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

Adel Jasim Mohammed, B.D.S., H.D.D.[(1)]
Ameena Ryhan Diajil, B.D.S., M.Sc., Ph.D.[(2)]

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 bioten 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

(1) Master Student, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.
(2) Assistant Professor, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.

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 histopatholgical 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.

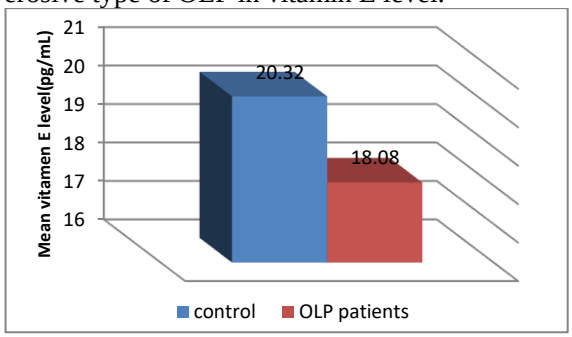

Figure 1: Mean salivary vitamin E in OLP patients and healthy subjects

Salivary uric acid

A significant difference in salivary uric acid level was fond 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), figure2.

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

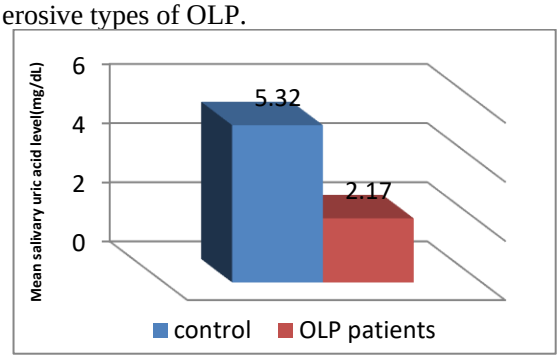

Figure 2: Mean salivary uric acid in OLP patients and healthy subjects.

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). Vitamen 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 vitamen E in OLP patients in comparism 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.

REFERENCES

1. Ropashree MR,Gondhalekar RV,Shashikanth MC,George J, Thippeswamy SH, Shukla A. Pathogenesis of oral lichen planus. A review. J Oral Pathol Med 2010; 39(10): 729-34.
2. Neville BW, Damm DD, Allen CM, Bouquot JE. Oral and maxillofacial pathology. 2nd edition. W.B. Saunders Company, 2002.
3. Daoud MS, Wakid MR. Lichen planus. In: Irwin MF, Eisen AZ, Wolf K, Austen KF, Goldsmith LA, Katz SI.(Eds.). Fitzpatrick's Dermatology in general medicine, sixth ed. McGraw- Hill, New York, 2003, p. 463-477.
4. Middel P, Lippert U, Hummel KM, Bertsch HP, Artuc M, Schweyer S, Radzun H-J. Expression of lymphotoxin- by keratinocytes: a further mediator for the lichenoid reaction. Pathobiology 2000; 68(4): 291-300.
5. Scrobotăl I, Mocan T, Cătoi C, Bolfă P. Mureşan A, Băciuţ G. Histopathological aspects and local implications of oxidative stress in patients with oral lichen planus. Rom J Morphol Embryol 2011; 52(4): 1305-9.
6. Nagler RM, Klein I, Zarzhevsky N, Drigues N, Reznick AZ. Characterizatin of the differentiated antioxidant profile of human saliva. Free Radic Biol Med 2002;32(3): 268-277.
7. Momen- Beitollahi J, Mansourian A, Momen-Heravi F, Amanlou M, Obradov S, Sahebjamee M. Assesment of salivary and serum antioxidant status in pateints with recuurent aphthous stomatitis. Med Oral Patol Oral Cir Bucal 2010; 15(4): 557-61.
8. Anshumalee N, Shashikanth MC. Efficacy of oral lycopene in management of lichen planus [dissertation]. Rajv Ghandi University of Health Sciences. Bangalore. 2007; 91-119.
9. Iqubal MAA, Khan M, Kumar P, Ajai K. Role of vitamin E in prevention of oral cancer: a review. J Clin Diagn Res 2014; 8(10): ZE05-ZE07.
10. de Gutierrez ER, Di Fabio A, Salomon S, Lanfranchi H. Topical treatment of orallichen planus with anthocynins. Med Oral Patol Oral Cir Bucal 2014; 19(5): 459-66.
11. Scott G. Antioxidants in science, technology, medicine and nutrition. Chichester, Abion Publishing,1997.
12. Kagan VE. Lipid peroxidation. Eur J Clin Nutr 1998; 4:759-764.
13. Nagao T, Warnakulasuria S, Ikeda N, Fukano H, Yamamuto S, Yano M, Miyazaki H, Ito Y. Serum antioxidant micronutrient levels in oral lichen planus. J Oral Pathol Med 2001 30: 264-7.
14. Agha-Hosseini F, Mirzaii-Dizgah I, Mikaili S, Abdollahi M, Abdolahi M. Increased salivary lipid peroxidation in human subjects with oral lichen planus. In J Dent Hyg 2009; 7(4): 246-50.
15. Updhay RB, Carmelio S, Shenoy RP, Gyawali P, Mukherjee M. Oxidative stress and antioxidant defense in oral lichen planus and oral lichenoid reaction. Scand J Clin Lab Invest 2010; 70(4): 225-8.
16. Khan A, Farah CS, Savage NW, Walsh LJ, Harbrow DJ, Sugerman PB. Th1 cytokines in oral lichen planus. J Oral Pathol Med 2003; 32:77-83.
17. Batu S, Ofluoğlu D, Ergun S, Warnakulasuria S, Uslu E, Güven, Y, Tanyeri H. Evaluation of prolidase activity and oxidative stress in patients with oral lichen planus and oral lichenoid contact reactions. J Oral Pathol Med 2016; 45(4): 281-8.
18. Scrobotă I, Mocan T, Cătoi c, Bolfă P, Mureşan A, BĂCIUŢ G.. Histopathological aspects and local implications of oxidative stress in patients with oral lichen planus. Rom J Morphol Embryol 2011; 52(4): 1305-1309.
19. Battino M, Greabu M, Tatan A, Bullon P, Bucur A, Palatos I, Spinu T, Totan C. Oxidative stress markers in oral lichen planus. Biofactors. 2008; 33(4): 301-10.
20. Serafini M, Villano D, Spera G. Pellegrini N. Redox molecules and cancer prevention: The importance of understanding the role of the antioxidant network. Nutr Cancer 2006; 56(2): 232-40.

