

Salivary vitamin E and uric acid in patients with OLP and healthy individuals

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ABSTRACT

Background: Oral Lichen planus (OLP) is a T-cell mediated chronic inflammatory oral mucosal disease of unknown etiology. Recent studies have reported an increased oxidative stress and lipid peroxidation in such patients. This suggests that reactive oxygen species may have a role in the pathogenesis of lichen planus. Oxidative stress in OLP release molecules consisting of granzymes resulting in local tissue damage in the effectors.

Antioxidants that can defend against oxidative stress in the body cells include enzymes, as well as non- enzymatic antioxidants, such as melatonin, uric acid, vitamin A and E.

Purpose: To study the level of salivary vitamin E and uric acid as antioxidant agents in patients with OLP and compared with healthy control.

Methods: Twenty five patients with OLP were enrolled in this study. Age, gender, occupation, smoking status (smokers or non-smokers), lesion types, duration, location and size were recorded for each patient. After an oral examination, salivary samples were collected and flow rates (ml/min) were recorded. The collected samples were centrifuged at 3000 rpm for 10 minutes; the clear supernatants were separated and stored frozen at (-20 c) until analysis. Then salivary vitamin E was investigated using ELISA kit based on biotin double antibody sandwich technology.

Uric acid was analyzed using a proprietary enzymatic reaction mixture that enables the detection of uric acid by the production of a red chromogen, which is quantitatively measured at a wavelength of 515 or 520 nm.

Results: The mean age of OLP patients was 48.3 years with a range of 30-60 years. Control group consisted of 35 healthy subjects who were age matched with OLP patients. Fourteen (56%) patients were with reticular and 11 (44%) were with erosive form, with the buccal mucosa was the most commonly affected site (88%), followed by tongue (8%) then gingiva (4%). A significantly lower salivary flow-rate, lower salivary vitamin E and uric acid level in OLP patients compared to control; while, no significant difference was seen between reticular and erosive type of OLP for both vitamin E and uric acid level. Regarding gender, no differences were found between males and females in salivary vitamin E. No significant correlation was found between vitamin E /uric acid and age. Similarly, no difference was found between males and females in uric acid.

Conclusion: Salivary anti-oxidant markers represented by vitamin E and uric acid decreased in OLP patients due to increase oxidative stress which may have an important role in the pathogenesis. Thus, it is recommended to give OLP patients anti-oxidant agents that may either help in healing process or decreased the severity.

Key words: OLP, Saliva, vitamin E, uric acid.. (Received: 10/1/2019; Accepted: 19/2/2019)

INTRODUCTION

Oral lichen planus (OLP) is a T-cell mediated chronic inflammatory mucosal disease of unknown etiology ⁽¹⁾. It is clinically presented as reticular, popular, plaque, erosive, atrophic or bullous types ⁽²⁾. The exact pathogenesis is unknown, but cell mediated and humoral immunity has been implicated ⁽³⁾. Activation of the cell- mediated immune response destined toward keratinocyte apoptosis is the prime event in the pathogenesis of OLP. The process involves three sequential stages; LP- specific antigen recognition, cytotoxic lymphocyte activation and keratinocyte apoptosis ^(3,4).

Oxidative stress in OLP release molecules consisting of granzymes that may result in local tissue damage in the effectors (5).

Antioxidants present in the mammalian cells can defend against oxidative stress. These include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzymatic antioxidants, including melatonin, uric acid, and vitamin A and E ^(6,7).

Increased oxidative stress and lipid peroxidation in patients with lichen planus has been reported ⁽⁸⁾. This may suggest a role of reactive oxygen species in the pathogenesis of lichen planus.

Antioxidant therapy strategies aimed to protect keratinocyte against damage, independent of disease progression, can be used on knowledge of the molecular aspects of oxidative stress in numerous diseases, including OLP ⁽⁹⁾. Some researchers suggest that oral use of antioxidants or antioxidant medications may successfully inhibit increased oxidative stress, and thus may help clinical improvement of disease ⁽¹⁰⁾.

