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SALIVARY LEVELS OF SOLUBLE FAS IN ORAL LICHEN PLANUS PATIENTS BEFORE AND AFTER TREATMENT WITH TOPICAL CORTICOSTEROIDS

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ABSTRACT

Purpose: Oral Lichen planus (OLP) is a chronic mucocutaneous disorder. One of the factors associated in induction of apoptosis in OLP is the Fas/FasL pathway. The aim of this investigation was to detect the salivary levels of apoptotic related marker soluble Fas (sFas) in OLP patients.

Methods: 30 individuals were divided into 2 groups: 15 patients suffering from OLP and 15 healthy individuals as controls. Clinical score for OLP lesions and pain assessment at exacerbation was collected. Saliva samples were collected twice from the OLP group at baseline and after topical corticosteroid treatment and once from control group.

Results: The mean salivary levels of sFas were significantly higher in OLP group at baseline than that of control group. After treatment sFas levels, pain and clinical scores decreased significantly in OLP group when compared to baseline.

Conclusion: sFas levels in saliva were higher in patients with OLP when compared to controls and decreased significantly after treatment which may emphasize its role in the pathogenesis of the disease.

KEY WORDS: Fas/FasL pathway, pathogenesis, apoptosis, mRNA, pain.

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INTRODUCTION

Oral Lichen planus (OLP) is a common chronic mucocutaneous disorder that have many clinical presentations; papular, plaque like, reticular, bullous-erosive, and atrophic. The bullous-erosive and atrophic forms cause discomfort and painful symptoms. In addition, OLP has a malignant potential [1].

Though OLP etiology is unknown, it is believed to be a result of an immune-mediated process with an unknown predisposing factor. However, genetic susceptibility, diet, infections, traumatic events, and chemicals are identified as the main risk factors [2-4]. Furthermore, it is a poorly understood and an intricate clinical disorder with periods of remissions and exacerbations [5].

OLP is characterized microscopically by elevated numbers of intra-epithelial lymphocytes, dense subepithelial lympho-histiocytic infiltrate, and degeneration of basal keratinocytes [6]. By light microscopy, apoptotic keratinocytes are detected as shrunken cells with eosinophilic cytoplasm and condensed nuclei. They also have been distinguished by the Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method [7, 8] and by electron microscopy [9].

Some factors were associated in initiation of apoptosis in OLP including p53 [10], the B-cell lymphoma 2 (BCL-2) family proteins [10, 11], tumor necrosis factor (TNF)-α, the Fas/ FasL pathway, proteases of the matrix metalloproteinase-9, granzyme B-perforin system (MMP-9) [12] and caspase-3 [9].

Fas/APO-1/CD95 is a type I membrane protein and a prototypical member of the nerve growth factor/tumor necrosis factor-R4 superfamily that causes apoptosis. Furthermore, The Fas/FasL system has a cytotoxic effect on natural killer (NK) cells and cytotoxic T cells and the interaction between cell-surface Fas and FasL induces apoptosis in sensitive cells [13].

Fas exists both as a soluble protein and as cell-surface protein. Cell-surface Fas is attached by a single membrane-planning domain that is broadly expressed in both normal and malignant cells. Soluble Fas (sFas), which is produced by an alternative mRNA splicing and lacks a transmembrane domain, is believed to block Fas-mediated apoptosis and inhibits the Fas/FasL binding. This apoptotic Fas/FasL pathway may play a role in tumorigenesis and disease progression [14].

Up to our knowledge no previous study has investigated the levels of apoptotic related marker sFas in saliva of OLP patients before and after treatment with topical corticosteroids. Therefore, the aim of the present investigation was to measure the salivary levels of sFas in OLP patients and to explain its role in disease pathogenesis and progression.

MATERIAL AND METHODS

Participants

Patients included in this study were recruited from the outpatient clinic of Oral Medicine and Periodontology Department, Faculty of Oral and Dental Medicine, Future University in Egypt in the period from December 2021 to May 2022.

Sample size calculation

A sample size was calculated and assessed to include an error of 5% and 85 % power with standard deviation of 1.0mm and a difference of 1.0 mm which was considered clinically significant between groups. It was assumed that a sample of 12 patients for each group would be needed. By considering the dropouts during the follow up we included 15 patients in each group:

OLP group: included (15) patients who were previously diagnosed (1 to 6 months prior to the beginning of the study) with OLP. For each patient salivary samples were collected twice before and after topical corticosteroid treatment.

