Evaluation of the Level of Melatonin, Cortisol and IgA in Saliva of Patients with Oral Lichen Planus Lesions

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ABSTRACT

Back ground: Oral lichen planus is a chronic inflammatory disease that affects the mucous membrane of the mouth. Several researchers suggest that oxidative stress is implicated in the pathogenesis of this disorder. It has been hypothesized that melatonin is powerful anti-oxidants and can easily cross the cell membrane and is considered a free radical scavenger of Hydroxid, Oxygen and Nitrogen dioxide, cortisol as a stress hormone and the immunoglobulin A as first line of defense and protection to the mucous membrane of the mouth are interrelated factors for the emergence of oral lichen planus. Aim of this study was to evaluate the level of melatonin, Cortisol and IgA in saliva of patients with oral lichen planus lesions in comparison with participants with normal oral mucosa.

Materials and methods: In this study seventy five subjects with age 18 and over were included. The subjects were divided into two groups; control group, this group comprised of 41 subjects with normal oral mucosa and the study group, which comprised of 34 subjects with clinically and histopathologically diagnosed oral lichen planus lesion. The samples were selected from patients attending College of Dentistry/University of Baghdad and cases that recruited to the AL-Emmam Ali hospital / in Baghdad /Dermatology Department. Samples collection started from 2/ may 2013 to 23/ January 2014.Saliva samples were collected between 10-12 Am. High performance chromatography (HPLC) analyzing technique was used for estimating the salivary melatonin and cortisol level. IgA level was measured by ELIZA method.

Results: The mean salivary melatonin level in patients with oral lichen planus was 4.786 µg/ml and the mean saliva melatonin level in normal person was 8.759 µg/ml. significant difference ($p<0.01$) was observed in the salivary melatonin levels between the study and control group. The mean salivary cortisol level in patients with OLP was 0.730 µg / ml and the mean saliva cortisol level in normal persons was 0.165 µg/ml. significant difference ($p<0.01$) was observed in the salivary cortisol levels between the study and control, group. The mean salivary IgA level in patients with OLP was 221.4 µg/ml and the mean saliva IgA level in normal person was 125.8 µg/ml. There was a high significant difference ($p<0.01$) the study and control groups.

Conclusions: The level of salivary melatonin was lower in patients with oral lichen planus, however cortisol and IgA was higher when comparing the salivary level with that of the control groups.

Key words: lichen planus, melatonin, cortisol, sIgA. (J Bagh Coll Dentistry 2016; 28(2):63-68).

INTRODUCTION

Certain diseases can cause alteration of the body tissues in general and the oral cavity in particular, one of these diseases is Lichen planus which is a mucocutaneous inflammatory disease of unknown origin. The skin and oral mucosa are the most frequently involved areas. Other mucous membranes (including the genitalia, esophagus, and conjunctiva) and skin Appendages (e.g. scalp hair and nails) can also be affected [(1)].

Oral lichen planus is a chronic inflammatory disease of unknown etiology that affects the mucous membrane of the oral cavity [(2)].

The reported prevalence rates of OLP vary from 0.5% to 2.2% of the general population. It is more frequently observed, in middle-aged women, female to male ratio is 1.4:1 [(3)]. It occurs more commonly in the mucosa than the cutaneous form and tends to be more persistent and more resistant to treatment. Oral lesion may accompany, precede or follow cutaneous lesion, also affect all racial groups [(4-7)].

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Many clinical forms of oral lichen planus are recognized, the reticular, erosive or ulcerative, Papular, Plaque-like, Atrophic, Bullous and pigmented [(8)].

The etiology of this cellular degeneration is believed to be attributed to sub epithelial infiltration of T-lymphocytes that contributes to the local production of cytokines which in turn can stimulate production of Reactive Oxygen Species (ROS) and cause oxidative damage to the tissues [(9)].

The WHO considers OLP as a precancerous condition; the premalignant potential of OLP is still debatable. Malignant transformation has been estimated to occur in 0.5 – 2.9% of the OLP patients [(4,10)]. Since etiology is unknown, there is no cure for lichen. The symptomatic treatment has been focused on reducing the subjective discomfort and to maintain or improve the quality of life [(11)].

In recent years, there has been an increasing research interest in oxidation of biological systems including free radicals, Reactive Oxygen Species (ROS), oxidative stress and antioxidant defence mechanisms in inflammatory and chronic degenerative diseases and during carcinogenesis

[12-14]. Oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of pro-oxidant/antioxidant reactions in living organisms [15].

It has been found that ROS produced by keratinocytes, fibroblasts and various inflammatory cells could result in disequilibrium between the pro-oxidants and antioxidants [16]. Reactive oxygen metabolites lead to destruction and damage to cell membranes by lipid peroxidation [12].