MATERIALS AND METHODS

Participants

Sixty subjects were participated in this study; twenty-five oral lichen planus patients who were

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diagnosed clinically and histopathologic ally as an oral lichen planus, and thirty-five healthy looking volunteers as a control group who were age matched with OLP patients. The study was approved by the Ethics Committee of Oral Diagnosis Department in the College of Dentistry –University of Baghdad.

Method of saliva collection:

Salivary samples collection and histopathological study was done in Imam Hussain Medical City/ Kerbala Dermatology Department during the period from January to May 2017.

Saliva collection was started after the clinical examination. Saliva was collected from all participants under the same circumstances (20). Saliva was allowed to accumulate in the mouth and to expectorate all saliva formed over five minutes period into a sterile graduated test tube. The saliva samples of all the participants were identified by a code number during the period of s collection and processing. After the disappearance of the salivary froth, the salivary flow rate was measured in millilitres per minutes. Samples were stored at - 80°C, until analyzed.

Samples collection was limited to the hours between 8:00 and 11:00 AM to minimize the effect of diurnal variations.

Salivary vitamin E was measure using Elisa kit (Shanghai Yuhua Biological Technology); salivary uric acid was estimated using enzymatic assay kit.

A description about the purpose and aim of the study was performed for participants.

The study was carried out using a structured case sheet; the first part was related to the demography regarding name, age, gender, occupation and marital statutes. The second part involved clinical oral examination that carried out under natural light using disposable plane mouth mirrors.

Oral examination was performed by the same examiner.

Inclusion criteria of the current study include patients who were clinically and histologically diagnosed as an OLP

Exclusion criteria were patients having any systemic treatment suppressing the immune system such as systemic steroids or other immunosuppressive agents, as well as NSAIDs, antimalarial, diuretics, antihypertensive, antibiotics, and antifungals for the last 4 weeks and topical medications for last 3 weeks prior to sample collection. Also, patients with a history of trauma or any surgery 4 weeks prior to sampling, and those who were suffering from any systemic or dermatological disease affecting the immune system or any malignancy.

Furthermore, smoker patients were also excluded from this study.

Statistical analysis

Statistical analysis was performed with SPSS version 19.0. Descriptive statistical analysis, student T-test, analysis of variance (ANOVA) and linear and multiple linear correlation were used. A p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Age and Gender:

Twenty -five patients were with OLP (14 were females and 11 were males).The mean age was 48.3 years with an age range of 30-60 years. The control group consisted of 35 healthy subjects (20 were females and 15 were males) with an age range of 38-60 years and a mean of 48.2 years.

Occupation:

The majority of OLP patients were workers (18; 72%) followed by house wives (4; 16%) and officers 3(12%). Similarly, in control group subjects, 26(74%) were workers, 6(17%) were house wives, and 3(8%) were officers.

Oral lichen planus findings:

Oral lichen planus patients were divided into two subgroups according to the clinical presentation of the lesions at first presentation, 14 patients were with reticular form (56%) and 11 patients were with erosive form of OLP (44%).

Location:

The present study showed that buccal mucosa was the most common affected site (88%), followed by tongue (8%) then gingiva (4%).

In reticular form, buccal mucosa represented 86 % of the affected site followed by tongue 14%. While in erosive form of OLP, buccal mucosa represented 91% of the affected sites followed by gingiva 9%.

Size

In this study, the size of the OLP lesion was divided into three categories: 1.5, 2.5 and 3.3 cm. The majority of the reticular type of OLP was 1.5 cm in diameter, followed by 2.5 cm. In relation to the erosive type, the majority of OLP lesions were 1.5 cm followed by 2.5 cm in diameter.

Salivary flow rate (F/R):

Regarding salivary F/R, the present study showed a significant difference between the control and OLP patients ($p=0.001$); salivary F/R is significantly lower in OLP patients (0.04 ± 0.06) compared to the control (0.05 ± 0.02) ($p=0.001$)

In relation to the subgroups, there is no significant difference between reticular and erosive type of OLP in salivary flow rate.

