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Increased salivary lipid peroxidation in human subjects with oral lichen planus

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Abstract: *Background:* Oral lichen planus (OLP) is a chronic inflammatory mucosal disease of unknown aetiology.

Recently, increased oxidative stress has been implicated in the pathogenesis of various diseases. The aim of this study was to evaluate the total antioxidant capacity (TAC) and lipid peroxidation level, in the saliva of patients with OLP.

Method: Thirty patients with OLP (15 men, 15 women; mean age 44.0, SD \pm 11.4 years) and 30 control subjects (16 men, 14 women; aged 23–67 years), matched for age and gender, were enrolled in this case and control study. This study was conducted at the Clinic of Oral Medicine of Tehran University of Medical Sciences in 2007. The unstimulated whole saliva malondialdehyde (MDA), as an indicator of lipid peroxidation and TAC levels were assayed by thiobarbituric acid and ferric reducing antioxidant potential (FRAP), respectively, in both groups. *Results:* Mean levels of saliva MDA, but not TAC, in patients with OLP was significantly higher than those of the control group. *Conclusion:* We conclude that OLP patients have more cellular lipid peroxidation than healthy subjects.

Key words: lipid peroxidation; oral lichen planus; saliva; total antioxidant capacity

Introduction

Oral lichen planus (OLP) is a chronic inflammatory mucosal disease (1) which has been clinically associated with the development of oral cancer (2, 3). It can cause symptoms ranging from a burning sensation to severe pain interfering with speaking, eating and swallowing (4). However, the mechanism involved in the pathogenesis of OLP, and the underlying mechanisms of OLP that initiate the development of oral cancer in OLP patients, have not been clearly established yet. Reactive oxygen

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species (ROS) are considered to play the key role in inflammation-mediated carcinogenesis.

Reactive oxygen species induce lipid peroxidations, with related effects on cells (5). When ROS interact with the polyunsaturated fatty acids in membranes or lipoproteins, the process of lipid peroxidation begins. Uncontrolled production of lipid peroxides can result in oxidative stress, with significant damage to cell integrity (6, 7). Because lipid peroxidation is an outcome of oxidative stress, numerous markers have been used to monitor this process. Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation that can be shown to increase following oxidative stress (7, 8).

Increased ROS and lipid peroxides have been implicated in the pathogenesis of various diseases like gastrointestinal tract (9), diabetes (10) and especially in periodontitis (11). A recent study indicated that excessive ROS may lead to inflammatory-mediated carcinogenesis in OLP (12). Chapple *et al.* (13) reported that periodontal disease was associated with reduced salivary antioxidant status and increased oxidative damage within the oral cavity.

Saliva is the first biological medium confronted by external materials that are taken into our bodies as part of food, drink or inhaled volatile ingredients. During evolution, various defense mechanisms have developed, so saliva is armed with various mechanisms, such as immunological and enzymatic defense systems (14, 15).

The aim of this study was to evaluate the total antioxidant capacity (TAC) and cellular lipid peroxidation in saliva of patients with OLP.

Materials and Methods

Patients and controls

Thirty patients with OLP clinically diagnosed with lichen planus (presence of bilateral lesions and presence of reticular lesions elsewhere in the oral) and in histopathological examination (presence of well-defined band like zones of inflammatory infiltration confined to the superficial part of the connective tissue, consisting mainly of mature lymphocytes – vacuolar alteration of the basal layer of the epithelium), comprised the case group (15 men, 15 women; aged 23–69 years), and 30 age- and sex-matched healthy volunteers (16 men, 14 women; aged 23–67 years), who did not have clinical signs of gingival inflammation, comprised the control group. In either groups, subjects with systemic and periodontal diseases and gingivitis, or who were taking medication (at all) at the time of the study, and

smokers were excluded. Subjects in case group were in atrophic and erosive forms and none of them had bleeding. This case–control study was conducted at the Clinic of Oral Medicine of Tehran University of Medical Sciences in 2007.