Melatonin is the principal secretory product of the pineal gland. It has immunomodulatory and antioxidant activities. Arising out of its antioxidant actions, melatonin protects against inflammatory processes and cellular damage caused by the toxic derivates of oxygen [17].

Steroid hormone cortisol released in response to stress which is one of the factors that leads to or triggering the oral lichen planus. Immunoglobulin A (IgA) is an antibody that plays a critical role in mucosal immunity [18]. IgA serves an important defending function at mucous membrane surfaces, which are the main entry sites for most pathogenic organisms.

Rabiei et al. found that the salivary cortisol and IgA levels are correlated with the incidence of OLP, and one may consider the salivary IgA and cortisol levels as a possible indicator in the creation or development of OLP lesions [19].

The following study aimed to evaluate the salivary melatonin, cortisol and IgA levels in patients with OLP and normal subjects.

SUBJECTS, MATERIALS AND METHODS

Seventy five subjects with age 18 and over were included in this study. The subjects were divided into two groups; control group, this group comprised of 41 subjects with normal oral mucosa and the study group, this group comprised of 34 subjects with clinically and histopathologically diagnosed oral lichen planus lesion. Thorough clinical history was obtained from the participants.

Oral cavity was examined by oral diagnosis set under artificial light. After proper clinical examination and required investigation (biopsy), clinical diagnosis was established.

The procedure of the study was explained to the recruited subjects and informed consent was obtained before starting the procedure. The participants were instructed not to eat, drink or take any medication 1hr before the sample collection, and then they were asked to rinse their mouth with tap water to remove any derbies before the saliva samples collection.

Unstimulated whole saliva was collected for the analyses in order to avoid any changes in the chemical composition of saliva initiated by stimulation [20]. Saliva was collected between 10-12 a.m.

The collected saliva samples were centrifuged at 3000 rpm for fifteen minutes. The supernatant was aspirated for estimating the salivary analysis. HPLC – UV detector was used for estimation the salivary melatonin and cortisol level. For evaluation the level of salivary IgA the ELISA method was used.

RESULTS

In this study, 34 patient with OLP aged 20-63 years and a mean age of 45.3 years and 41 subject with normal oral mucosa aged 18-65 years old with mean age of 54.7 years were studied.

Majority of OLP cases (24 cases) 75% were above 30 years of age.

The predominance of OLP was among female patients who showed 29 cases (85.3%); and 5 cases (14.7%) of OLP were male, with a female to male ratio was 5.8:1.

The distribution result of the study groups regarding gender and lesion form with comparisons significant has shown a non significant different between the two groups ($P<0.05$), since 24.1% of the female and 0.0% of male group were having plaque like lesion figure (3), while 48.3% of female and 80% of male were having reticular form of lesion, figure (4) and the leftover of 27.6% female and 20.0% of male were having erosive form of lesion, figure (5) ,these data illustrated in table (1).

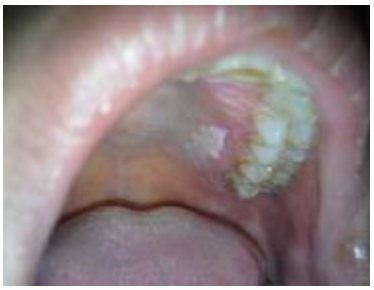

Figure 3: Plaque like OLP lesion at left side of palate

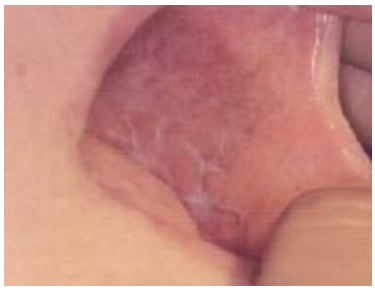

Figure 4: Reticular form of OLP at the left side of the buccal mucosa

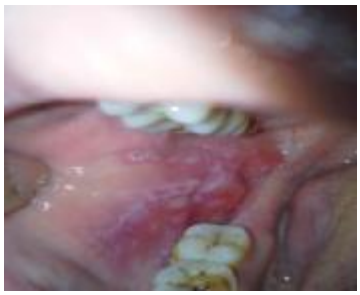

Figure 5: Erosive form of OLP at the right buccal mucosa

Table 1: Distribution of lesion form factor and gender with Comparisons Significant