Oral health status

Oral health status was divided into three scores: fair, moderate and good. The majority of patients with reticular form of OLP were seen with fair oral hygiene 6(43%) followed by 4(28.5%) moderate with similar number with good hygiene status. In relation to erosive form of OLP, 6 (43%) were observed with a fair oral hygiene, 3 (27 %) with moderate, and 2 (18 %) were seen with a good oral hygiene status.

Salivary vitamin E (pg/mL):

Regarding salivary vitamin E, the present study showed a significant difference between OLP patients and control group; OLP patients showed lower salivary vitamin E level (18.08 pg/mL) compared to control subjects (20.32 pg/mL) ($p=0.001$), figure 1.

In relation to OLP subgroups, there is no significant difference between reticular and erosive type of OLP in vitamin E level.

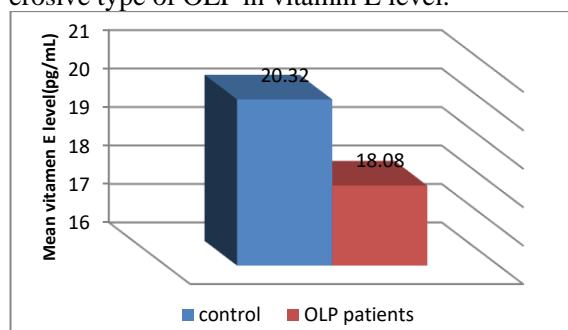


Figure 1: Mean salivary vitamin E in OLP patients and healthy subjects

Salivary uric acid

A significant difference in salivary uric acid level was found between control and OLP patients; patients with OLP showed lower mean levels of salivary uric acid (2.17 mg/dl) compared to healthy control subjects (5.32 mg/dL) ($p=0.0001$) or ($p<0.01$), figure 2.

Regarding OLP subgroups, there was no significant difference between reticular and erosive types of OLP.

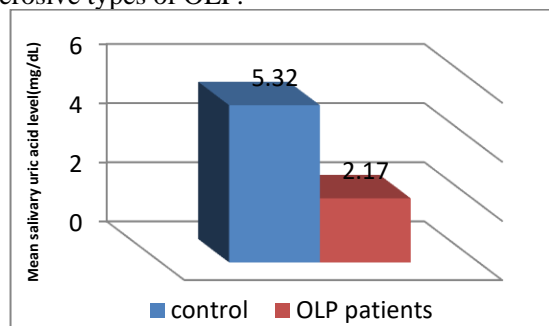


Figure 2: Mean salivary uric acid in OLP patients and healthy subjects.

DISCUSSION

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defense system

and is exclusively obtained from the diet. The term "vitamin E" refers to a family of eight naturally occurring homologous that are synthesized by plants from homogentistic acid⁽¹¹⁾.

The major biologic role of vitamin E is to protect poly unsaturated fatty acids and other components of cell membranes and low-density lipoprotein (LDL). Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly deterioration of poly unsaturated fatty acids. Elevated levels of lipid peroxidation associated with numerous diseases and clinical conditions⁽¹²⁾.

The results showed statistically significant reduction in the level of vitamin E in OLP patients in comparison with healthy subjects. Many studies have been done to determine the level of antioxidant in patients with Lichen Planus and other autoimmune disease^(13;14;15). In many of them, decrease in the production of antioxidants has been implicated in the etiology of lichen planus and lichenoid reactions which is agreed with the present study.

Pathophysiology of OLP is multifarious associated with pathognomonic characteristics and degeneration of cells which is supported to be certified to associate epithelial permeation of T-lymphocytes leading to local production cytokines⁽¹⁶⁾. Recently, it has been stated that imbalance in free radical and ROS with antioxidants may play an important role in the initiation of several inflammatory oral disease⁽¹⁴⁾. ROS and tissues oxidative damage, following extend a lack of antioxidants may result in appearance of this disease^(15; 17). The age incidence of OLP as stated in selected previous studies suggests that this disease is most commonly seen in the fifth decade of life^(15; 18).

Uric acid is one compound of catabolism purine nucleotides is a main significant antioxidants and also a powerful free radical scavenger in human biological fluids.

