidant/ antioxidant status, producing oxidative stress. The resulting increased oxidative damage to biomolecules may play an important role in the pathology of several human diseases and is amenable to therapeutic intervention with appropriate antioxidants [5].

Increasing appreciation of the causative role of oxidative injury in many disease states places great importance on the reliable assessment of lipid peroxidation. Malondialdehyde (MDA) is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products [6].

The purpose of this study is to evaluate the oxidative stress and its role in the pathogenesis of OLP through the study of serum and saliva MDA.

MATERIALS AND METHODS

The study included 48 patients with OLP which divided into 21 patients with the reticular form and 27 patients with erosive form and 32 healthy looking volunteers that were age- and sex-matched with the patients.

All of the OLP patients were diagnosed clinically, and the diagnoses were confirmed through histopathologic examination according to the modified WHO diagnostic criteria for OLP by Van der Meij and Van der Waal [7].

(1) Ph.D. student, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.
(2) Professor, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.
(3) Assistant Professor, Department of Microbiology, College of Medicine, Al-Mustansiriya University

Blood and saliva were collected from each subject then the supernatant serum and saliva was aspirated and transferred immediately into Eppendorf tubes and frozen at -20 °C for subsequent analysis.

Malondialdehyde (MDA), lipid peroxidation end products, react with thiobarbituric acid under acidic conditions and heating to a pinkish color that measured spectrophotometricaly at 532 nm [8].

RESULTS

The mean age of the patient group was 50.96 ±10. 55 with female predilection 52.1%.

The present study showed that the mean level of serum MDA in patients with OLP (4.725±1.634 µmol/l) was significantly higher ($p<0.001$) by using t-test than that of control group (1.626±0.712 µmol/l). (Table 1) (Figure 1)

The mean level of saliva MDA in patients with OLP (0.972±0.433 µmol/l) was significantly higher ($p<0.05$) by using t-test than that of control group (0.732±0.358 µmol/l). (Table 2) (Figure 1).

Statistically, there was no-significant difference in serum and saliva MDA level between erosive and reticular OLP patients groups. (Table 3).

This study showed that there was no statistically significant correlation ($r = 0.053$, $p>0.05$) between serum and saliva measurements of MDA in patients with OLP. (Figure 2)

Table 1: Mean of serum MDA in OLP patients and controls

Serum MDA (µmol/l)	Patients	Controls
No.	48	32
Mean±SD	4.725±1.634	1.626±0.712
Standard Error of Mean	0.236	0.126
Mode	2.88	1.88
Range	2.68-9.48	0.52-3.48
Percentile 05th	2.88	0.60
25th	3.44	1.04
50th (Median)	4.32	1.62
75th	6.10	1.98
95th	7.20	3.44
99th	9.48	3.48
P value	0.0001**	

** Highly Significant using Students-t-test for difference between two independent means at 0.01 level

Table 2: Mean of saliva MDA in OLP patients and controls

Saliva MDA (µmol/l)	Patients	Controls
Mean±SD	0.972±0.433	0.732±0.358
Standard Error of Mean	0.074	0.070
Mode	0.88	0.56
Range	0.48-2.12	0.12-1.40
Percentile 05th	0.52	0.32
25th	0.64	0.48
50th (Median)	0.84	0.58
75th	1.24	1.04
95th	2.00	1.40
99th	2.12	1.40
P value	0.026*	

*Significant using Students-t-test for difference between two independent means at 0.05 level.

Table 3: Mean and significant level of serum and saliva MDA between reticular and erosive forms of OLP patients group

MDA (µmol/l)	Type		P value
	Reticular	Erosive	
Serum	4.703±1.818	4.742±1.512	0.935
Saliva	0.994±0.426	0.956±0.448	0.804

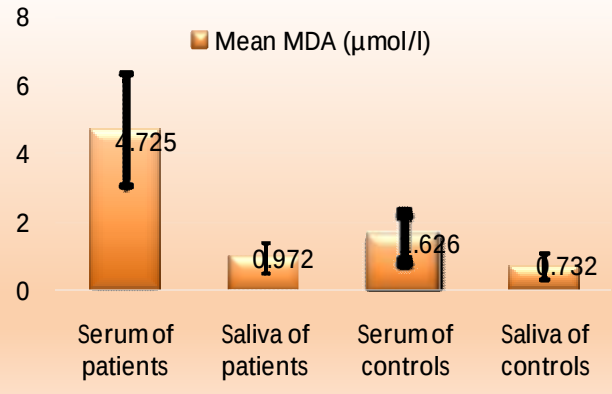

Figure 1: Mean of MDA conc. in serum and saliva of OLP patients and control groups

