Assessment of Salivary MDA and Antioxidant Vitamins in Patients with Erosive Type of Oral Lichen Planus and Lichenoid Reaction

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Abstract

Background: oral Lichen planus (OLP) is a chronic inflammatory disease with an unknown etiology. Reduced level of antioxidant and oxidative stress is implicated in pathogenesis of OLP.

Methods: 80 patients (40 OLP patients and 40 OLR patients), were included in this cross sectional study and compared with 40 normal subjects. Unstimulated whole saliva (UWS) was collected and vitamin A, E, C and MDA (malondealdehyde) concentrations were measured using human Elisa kits. One way variances, ANOVA and post hoc were used to analyze the data (SPSS ver. 18) (α=0.05).

Results: Mean levels of vitamin A antioxidants in OLP (0.16 ± 0.06 Nmol/mg)and OLR(0.14 ± 0.05 Nmol/mg) patients were significantly lower than those of the control group(0.54 ± 0.20 Nmol/mg) (P value<0.001). mean levels of vitamin E antioxidants only in OLP patients(7/82 ± 2.94 Nmol/mg) were significantly lower than those of the control group(10.80 ± 4.40)(P value=0.03).but with no difference in mean level of vitamin C between two groups and with control group (P value=0.619).

There wasn't any significant difference in mean level of MDA between OLP patients (2.46 ± 1.21 Nmol/mg), OLR patients (2.53 ± 1.36 Nmol/mg) and control group (2.62 ± 1.15 Nmol/mg) (P value=0.925).

Conclusion: Considering the results of the present study and reported role of antioxidant deficiency and oxidative stress in OLP and OLR pathogenesis, measurement of salivary vitamins (A, E, C) and MDA concentrations may help in treatment planning and preventive strategies.

Key Words: Antioxidant, Lichenoid eruption, malondealdehyde, Oral lichen planus, Saliva, Vitamin A, E

Introduction

Lichen planus is a chronic inflammatory disease of skin and oral mucosa with an unknown etiology. Subepithelial autoreactive T lymphocytes infiltration and liquefaction degeneration may be of primary importance for oral lichen planus development [1]. Oral mucosa is one of the most common affected sites and is more prevalent in middle-aged patients [2-4]. The prevalence of OLP is 1-2% in the general population while its prevalence in asian population is 2.6% [5].The imbalance in antioxidants is also thought to play an important role in this disease [6-8].Increased level of oxidative stress, lipid peroxidation and serum or salivary oxygen free radicals lead to inflammatory process and damage to cellular membrane [9].

Reduced antioxidant defense and increased oxidative stress may be play an important role in etiology of many disease such as: diabetes [10], periodontitis [11], psoriasis [12], vitiligo [13] and digestive system disorders [8].

Antioxidant activity of Vitamin A and E produce free radicals that protect mucosal membranes from damage [14-16]. MDA (Malondealaldehyde) is one of the end-products of lipid peroxidation.

In this process free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage.epithelial cells structures specially oral mucosa can be damaged by this process [17-18] it is acceptable that oral lichen planus (OLP)potential for malignant transformation and Some patients with a diagnosis of OLP eventually developed SCC.It is prudent for clinicians to pursue continued regular observation and follow-up in patients with these conditions [1].

Aghahoseini et al [19] in 2009 compared salivary lipid peroxidase level in 30 OLP patients and 30 control subjects.in this study salivary lipid Peroxidase in OLP group. Mean levels of saliva MDAwas significantly higher than those of the control group.

If oxidative - reduction system disorder in OLP be confirmed, can resolve this problem by administration of multivitamin Compounds and step toward better understanding and treatment of lichen planus. The aim of this study was comparision of salivary MDA level with oxidative activity and salivary vitamin A, E and C levels with antioxidant activity in OLP and OLR patients with control subjects.

Method and Materials

The study subject population consisted of 80 patients (40 OLP patients and 40 OLR patients) who selected by convenience method from referring patients to the dental school of the University of Isfahan, Iran between April 2010 and October 2011. Also 40 healthy subjects without any oral and systemic disease were recruited as control group. The Ethics committee of the University approved the study protocol (no: 390274) and informed constant obtained prior to participation in this cross-sectional study.