The protocol was approved by the Review Board of Tehran University of Medical Sciences, and written informed consent was obtained from all patients and control subjects.

Saliva collection

Five millilitre of unstimulated whole salivary samples were obtained by expectoration, in the absence of chewing movements, in dry plastic vials with the test subject sitting in a relaxed position. The collected saliva samples were centrifuged (2000 *g* for 10 min). The supernatants were stored at -70°C until further analysis. Samples were collected at the same time of day (10–12 a.m.) and at least 2 h after the last intake of food or drink. Saliva malondialdehyde (MDA) and TAC levels were assessed in both patient and control groups.

Measurement of saliva MDA and TAC

The saliva MDA levels, as the end product of the oxidation of polyunsaturated fatty acids, were determined by a method based on reaction with thiobarbituric acid (TBA) at $90\text{--}100^{\circ}\text{C}$ (16). MDA and TBA react together, in the TBA test reaction, to produce a pink pigment having an absorption maximum at 532 nm. The reaction was performed at pH 2–3 at 90°C for 15 min. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was palliated by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm using a UV-160-Shimadzu double beam spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed as $\mu\text{mol l}^{-1}$ according to a standard curve, which was prepared with a serial dilution of standard 1,1,3,3-tetramethoxypropane.

The TAC of saliva was determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} using the FRAP test (17). Briefly, in this test, the medium is exposed to Fe^{3+} and the antioxidants present in the medium produce Fe^{2+} as a result of antioxidant activity. The reagent included a 300 mmol l^{-1} acetate buffer with a pH of 3.6 and 16 ml of $\text{C}_2\text{H}_4\text{O}_2$ per litre of buffer solution along with 10 mmol l^{-1} of 2,4,6-tripyridyl-1,3,5 triazine (TPTZ) in 40 mmol l^{-1} HCl, 20 mmol l^{-1} $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$. The working FRAP reagent was prepared as required by combining

25 ml of acetate buffer, 25 ml TPTZ solution and 2.5 ml $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution. Ten microlitre of H_2O diluted samples was then added to 300 μl freshly prepared reagents and warmed at 37°C . The complex between Fe^{2+} and TPTZ gives a blue colour with absorbance at 593 nm.

Statistical analysis

Student's *t*-test for independent samples was used to determine the statistical significance of unstimulated whole saliva MDA and TAC levels within both patient and control groups, with significance defined as $P < 0.05$. Data were expressed as mean \pm SEM.

Results

The mean \pm SD age of the patients and control group were 44.0 ± 11.4 and 44.5 ± 12.1 years respectively. There was no statistically significant difference between ages of the patients and of the controls. Of the 30 patients, nine and 21 suffered plaque and erosive forms of OLP respectively.

The mean level of unstimulated whole saliva MDA in patients with OLP was significantly higher than that of the control group ($t = 2.34$, $P < 0.05$; Fig. 1a). The mean saliva TAC in patients with OLP was higher than those of healthy controls, but it was not statistically significant ($t = 1.44$, $P > 0.05$; Fig. 1b).

Discussion

Lichen planus is a chronic autoimmune, mucocutaneous disease, which can affect the oral mucosa, skin, genital mucosa, scalp and nails. The aetiopathogenesis of OLP appears to be complex, interactions with genetic, environmental and lifestyle factors are reported (1, 18). In the present study, we observed that unstimulated whole saliva level of MDA, an indicator of lipid peroxidation, was higher in patients with OLP than in a matched group of healthy control subjects. Our findings are in accordance with several reports on increased circulatory oxidant levels in lichen planus (19, 20), inflammatory bowel disease (9) and cancer (21). Numbers of previously published studies showed a decline in circulating antioxidant capacity in patients with lichen planus (19, 20). However, our data shows that saliva antioxidant capacity of OLP subjects was higher, but not significantly, than the healthy control individuals. This is in agreement with the report of Nagao *et al.* (22) that low serum antioxidant micronutrients cannot be risk factors for occurrence of OLP.