Control group: included 15 healthy individuals from whom salivary samples were collected once.

Both groups were systemically free as assessed by the assistance of the Dental modification of the Cornell medical index to standardize their systemic condition 15. Informed consent from all patients

was obtained. The study protocol was permitted by the Ethics Committee of Faculty of Oral and Dental Medicine, Future University (FUE.REC (27)/12-2021).

Patients were included in the study if they were diagnosed with OLP after clinical examination in accordance with the World Health Organization (WHO) diagnostic criteria revised in 2009 [16], presented with acute exacerbation at time of specimen collection and did not receive any treatment, especially corticosteroids for at least 6 months prior to the start of the study.

Excluded criteria included participants who were taking any drugs inducing hyposalivation, had extraoral lichen planus lesions, had any visible oral lesion other than OLP or presented with histological signs of dysplasia. Smokers, pregnant or lactating patients were also excluded from the study.

Methods

All the patients and controls were subjected to the following.

Case history:

Comprehensive oral diagnosis was carried out using the department's oral diagnosis chart. Pain was assessed for OLP group at baseline (during exacerbation) and after treatment by present pain intensity (PPI) of McGill Pain Questionnaire [17] as follows: (0) No pain; (1) Mild pain; (2) Discomforting; (3) Distressing; (4) Horrible; (5) Excruciating.

Clinical examination:

A clinical score was given to all OLP lesions during exacerbation according to the clinical severity on a scale that ranged from 0-5 according to the criteria set by Thongprasom et al. [18] as follows: (0) No lesion, normal mucosa; (1) Mild white striae, no erythematous area; (2) White striae with atrophic area less than $1cm^2$, (3) White striae with atrophic area more than $1cm^2$, (4) White striae with erosive area less than $1cm^2$, (5) White striae with erosive area more than $1cm^2$.

The clinical score for each patient was calculated via recording each lesion's score separately, followed by calculating the average of these scores.

In OLP group topical triamcinolone acetonide 0.1% in orabase (Kenalog in Orabase: Bristol-Myers, Squibb, Spain) was prescribed to be applied topically 4 times a day i.e. following each meal and at bed time for one month [19] (Figure 1).

Salivary sample collection: Salivary samples were collected twice from OLP group at baseline and after topical corticosteroid treatment (after 5 weeks from baseline) and once from control group. Whole unstimulated saliva (WUS) was collected via standard techniques as described by Navazesh [20]. Briefly, patients were asked to stop drinking, eating, using chewing gum etc. for at least one and half hour former to the evaluation. Patients were requested to swallow first, incline their head forward and expectorate all saliva in a sterile tube for 5minutes without swallowing. Afterwards, all samples were instantly stored at -80° C until assessed.

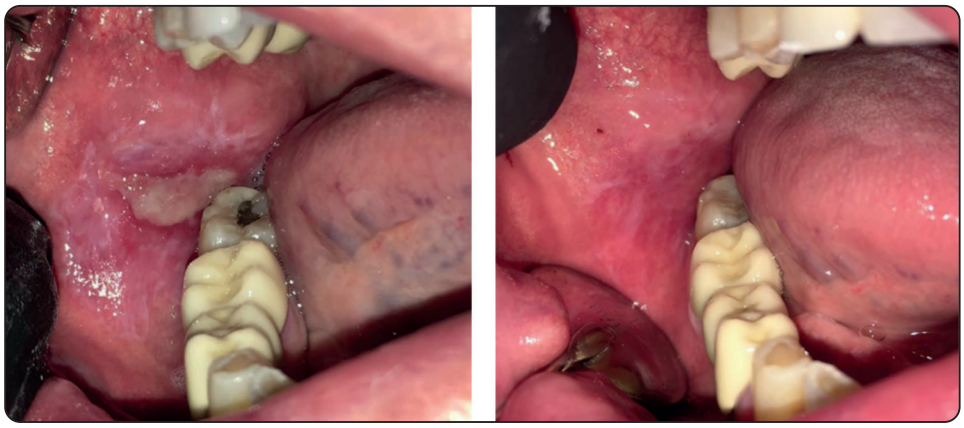

Fig. (1) Showing a bullous erosive lichen planus case (a) before and after (b) treatment.