Lesion Form	Freq. & Percents	Gender		Total	C.S. (*) P-value
		Male	Female		
Plaque	Freq.	0	7	7	C.C.= 0.242 P=0.348 NS
	% Lesion Form	0.0%	100%	100%	
	% Gender	0.0%	24.1%	20.6%	
Reticular	Freq.	4	14	18	
	% Lesion Form	22.2%	77.8%	100%	
	% Gender	80%	48.3%	52.9%	
Erosive	Freq.	1	8	9	
	% Lesion Form	11.1%	88.9%	100%	
	% Gender	20.0%	27.6%	26.5%	
Total	Freq.	5	29	34	
	% Lesion Form	14.7%	85.3%	100%	
	% Gender	100%	100%	100%	

(*)NS: Non Sig. at P>0.05

Table 2: levels of the three parameters (µg/ml) for the study and control groups

Parameter	Group	No	mean	median	SD	Mann-Whitney U	Z-value	C.S.(*)
Melatonin (µg/ml)	Study	34	4.786	5.001	2.344	451	-2.618	HS
	Control	41	8.759	5.800	6.235			
Cortisol (µg/ml)	Study	34	0.730	0.280	0.691	306	-4.163	HS
	Control	41	0.165	0.140	0.132			
sIgA (µg/ml)	Study	34	221.4	176.9	151.3	399	-3.172	HS
	Control	41	125.8	82.4	69.4			

(*) HS: Sig p<0.01

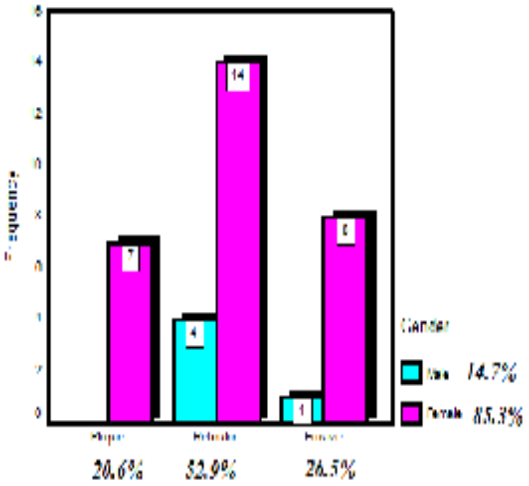

Figure 6: Cluster Bar Chart of the studied distribution of gender and Lesion Form factors

The result has shown a non normal distribution data of melatonin, cortisol and sIgA. Mann – Whitney U test was used for all readings of the studied parameters and the median values assessed to compare between the groups (table 2). High significant difference at (P<0.01) observed between the study and control groups. Control group has shown higher readings of Melatonin level (µg/ml) compared with the study groups (Fig. 7).

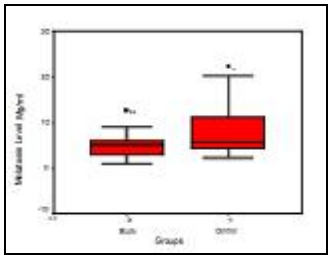

Figure 7: Melatonin of the study and control groups

There was a high reading of cortisol level in saliva compared with that of the control group. The results showed that there was a highly significant different (P<0.01) between the study group and control group (Fig. 8).

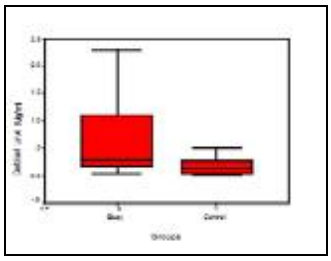

Figure 8: Cortisol of the study and control groups

Based on the ELISA test the results have shown that the study group reported a high readings of sIgA compared with the control group (Fig. 9) with highly significant difference (p<0.01).

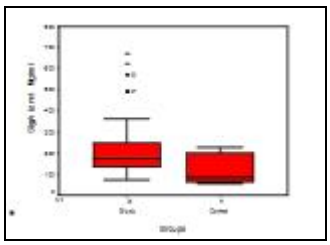

Figure 9: Salivary IgA of the study and control groups

DISCUSSION

In this study the patients under steroid or immunomodulation or cytotoxic drug, pregnant or breast feeding women and patient under medication that might cause lichenoid lesion like (antihypertensive, diabetic and sulfa drug) were excluded. Lichen planus is a common, chronic, autoimmune, mucocutaneous disease affecting the skin, genital mucosa, scalp, nails, as well as the oral mucosa.

Studies and researches have been going on to elucidate the main causes of disease in addition to explore the factors that may initiate the emergence of the disease. Identifying the causes will enhance the treatment or reaching to a specific therapy. Furthermore avoiding the occurrence of the precipitating factors and the appropriate treatment will be in hand to treat the lesion radically rather than just symptomatic relieve (soreness, burning sensation or discomfort).Melatonin, cortisol,and IgA level in saliva were measured in patients with oral lichen planus and compared with that of the control group to determine if they could be used as marker of existence of the lesion or assess the activity of the disease.