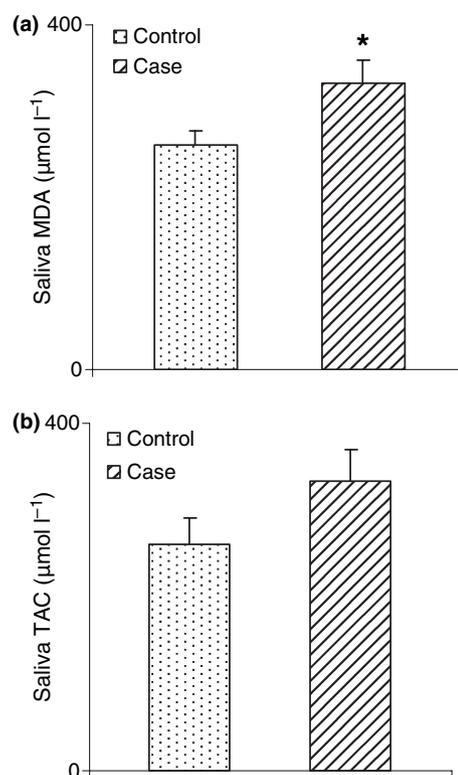


Fig. 1. The unstimulated whole saliva concentrations of (a) malondialdehyde (MDA) and (b) total antioxidant capacity (TAC) in case (patients with oral lichen planus) and control groups (healthy individuals). * $P < 0.05$ was considered statistically significant.

A possible relation between the inflammatory process and free radical (FR) metabolism has been reported in several studies (23). Recently, it has been claimed that the imbalances in levels of FR and ROS with antioxidants may play an important role in the onset and development of several inflammatory oral pathologies (11, 24, 25). Physiologically FR/ROS in the mouth are derived mainly from polymorphonuclear neutrophils, which may also help to control bacterial growth by the well-known 'respiratory burst'. Such physiological processes are usually efficiently counteracted by intrinsic antioxidant systems; if such systems fail, tissue damage can result.

Saliva may constitute a first line of defense against FR-mediated oxidative stress, because the process of mastication promotes a variety of such reactions, including lipid peroxidation. The increased membrane lipid peroxidation is considered to evoke immune and inflammatory responses and to activate gene expression and cell proliferation (26, 27).

The FR/ROS production and actions are rather complex and their interaction is frequent (25). The antioxidant defense systems are also highly complex (13, 25), constituting an effective network capable of counteracting FR/ROS effects. It is essential to evaluate the amounts and/or the activities of the

different antioxidants when assessing antioxidant status *in vivo*. However, as FR/ROS and antioxidant systems appear to act in concert rather than alone, investigations of individual antioxidant activity may be misleading and the measurement of any individual antioxidant may be less representative of the whole antioxidant status. Moreover, the number of different antioxidants makes it difficult, and also expensive, to measure each antioxidant separately, especially during daily clinical treatments. For these reasons, we evaluated the TAC of saliva.

Reactive oxygen species play important roles in physiological and immunoinflammatory reactions. In the human body, there is an antioxidant mechanism to maintain the balance of oxidation–reduction (28–30). The breakdown of this balance (i.e. increased ROS) could lead to increased damage directly by ROS. Indeed, several diseases have been correlated to an imbalance of oxidation–reduction or oxidative stress (31–34). There are two possible causes for the imbalance of oxidative stress: increased ROS with no concomitant or less increased antioxidant, or decreased antioxidant with no marked change of ROS. In this study, the levels of lipid peroxidation increased in patients with lichen planus without significant increase in antioxidant activity. So, it seems that there is an imbalance of oxidative stress in patients with OLP.

Conclusions

We conclude that OLP patients have more cellular lipid peroxidation than healthy subjects. It is suggested that patients with OLP are more susceptible to an imbalance of antioxidant–oxidative stress situations.

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