Detection of sFas in salivary samples: Samples were assayed for salivary levels of sFas by enzyme linked immunosorbent assay (ELISA). In the lab, the salivary samples were thawed in ice and then subjected to centrifugation at 3500 rpm (2600g) for 15 minutes at 4° C to obtain the supernatant fluid phase. Supernatant were drawn off and used in the ELISA assays. sFas concentration was determined in saliva samples from each patient using ELISA kits provided by DIA Source, Nivelles, Belgium.

Calculation of results: For each set of duplicate calibrators, the average absorbance values were calculated. The calibration curve was formed by plotting the mean absorbance for each calibrator concentration on the ordinate against the sFas concentrations on the abscissa. The best fit curve was drawn through the points of the graph (a 5-parameter curve fit is recommended). To detect the concentration of circulating sFas for each sample, the mean absorbance value was first found on the ordinate and extends a horizontal line to the calibration curve. At the point of intersection, a vertical line was extended to the abscissa and the corresponding sFas concentrations were read.

Statistical analysis of collected data:

The recorded values were charted and studied statistically using SPSS statistical program. Shapiro test was used to assess the normality of the data. Statistical analysis was performed using Student t-test and Paired t-test. Results were considered significant at probability value (p) ≤ 0.05.

RESULTS

This study was carried out on 30 subjects, 15 patients with OLP and 15 controls. sFas level was detected in saliva of OLP patients at baseline and after treatment with topical corticosteroids and it was compared with that of 15 controls. Clinical data included age in years, the sex distribution, clinical score and pain score for OLP lesions.

In OLP group, patients age ranged from 33-50 years with a mean value of 38.9 years (±11.2) and in control group, it ranged from 30-45 years with a mean value of 35.8 years (±2.2). In OLP group, there was a predilection for females where 80% of the total numbers of cases were females (12) and 20% were males (3). The same gender predilection was found in the control group (10 females and 5 males).

In OLP group, the mean salivary sFas (pg/ul) at baseline was statistically significantly higher than in control group with p <0.05 (Table 1). sFas levels decreased significantly after treatment (Table 2).

Pain and clinical scores were statistically significantly higher in OLP group at baseline and decreased significantly after treatment (Table 3).

TABLE (1): Mean levels of sFas (pg/ul) in both groups before treatment

	OLP group mean (±SD)	**Control group** mean (±SD)	**95% CI** (Lower bound- upper bound)	***P*** value
sFas level in saliva before treatment	5271.5 (±19.2)	3603.8 (±17.5)	1667.7 (1653.96- 1681.44)	< 0.05*

**P value < 0.05 is considered significant*

TABLE (2): Mean levels of sFas (pg/ul) in OLP group before & after treatment

	Before treatment mean (±SD)	**After treatment** mean (±SD)	**95% CI** (Lower bound- upper bound)	***P*** value
sFas level in OLP group	5271.5 (±19.2)	3833.8 (±9.5)	1437.700 (1426.370- 1449.030)	< 0.0001*

**P value < 0.05 is considered significant*

TABLES (3): Pain & clinical scores in OLP group (at baseline and after treatment).

Point of comparison		Mean	SD	Median	Range	Minimum	Maximum
Pain score in OLP group	Before treatment	2.6	±0.55	2	4	1	5
	After treatment	0.40	0.43	0	1	0	1
	P value < 0.0002*						
Clinical score in OLP group	Before treatment	3.6	±0.33	3	2	3	5
	After treatment	0.6	0.24	0	2	0	2
	P value < 0.0001*						

**P value < 0.05 is considered significant*

DISCUSSION

Oral lichen planus is a chronic inflammatory T-cell–mediated disorder of the oral mucosa. The pathogenesis of OLP is still obscure but apoptosis has been reported in epidermal cells, suggesting its role in epithelial destruction [21].