The samples were collected from participants; their age was ranged between 18 years and over. The first group was the study group, they were patients having oral lichen planus lesion and their mean age was 45.3 years.

The control group was the second group consisted of participants having normal oral mucosa and their mean age was 54.7 years.

The study revealed that the majority of cases (24 cases) 82% were above 30 years of age and this was in consistent with the other reported studies [21].

Predominance of OLP among female patients was observed, which showed 29 cases (85.3%) of oral lichen planus were females; and 5 cases (14.7%) of OLP were males, with a female to male ratio was 5.8:1 and this was in agreement with other studies [22-24].

The reticular form was the most frequent clinical form observed, they were 18 cases (52.9%) followed by the erosive form, 9 cases (26.5%) then the plaque like form, 7 cases (20.6%). This finding was also noticed in harmony with result reported by other investigators [21,23,25].

Melatonin, known chemically as *N*-acetyl-5-methoxytryptamine, is a hormone found in animals, plants, and microbes [26, 27]. In animals, circulating levels of melatonin vary in a daily cycle, thereby allowing the entrainment of the circadian rhythms of several biological functions [28].

The hormone is synthesized by the pineal gland and a variety of other body organs; Melatonin had been reported to play a role in protecting the oral cavity from tissue damage through its antioxidant, anti-inflammatory or immunomodulator activity [29].

Cortisol is a natural steroid hormone produced by the adrenal gland cortex. It has a strong diurnal variation, generally high early in the early morning and falling during the day time.

Stress induces increased cortisol secretion that counteracts inflammatory reactions. In this study, the correlation between the level of salivary cortisol and the OLP was analyzed.

IgA is the secretory immunoglobulin exists in the saliva as well as other body secretory fluid. It is the first line of the host defense against pathogens that invades the mucosal surfaces. Salivary IgA is antibody responsible for oral immunity by preventing microbial adherence, neutralizing enzymes, toxins and viruses [30]. It was theorized that serum level of immunoglobulin may play a role in the pathogenesis of oral mucosal diseases, or reflect clinical changes in these conditions [31]. Increased levels of serum IgA in patients with OLP were reported [32, 33].

The evaluation of IgA in saliva was performed by ELISA assay through which the optical density (OD) was measured and the concentrations calculated by using the quadratic equation.

HPLC system has been used to estimate the salivary melatonin and cortisol level. HPLC system was used for its advantages which include; speed (allow analysis to be done in a shorter time), sensitivity, accuracy and high resolution.

The results of melatonin level have shown highly significant difference between the study and control groups ($P<0.01$), since the control group has shown higher readings of Melatonin level (µg/ml) compared with the study groups. Up to our knowledge, there is no study considering the level of melatonin in patients with oral lichen planus lesion in order to compare it with the results of the present study. However, these results support the previous findings of the researchers which revealed the protective role of melatonin through its antioxidant and free radical scavenger, anti inflammatory or through immunomodulatory activity of this hormone [34,35].

Psychological stress which is one of the significant causal factors of oral lichen planus may have an effect on the interpretation of this difference between the levels of melatonin of two groups.

Raff and Benloucif and their colleagues have reported that chronic stress deplete melatonin and causes the production of too much cortisol which lowers melatonin, therefore Melatonin can be considered a stress protective hormone act by keeping down the hyper production of cortisol (the stress hormone) since melatonin can help control excess cortisol production, this may also be involved in lowering adrenal function [36, 37]. It was also suggested that melatonin could be used therapeutically, for instance locally in the oral cavity to treat diseases such as bacterial and viral lesions, postsurgical wounds, and oral surgeries, Lichen planus and oral cancers [38].

About the cortisol level the results showed that there was a highly significant difference ($P<0.01$) between the study group and control group. There was a high reading of cortisol level in saliva compared with that of the control group. This result is in agreement with previous results from Koray *et al.* who studied the relation between levels of cortisol (the stress hormone) in the saliva of fourty patients with oral lichen planus. The analysis compared salivary cortisol levels in these patients with the control group [39]. The authors concluded that salivary cortisol level were considerably higher in patients with oral lichen planus compared to those without disease, further strengthening the ties between salivary cortisol levels and oral lichen planus, Ivanovski *et al.* [40] have confirmed this conclusion after finding the same results in a basically similar study.

The IgA results have shown that the study group reported a high readings of sIgA (mean 221.4) µg/ml compared with the control group (mean 125.8) µg/ml, this finding is in consistant with many reported studies which have shown increased level of secretory IgA in saliva of patients with OLP lesion [31,41], therefore this difference in results between the two groups stressed the need to conduct further research analysis to include more immunoglobulines.

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