The present study showed a significant decrease in salivary uric acid for OLP patients when compared with healthy individuals which is agreed with previous studies⁽¹⁹⁾. The problem of oxidative stress and free radicals in OLP should be examined in studies with larger number of patients, and with other indicators of oxidative stress and antioxidants to support the present study findings. Also, the results of this study may indicate that OLP may be related to decrease of UA concentration in saliva. UA may be considered as a useful biomarker of antioxidant status for difficulty of treatment strategy and monitoring process in OLP patients. Low levels of total antioxidant are known to disturb its balance

to oxidative stress parameter ⁽²⁰⁾. Thus, further studies are required to see if UA level in saliva may play a role in treatment of OLP.

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الخلاصة:

الخلفية: الحزاز المسطح الفمي هو مرض التهابي مزمن يصيب الطبقة الطلائية من الفم ويحدث لأسباب غير معروفة. لوحظ في الدراسات الحديثة أن هنالك زيادة في الجهد التأكسدي لدى مرضى الحزاز المسطح. وكذلك نوع الاوكسجين التفاعلي لربما له دور في نشوء المرضي. الجهد التأكسدي يحرر انزيمات والاي قد يؤدي الى ضرر النسيج المحلي. مضادات الاكسدة التي يمكن ان تعمل ضد الجهد التأكسدي في خلايا الجسم تتضمن انزيمات مثل catalase, superoxide dismutase و Glutathione بالإضافة الى مضادات الاكسدة غير الانزيمية والتي تشمل melatonin, uric acid, فيتامين A

الغرض: لدراسة مستوى فيتامين اي و حامض اليوريك في اللعاب كعناصر مضادة للاكسدة في مرضى الحزاز المسطح و مقارنة ذلك بالمجموعة الضابطة

طريقة العمل: شارك في هذه الدراسة خمسة وعشرون مريضاً خضعوا للفحص النسيجي لتأكيد التشخيص وتم تقسيمهم الى مجموعتين اعتماداً على الوصف السريري للحالة، اربعة عشر يمثلون الحزاز المسطح الفمي الشبكي و احد عشر يمثلون الحزاز المسطح الفمي التآكلي بالإضافة الى المجموعة الضابطة والتي تتكون من خمسة وثلاثون شخصاً من الافراد الاصحاء المطابقين من حيث العمر. وقد تم تجميع عينات اللعاب لغرض تحليل النتائج.

النتائج: متوسط عمر مرضى الحزاز المسطح كان 48,3 ويتراوح بين 30-60 سنة. المجموعة الضابطة شملت 35 شخص سليم. طبقاً للاعراض السريرية للمرض، 56(14%) كانوا من النوع الشبكي، و 44(11%) من النوع التآكلي، بطانة الفم هي الموقع الأكثر تأثراً (88%) يليها اللسان (8%) ثم اللثة (4%). أغلبية قياس كلا النوعين 1,5 سم في القطر ثم 2,5 سم و بعدها 3,5 سم. أغلبية مرضى الحزاز المسطح كانوا عمال و غير مدخنين و لا توجد اي علاقة هامه بين التدخين وحجم الحزاز احصائياً. لوحظ ان مستوى كمية فيتامين اي و حامض اليوريك اقل في المرضى مقارنة بالاصحاء، بينما لا توجد اختلافات بين النوع الشبكي والتآكلي لكلا مضادات الاكسدة (فيتامين اي، حامض اليوريك). بالنسبة للجنس، لا اختلافات توجد بين الذكور والاناث في كمية فيتامين اي وكذلك حامض اليوريك في اللعاب، ولا يوجد ارتباط مهم بين فيتامين اي والعمر.

الختامه: كمية مضادات الاكسدة في اللعاب بالنسبة لفيتامين اي، حامض اليوريك تقل في مرضى الحزاز المسطح بسبب زيادة الاجهاد التأكسدي (زيادة في نوع التفاعل الاوكسجيني) وذلك لربما له دور مهم في نشوء مرض الحزاز المسطح. ولذلك يوصى بان يدعم مرضى الحزاز المسطح بمضادات الاكسدة والتي تساعد في الشفاء او التقليل من حدة المرض.