Fas is a member of the tumor necrosis factor (TNF) receptor family, that was initially described as a membrane protein of the cell surface (mFas). Soluble Fas (sFas) that has been isolated in the serum, due to alternative splicing, lacks the transmembrane domain. sFas blocks apoptosis by obstructing the binding of Fas ligand (FasL) to Fas on the cell membrane [22].

Special interest of scientists has been focused upon apoptosis when investigating the pathogenesis of OLP in recent years. It is still unidentified which molecules are involved in the interaction between lymphocytes and basal keratinocytes, and if the molecular mechanisms that are responsible for cell death in lymphocytes are the same as those causing cell death in keratinocytes [23]. Therefore, the purpose of the current study was to measure the salivary levels of sFas in OLP patients before and after treatment and to elucidate its role in disease pathogenesis and progression.

The results of the present study showed that the mean salivary levels of sFas in OLP group at baseline were statistically significantly higher than that of control group. After treatment it decreased significantly when compared to baseline values. Furthermore, in OLP group pain and clinical scores decreased significantly after treatment.

This is in accordance with Sklavounou et al., who reported significantly higher serum levels of sFas in OLP patients compared to healthy controls and concluded that a supposed disfunction in the Fas/FasL mediated apoptosis might be involved in OLP pathogenesis [24, 25].

As only few studies have investigated the serum levels of sFas in OLP and as far as we know no studies investigated the salivary levels of sFas we needed to extend our comparisons to studies investigating sFas in other immunologically mediated diseases. Tokano et al., found that the level of sFas increased significantly in patients with systemic lupus when compared to control subjects. The level of sFas and $CD4^+$ T cells was significantly correlated, suggesting a connection between sFas and the activation of $CD4^+$ T cells. They added that low levels of sFas were found in patients with lymphopenia, inferring that sFas protects against the apoptosis of $CD4^+$ cells and thus enhancing the activation of $CD4^+$ cells leading to persistence of inflammatory infiltrate[26]. This may explain the elevated pain and clinical scores in the OLP group before treatment.

Moreover, it has been found that serum levels of sFas were elevated in patients with active stages of Behcet's disease [27], SLE, rheumatoid arthritis [28, 29] and multiple sclerosis [30] compared with those in inactive stages. Jodo et al., found a correlation between elevated serum levels of sFas, and clinical and laboratory findings in patients with SLE and concluded that sFas serum levels could be considered a proper marker to determine disease activity in SLE [28].

However, the elevation of sFas found in this study is in contrast with Knipping et al., and Goel et al., who reported no elevation in serum levels of sFas in SLE. In their studies the range and means of sFas/APO-1 levels in active SLE patients, did not differ from those of normal controls [31, 32]. The differences found between studies may be related to differences in anti-Fas antibodies used in each sandwich ELISA system to measure the sFas as suggested by Jodo et al., [28]. They further added that sFasL seems to be rapidly generated by matrix metalloproteinases from the cell surface of activated T cells and may become complexed with sFas in SLE sera making its detection by antiFas MoAb difficult [33].

In the present study the use of topical steroids resulted in improvement of clinical signs and symptoms as well as decrease in levels of serum sFas. Tokano et al., examined the association between the level of sFas and steroid therapy and reported a reduction in sFas with therapy that was accompanied by a high level of CD4. They found that sFas level was reduced by steroid therapy only in patients with activated $CD4^+$ cells which again confirms the contributory role of sFas to the dysregulated activation of T cells in various diseases [26].

Several studies proposed that apoptosis is a phenomenon of little quantitative importance in OLP infiltrate [34,35]. Trying to explain the high expression of the apoptosis-inducing Fas R/Fas L complex, in comparison to a lack of lymphocyte apoptosis in OLP, Neppelberg et al., proposed that there may be no transmission of the signal for apoptotic death to the inside of the cell, despite the initiation for apoptosis mediated by the Fas R/Fas L system [7].

Upregulation of sFas antagonizes the Fas membrane-bound form in OLP lesions and tends to block the removal of putative autoreactive lymphocytes through Fas/FasL mediated apoptosis [25]. Therefore, sFas release may be one probable mechanism that involved in the escape of T cells from apoptosis leading to their increase locally causing massive inflammatory infiltrates formation in OLP. In conclusion, sFas seems to play a role in disease pathogenesis of OLP and could be used as marker of disease activity.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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