The inclusion criteria were a biopsy-proven diagnosis of erosive OLP or OLR and exclusion criteria for both groups was no history of graft,smoking and tobacco or alchol consuming, diabetes, malignancy, hepatitis and any other

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systemic or infection diseases. Also patients using any oral drugs and vitamins in last 3 months,History of trauma, surgery about 1 month ago was other exclusion criteria [20]. 18 patients with smoking, 14 patients with systemic disease and 12 patients without erosion in clinical manifestation were excluded and finally 80 patients were enrolled in this study.

We defined erosive type of lichen planus or lichenoid reaction based on clinical presentation. Any sign of a non-traumatic ulceration in the oral mucosa associated with typical lichen signs indicated an erosive type [21].

The differential diagnosis between lichen planus and lichenoid reaction was determined by a combination of clinical and histological criteria [22]. Cases of lichen planus must be had all of the clinical and histological criteria. Whereas, lichenoid reaction includes: patients with typical lichen planus clinically but not histologically, patients with typical lichen planus histologically but not clinically, and patients who are both clinically and histologically only compatible with lichen planus.

Biopsies were taken from reticular border of lesions on buccal mucosa. Specimens were rapidly placed in a 10% formalin-buffered solution to avoid autolysis. Specimens were processed and embedded in paraffin using standard procedures. Then, from each block, a section was stained with hematoxylin and eosin (H&E) to allow histopathology examination. Finally the definitive diagnosis was done based on WHO clinical and histopathological criteria for oral lichen planus and lichenoid reaction [23].

Saliva sampling

Participants were asked not toeat, drink or use saliva stimulators like chewing gum, or mint for at least one hour before evaluation.Unstimulated whole saliva (UWS) was collected between 10:00 and 11:00 a.m. using standard technique [24]. Participants were

Asked to swallowfirst, then tilt their head forward and expectorate all saliva into a 50 ml centrifuge tubes for 5 min without swallowing. Thesaliva samples were frozen at -70°C, until analysis. All samples were centrifuged at 4500 g for 15 minutes.

ELISA test was used to determine salivary vitamin E (Elisa kit katalog No.CSB-E07893h USA), vitamin A (Elisa kit katalog No. CSB-E07889h USA), vitamin C (Elisa kit katalog No. ABIN777846 USA) and MDA (Elisa katalog No. CSB. A082431 h USA).

Statistical analysis

One way variances, ANOVA and post hoc tests were used to analyze the data (SPSS ver. 18) (α=0.05).

Results

120 subjects were included in the analysis. 40 patients in OLP group (22 female and 18 male with mean age of 43.27±1. 96), 40 patients in OLR group (26 Female and 14 male with me an age of 41.17±3.25) and 40 people in control group (26 female with mean age of and 14male 32. 2 ± 4.1) were examined.

In order to obtain results and relations between variables at first ANOVA test was done. Based on the results it was found that there is significant difference in vitamin E and A between control group, OLR and OLP groups. For two by two comparison between groups we used of Tucky test only in groups that ANOVA test was significant.

one Way variance showed there was significant difference in saliva level of vitamin A and E Between groups(p-value<0.001) but in other variables there wasn't any significant difference *(Table 1)*.

Table 1. Mean and standard deviation of A, E, C vitamins and MDA enzyme in Lichen planus and Lichenoid reaction and control group and comparison of them according to p-value.

Variable	Groups	Number	Standard deviation	Mean	p-value
vitaminA Nmol/mg	Lichen planus	20	0.06	0.16	<0.001
	Lichenoid reaction	20	0.05	0.14	
	control	20	0.20	0.54	
vitaminE Nmol/mg	Lichen planus	20	2.94	7.82	0.048
	Lichenoid reaction	20	3.54	9.47	
	control	20	4.40	10.80	
vitaminC Nmol/mg	Lichen planus	20	0.64	0.93	0.619
	Lichenoid reaction	20	0.89	1.11	
	control	20	0.37	1.12	
MDA Nmol/mg	Lichen planus	20	1.21	2.46	0.925
	Lichenoid reaction	20	1.36	2.53	
	control	20	1.15	2.62	

Table 2. Two by two comparison of groups according to Tukey HSD criteria.

Variable	Groups		Mean difference	P value
Vitamin A Nmol/mg	Lichenoid reaction	Lichen planus	0.02	0.84
	Control	Lichen planus	-0.38	<0.001
	Control	Lichenoid reaction	-0.40	<0.001
Vitamin E Nmol/mg	Lichenoid reaction	Lichen planus	-1.65	0.33
	Control	Lichen planus	-2.98	0.03
	Control	Lichenoid reaction	-1.33	0.49

According to Tucky HDS test there wasn't any significant difference in vitamin A between OLR and OLP groups (p value=0.84). But it was significant relation between OLP, OLR and control group (p-value <0.001).

