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Original Article

Salivary tissue factor concentration and activity in patients with oral lichen planus☆

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ABSTRACT

Purpose: This study aimed to evaluate the tissue factor (TF) concentration and activity in patients with oral lichen planus (OLP) under oxidative stress.
Methods: Twenty patients with OLP were selected from the patients who were referred for treatment to the Marmara University, Faculty of Dentistry, Oral and Maxillofacial Radiology Department. Twenty healthy subjects from faculty staff and their family members were selected for the study. Salivary TF concentration and TF activity; total oxidant capacity; total antioxidant capacity; total thiol, malondialdehyde and glutathione concentrations; and oxidative stress index were measured in saliva samples.
Results: Salivary total oxidant capacity, oxidative stress index and malondialdehyde and TF concentration and activity were significantly increased in the patient group compared to those in the control group. Total thiol and glutathione concentration and total antioxidant capacity were significantly decreased in the patient group.
Conclusion: Impaired oxidant-antioxidant balance and inflammatory features of OLP might cause an increase in the salivary TF concentration and activity. As TF factor plays a critical role in inflammation progress, the use of an antioxidant agent in OLP may decrease the salivary TF concentration by decreasing oxidative stress. The findings of this study might represent a novel approach to OLP monitoring and treatment in terms of TF as the measurement of TF is easy and cost-effective.

1. Introduction

Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous disease of unknown aetiology. Characteristic histological features include a subepithelial band-like inflammatory cell infiltrate, variable numbers of intraepithelial mononuclear cells focused to the basal keratinocytes and epithelial basal cell degeneration/destruction. Changes in the basal cell compartment have gained much attention in recent research on OLP. In addition, it is characterized by relapses and remissions, and six clinical variants such as reticular, plaque-like, erosive, papular, atrophic, and bullous have been described [1]. The oral sites generally involved are the buccal mucosa, tongue and gingiva. It is characterized by a heavy lymphocytic infiltrate in the oral submucosa [2]. In OLP, lipid peroxidation levels increase in saliva and/or serum. This can be the indicator of premalignancy because of local or systemic oxidative stress [3–5]. There is an imbalance between the production of free radicals and antioxidative status in oxidative stress [6]. Therefore, some biomolecules and cells can be damaged, and many metabolic pathways can be affected [7]. Increased oxidative stress promotes the formation of the prothrombinase complex and subsequently generates thrombin. The aetiology of OLP remains unknown as it depends on several factors involved in its pathogenesis.

Oxidative stress increases the oxidation of plasma lipoprotein, which leads to the generation of the prothrombinase complex promotes and initiates blood procoagulation state [8]. Thus far, no study has explained the relationship between tissue factor (TF) concentration and oxidative stress in OLP. Galino et al. have revealed that oxygen free radicals induce TF messenger RNA transcription and expression of TF procoagulant activity in endothelial cells in culture [9]. TF is the main initiator of thrombogenesis as a cellular receptor and cofactor for plasma factor VII(a), and TF-FVII(a) complex leads to the generation of thrombin and fibrin [10]. TF is not highly expressed on endothelial cells under normal physiological conditions, but surface expression is induced by various stimuli,

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including inflammatory cytokines, endotoxin and hypoxia [11]. Various tissues, cells and saliva have TF activity [12,13]. Recently, Shin et al. reported that OLP is considered to have malignant potential, and it is unclear regarding which types of molecules may cause malignant transformation of OLP [14]. Therefore, this study aimed to evaluate whether salivary oxidative stress-based TF level and activity changes could be a link between OLP and its pathogenesis. Because the role of salivary TF in OLP has not been specified yet, the findings of this study are novel.

2. Materials and methods

The study protocol was confirmed by Marmara University ethics committee, and written informed consent was obtained from all patients and healthy subjects.

2.1. Subjects

Twenty OLP patients and 20 healthy individuals were included in this study. The biopsy confirmation was made for the diagnosis of OLP as recommended by the World Health Organization [15]. Medical history of each patient was taken in detail. Written informed consent was obtained from the OLP patients. OLP patients with any systemic disease or with suspected restoration-related reaction or gingival inflammation and/or under medical therapy that could cause lichenoid reaction during the 3 months were excluded from the study. Biopsy samples were collected from the most prominent area of the OLP lesion that also includes the healthy tissue by an experienced oral medicine specialist. An incisional biopsy was performed under local anaesthetic at the Oral Diagnosis and Radiology Department, Faculty of Dentistry, Marmara University. The specimen was sent for histopathological examination at the Department of Oncologic Cytology and Tumor Pathology, Institute of Oncology, Istanbul University. Twenty control participants included faculty staff and their family members after obtaining their informed consent. They also did not have any systemic disease or inflammatory oral lesions. The study was carried out according to the recommendations of the Declaration of Helsinki, and the study protocol was approved by the Local Committee of Research and Ethics of Marmara University, Istanbul.