الخلاصه:

الخلفيه: الحزاز المسطح الفمي هو مرض التهابي مزمن يصيب الطبقه الطلائيه من الفم ويحدث لاسباب غير معروفه. لوحظ في الدراسات الحديثه ان هنالك زياده في الجهد التاكسدي لدى مرضى الحزاز المسطح, و ك\لك نوع الاوكسجين التفاعلي لربما له دور في النشوء المرضي. الجهد التاكسدي يحرر انزيمات وال\اي قد يؤدي الى ضرر النسيج المحلي. مضادات الاكسده التي يمكن ان تعمل ضد الجهد التاكسدي في خلايا الجسم تتضمن انزيمات مثل catalase,superoxide dismutase,Glutathione بالاضافه الى مضادات الاكسده غير الانزيميه والتي تشمل melatonin,uric acid,فيتامين اي و فيتامين A

الغرض: لدراسة مستوى فيتامين اي و حامض اليوريك في اللعاب كعناصر مضاده للاكسده في مرضى الحزاز المسطح و مقارنة دلك بالمجموعه الضابطه

طريقة العمل: شارك في هده الدراسه خمسه و عشرون مريضا خضعوا للفحص النسيجي لتلكيد التشخيص وتم تقسيمهم الى مجموعتين اعتمادا على الوصف السريري للحاله, اربعة عشر يمثلون الحزاز المسطح الفمي الشبكي و احد عشر يمثلون الحزاز المسطح الفمي التاكلي بالاضافه الى المجموعه الضابطه والتي تتكون من خمسه وثلاثون شخصا من الافراد الاصحاء المطابقين من حيث العمر.وقد تم تجميع عينات اللعاب لغرض تحليل النتا ئج.

النتائج: متوسط عمر مرضى الحزاز المسطح كان48,3 ويتراوح بين 30-60 سنه.المجموعه الضابطه شملت 35 شخص سليم. طبقا للاعراض السريريه للمرض,14)56%(كانوا من النوع الشبكي, و 11(44%) من النوع التاكلي, بطانة الفم هي الموقع الاكثر تاثرا (88%) يليها اللسان(8%) ثم اللثه(4%). اغلبية قياس كلا النوعين 1,5 سم في القطر ثم 2,5 سم و بعدها 3,5 سم. اغلبية مرضى الحزاز المسطح كانوا عمال و غير مدخنين و لا توجد اي علاقه هامه بين التدخين وحجم الحزاز احصائيا. لوحظ ان مستوى كمية فيتامين اي و حامض اليوريك اقل في المرضى مقارنة بالاصحاء, بينما لا توجد اختلافات بين النوع الشبكي والتاكلي لكلا مضادات الاكسده(فيتامين اي,حامض اليوريك). بالنسبه للجنس, لا اختلافات توجد بين الدكور والاناث في كمية فيتامين اي وكدلك حامض اليوريك في اللعاب, ولا يوجد ارتباط مهم بين فيتامين اي والعمر.

الخاتمه: كمية مضادات الاكسده في العاب بالنسبه لفيتامين اي, حامض اليوريك تقل في مرضى الحزاز المسطح بسبب زيادة الاجهاد التاكسدي (زياده في نوع التفاعل الاوكسجيني) ودلك لربما له دور مهم في نشوء مرض الحزاز المسطح. ولدلك يوصى بان يدعم مرضى الحزاز المسطح بمضادات الاكسده والتي تساعدهم اما في الشفاء او التقليل من حدة المرض.