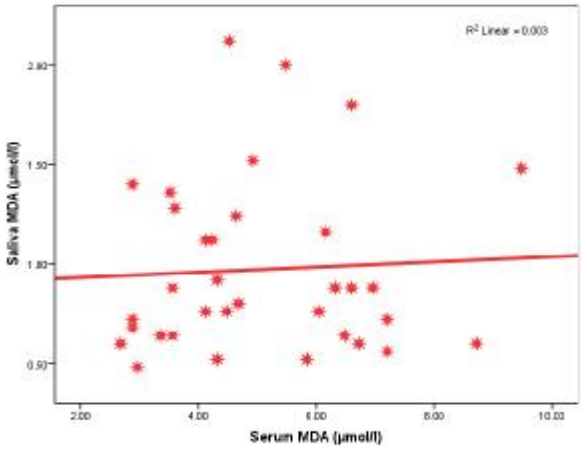

Figure 2: Correlation between serum and saliva MDA in OLP patients group

DISCUSSION

Chronic inflammation is a pathological condition characterized by continued active inflammation response and tissue destruction. Inflammatory process induces oxidative stress and reduces cellular antioxidant capacity. Excessive production of free radicals react with cell membrane fatty acids and proteins impairing their function permanently [9].

It has been proven that the imbalances in free radical levels and reactive oxygen species with antioxidants may play a key role in the onset and evolution of several inflammatory oral pathologies [10].

At the cellular level, oxidant elicits responses ranging from enhanced survival to cell death [11].

Several lines of experimental evidence recognize the mitochondrial dysfunction as one of the important mediators of apoptosis [12]. The mitochondria are sensitive to changes in the redox state of the cell. Several studies have shown that the global shutdown of mitochondrial function under conditions of the oxidative stress could contribute to apoptosis [13].

Reactive oxygen species also appear to play an important role in mediating Fas-dependent apoptosis [14] sustained by the observation that Fas-induced apoptosis were completely abolished by antioxidants such as glutathione [15].

Anshumalee et al. [16]; Sezer et al. [17] and Anshumalee and Shashikanth [18] reported that ROS may be involved in the pathogenesis of the LP.

The present study demonstrated that serum MDA levels were significantly higher in OLP than in controls. These findings are in accordance with studies by Sezer et al. [17], Rai et al. [19], Aly and Shahin [20], Upadhyay et al. [21] and Scrobota et al. [22], and supported the concept that free radical mediated lipid peroxidation may be involved in the patho-physiologic mechanisms of OLP.

From the other hand, the present study revealed that salivary MDA levels were significantly higher in OLP than in controls, which were consistent with previous studies by Agha-Hosseini et al. [23] and Ergun et al. [24].

Saliva offers an alternative to serum as a biological fluid that can be analyzed for diagnostic purposes. Saliva contains locally produced as well as serum-derived markers that have been found to be useful in the diagnosis of a variety of systemic disorders. Whole saliva can be gathered in a non-invasive manner by individuals with modest training, including patients. However, levels of certain markers in saliva are not always a true reflection of their levels in serum. The transfer of serum constituents which are not part of the normal salivary constituents into saliva is related to the physicochemical characteristics of these molecules. Salivary composition can be determined by the method of collection and the degree of stimulation of salivary flow. Furthermore, salivary proteolytic enzymes can affect the stability of certain diagnostic markers. Some particles are also degraded during intracellular diffusion into saliva [25]. These limitations opposed the diagnostic potential of saliva and may explain what the present study showed that there was no significant correlation between serum and saliva MDA levels in OLP patients group.

REFERENCES

1- Schlosser BJ. Lichen planus and lichenoid reactions of the oral mucosa. Dermatologic Therapy 2010; 23: 251–67.