Also there wasn't any significant difference in vitamin E between OLR and OLP. (P-value = 0. 33) and also between

OLR and control (p-value =0.49) but it was significant among OLP and control (P-value=0.03).

T-Test showed that there was significant difference between sexes only in mean of vitamin C (p-value <0.001) *(Table 2)*.

Discussion

The present study examined salivary level of antioxidant vitamins and MDA as an oxidizing agent in three group of OLP, OLR patients and control subjects. There wasn't any similar study witch simultaneously measure these two important factors in three groups of OLP , OLR and control group.

The results showed statistically significant reduction in the level of vitamin A in OLP and OLR patients in Comparison with control group also there was a significant reduction in vitamin E only in OLP patients but there wasn't any significant difference in vitamin C and MDA between OLP and OLR with each other and also in comparison With control group. Many studies have been done to determine the level of antioxidant in patients with Lichen Planus and other autoimmune disease [19,25-26]. In many of them, decrease in the production of antioxidants has been implicated in the etiology of lichen planus and lichenoid Reactions [25-28].

Upadhay et al in a similar study measures [25] the level of MDA in 22 OLP and 10 OLR patients in compare With 15 control subjects. According to the results of their study, there was significant increase in serum level of MDA in OLP and OLR patients in compared with control group.

Lopez and ergun in two separate studies also found elevated level of salivary MDA in OLP patients in compared with control subjects [29-30]. But the present study found no difference between three groups in the salivary level of MDA. Also this study shows the presence of reactive oxygen and free radicals in lichen Planus and lichenoid reaction patients.

In other study, Rai et al [15], compared the salivary level of vitamin E and C antioxidants in 10 OLP patients and control subjects. And the reduction in the amount of both vitamins was observed in case group. But in the present study no significant difference was found in salivary vitamin C level among any of the groups and salivary vitamin E level were significantly decreased only in patients with lichen Planus.

Abdolsamadi et al, compared total antioxidant capacity and MDA and antioxidant vitamin (vitamin s A, C and E) levels in patients with erosive OLP and healthy individuals and found salivary level of MDA was significantly higher in patients than in controls and antioxidant vitamins were significantly decreased in patients with OLP [31]. Decreasing salivary vitamin C and increasing MDA levels in OLP patients are in contrast with our findings.

Barikbin et al [28], compared the serum vitamin C, selenium, bilirubin, uric acid and glutathionperoxidase (GTX) level in OLP patients and healthy subjects. Based on their result serum vitamin c level decreased in lichen Planus group. But in present study there wasn't any significant difference in salivary vitamin c amounts.

The distinction of present study from other studies [15,25 and 28] is that in addition to lichen planus patients and healthy subjects, patients with lichenoid reaction were examined. Also the result showed no significant difference in levels at antioxidant vitamins A, E, C and MDA in OLP patients in Comparison with lichenoid reaction patients.

In addition most previous studies [25,28] investigated serum level of MDA and antioxidant vitamin A, E, C and there isn't enough information about salivary level of these factors.

Inconsistent findings may be due to differences in sample size, study design, ethnicity, environmental factors and Physiological Characteristic (32).

Also some studies have been done on serum and some on saliva.

Saliva is a collection of oral liquid that is directly affected by diet and indirectly affected by amount of secretion, emotional condition, physical activity, ambient Temperature, drug consumption and in general term environmental condition. Thus, despite many attempts to control confounding factors; some factors cannot be competently controlled.

One of our limitations in this study was sample size and larger groups of patients are needed in future studies.

Vitamin C is a compound with rapid environmental degradation. Perhaps one of the reasons for the lack of significant result in vitamin C level was degradation during sample Collection and maintenance. So it is recommended to measure vitamin C in highly controlled condition.

In the present study non enzymatic antioxidant vitamins were measured it is recommended in future studies to measure enzymatic Antioxidants.

Conclusion

Considering the results of the present study and reported role of antioxidant deficiency and oxidative stress in OLP and OLR pathogenesis, measurement of salivary vitamins (A, E, C) and MDA concentrations may help in treatment planning and preventive strategies.

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