2- Kilpi AM. Activation marker analysis of mononuclear cell infiltrates of oral lichen planus in situ. Scand J Dental Res 1987; 95:174-80.
3- Neville BW, Damm DD, Allen CM, Bouquot JE. Dermatologic diseases. Oral and maxillofacial pathology. 2nd ed. WB Saunders Co.; 2002.
4- Greenberg M S. Ulcerative, vesicular, and bullous lesions. In: Greenberg M S, Glick M. Burket's oral medicine diagnosis and treatment. 10th ed. BC Decker Inc; 2003
5- Halliwell B. Free radicals and other reactive species in disease. In: encyclo of Life Sciences; 2005. Available from: http://www.els.net
6- Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidatin and peroxidative tissue injury. Free Radical Biology and Medicine 1990; 9(6): 515-40.
7- van der Meij EH, van der Waal I. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. J Oral Pathol Med 2003; 32:507–12. (Cited by: Hiremath SKS, Kale AD, Charantimath S. Oral lichenoid lesions: Clinico-pathological mimicry and its diagnostic implications. Indian J Dental Res 2011; 22 (6): 827-34).
8- Shah SV, Walker PD. Evidence suggesting a role for hydroxyl radical in glycerol induced acute renal failure. Am J Physiol 1988; 255(3): F438-43
9- Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent Pat Inflamm Aller Drug Discov 2009; 3: 73-80
10- Battino M, Ferreiro M S, Gallardo I, Newman H N, Bullon P. The antioxidant capacity of saliva. J Clin Periodontol 2002; 29(3):189-94. (Cited by: Abdolsamadi HR, Goodarzi MT, Mortazavi H, Robati M, Motemaye F. Comparison of salivary antioxidants in healthy smoking and non-smoking men. Chang Gung Med J 2011; 34(6): 607-11)
11- Martindale JL, Holbrook NJ. Cellular response to oxidative stress: signaling for suicide and survival. J Cellular Physiol 2002; 192(1):1-15.
12- Marzo I, Susin SA, Petit PX, Ravagnan L, Brenner C, Larochette N, Zamzami N, Kroemer G. Caspases disrupt mitochondrial membrane barrier function. FEBS Lett 1998; 427: 198-202.
13- Zamzami N, Susin SA, Marchetti P, Hirsch T, Monterrey I G, Castedo M, Kroemer G. Mitochondrial control of nuclear apoptosis. J Exp Med 1996; 183 1533-1544. (Cited by: Kannan K, Jain SK. Review, oxidative stress and apoptosis. Pathophysiol 2000; 7(27): 153-63).
14- Aronis A, Melendez J A, Golan O, Shilo S, Dicter N, Tirosh O. Potentiation of Fas-mediated apoptosis by attenuated production of mitochondria-derived reactive oxygen species. Cell Death and Differentiation 2003; 10(3): 335–44.
15- Gulbins E, Brenner B, Schlottmann K, Welsch J, Heinle H, Koppenhoefer U, Linderkamp O, Coggeshall KM, Lang F. Fas-induced programmed cell death is mediated by a Ras-regulated O2-synthesis. Immunology 1996; 89: 205–12.
16- Anshumalee N, Shashikanth MC, Sharma S. Oxidative stress and oral lichen planus: A possible association? Cusp 2007; 4(2): 31–4. (Cited by: Aly DG, Shahin R. Oxidative stress in lichen planus. Acta Dermatoven Alpina Panonica et Adriat 2010; 19 (1): 3-11).
17- Sezer E, Ozugurlu F, Ozyurt H, Sahin S, Etikan I. Lipid peroxidation and antioxidant status in lichen planus. Clinical and Experimental Dermatology 2007; 32:430–4.
18- Anshumalee N, Shashikanth MC. Efficacy of oral lycopene in management of lichen planus [dissertation]. [Bangalore]: Rajv Ghandi University of Health Sciences, Bangalore. April 2007; 91-119. (Cited by: Aly DG, Shahin R. Oxidative stress in lichen planus. Acta Dermatoven Alpina Panonica et Adriat 2010; 19 (1): 3-11).
19- Rai B, Kharb S, Jain R, Anand SC. Salivary lipid peroxidation product malonaldehyde in pre-cancer and cancer. Adv Med Dent Sci 2008; 2(1): 7–8.
20- Aly DG, Shahin R. Oxidative stress in lichen planus. Acta Dermatoven Alpina Panonica et Adriat 2010; 19(1): 3-11
21- Upadhyay R B, Carnelio S, Shenoy R P, Gyawali P, Mukherjee M. Oxidative stress and antioxidant defense in oral lichen planus and oral lichenoid reaction. Scand J Clin Lab Invest 2010; 70: 225-8.
22- Scrobota I, Mocan T, Catoi C, Bolfa P, Muresan A, Baciut G. Histopathological aspects and local implications of oxidative stress in patients with oral lichen planus. Rom J Morphol Embryol 2011; 52(4):1305–9
23- Agha-Hosseini F, Dizgah IM, Mikaili S, Abdollahi M. Increased salivary lipid peroxidation in human subjects with oral lichen planus. Inter J Dental Hygiene 2009; 7(4): 246-50
24- Ergun S, Troşala SC, Warnakulasuriya S, Özel S, Önal A E, Ofluoğlu D, Güven Y, Tanyeri H. Evaluation of oxidative stress and antioxidant profile in patients with oral lichen planus. J Oral Pathology and Medicine 2011; 40: 286–93
25- Kaufman E, Lamster IB. The Diagnostic Applications of Saliva-A Review. Critical Reviews in Oral Biology Medicine 2002; 13(2):197-212.