2.2. Collection of saliva samples

Unstimulated whole saliva samples were obtained by expectoration without chewing movements in dry plastic vials. All subjects refrained from eating, drinking or smoking for a minimum of 2 h before saliva collection. Patients were asked to rinse their mouth with water before saliva collection. They were comfortably seated; after a few minutes of relaxation, they were trained to avoid swallowing saliva and asked to lean forward and expectorate all the saliva they produced for 5 min into a 50-mL sterile tube. The collected saliva samples were centrifuged ($1500 \times g$ for 10 min). The supernatants were aliquoted and stored at $-20\,^{\circ}C$ until the date of experiment.

2.3. Measurement of total oxidant capacity

Salivary total oxidant capacity (TOC) was determined using Erel's method [16]. The assay is calibrated with H_2O_2, and the results are expressed in terms of μmol H_2O_2 eq/L.

2.4. Measurement of total antioxidant capacity

The salivary antioxidative effect against the potent-free radical reactions was measured by Erel's method [17]. The results are expressed as mmol Trolox eq/L.

2.5. Measurement of oxidative stress index

As oxidative stress index (OSI) is an indicator of the degree of oxidative stress, and it was calculated according to the formula given below [18].

$$\text{OSI(arbitrary unit)} = \text{TOC}(\mu\text{mol } H_2O_2\text{ eq/L})/10 \times \text{TAC}(\text{mmol Trolox eq/L}).$$

2.6. Measurement of total thiol concentration

Total thiol concentration was measured using the total thiol measurement assay kit. (Rel-Assay Diagnostics, Turkey)

2.7. Measurement of salivary TF activity and TF concentration

Salivary TF activity was determined by the Quick's one-stage method [19]. The test was performed by mixing 0.1-ml saliva with 0.1 ml of 0.02 M $CaCl_2$. The clotting reaction was started after the addition of 0.1 ml of plasma.

Salivary TF concentration was measured by competitive ELISA kit (IDEL-F108) according to the manufacturer's instructions. The sensitivity of the method was 1 ng/mL.

2.8. Malondialdehyde and glutathione concentrations

Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) concentrations in saliva according to the method of Yagi [20]. Saliva glutathione (GSH) concentration was measured by Beutler's method [21].

2.9. Statistical analysis

Results are expressed as mean ± SD. Differences were considered significant at a probability level of $P < 0.05$. Student's t-test was used to compare the patient and control groups.

3. Results

3.1. Characteristics of the study group

Twenty patients were diagnosed with OLP, and 20 matched healthy controls were studied. The mean ages of the control group and the OLP group were 51.15 + 11.03 and 50.80 + 11.57 years, respectively. In both the OLP and control groups ($n = 20$ for each), 11 (55%) were females and 9 (45%) were males. The two groups did not differ for age and gender ($p > 0.05$). No significant differences in the salivary flow rate and age were observed between the patients with OLP and controls ($p < 0.05$). Characteristics of the patients with OLP are listed in Table 1. Ulceration of the buccal mucosa showed peripheral radiating keratotic striae, which is characteristic of oral erosive lichen planus (Fig. 1). In OLP biopsy specimen, hyperkeratosis, saw-toothed rete ridges and a band-like infiltrate of lymphocytes immediately subjacent to the epithelium were detected (Fig. 2).

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Table 1
Clinical features of the study patients.

		OLP	
		n	%
Affected site	Buccal mucosa	11	55
	Gingiva	1	5
	Tongue + buccal mucosa	4	20
	Lip + palatal mucosa	1	5
	Tongue + buccal mucosa + floor of the mouth	1	5
	Tongue + gingiva + buccal mucosa	1	5
	Tongue + buccal mucosa + palatal mucosa	1	5
Present site	Unilateral	2	10
	Bilateral	18	90
Clinical form	Reticular	14	70
	Erosive	2	10
	Hypertrophic	2	10
	Erosive + Atrophic	1	5
	Reticular + Erosive	1	5

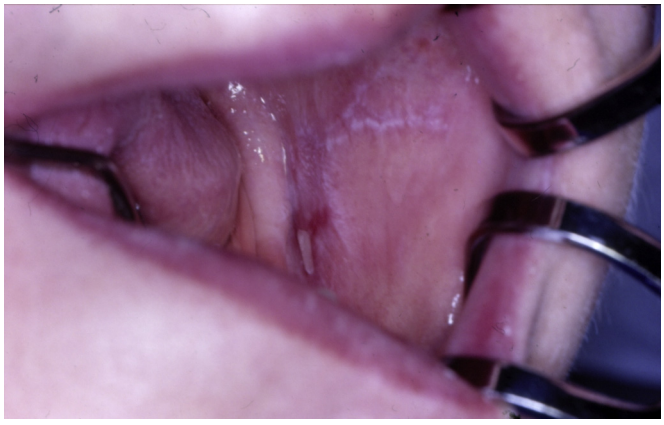

Fig. 1. Ulceration of the buccal mucosa shows peripheral radiating keratotic striae, characteristic of oral erosive lichen planus.

Table 2
Saliva MDA, GSH and total thiol concentrations.

	Control group ($n = 20$)	OLP group ($n = 20$)	*p*
MDA (nmol/mL)	2.09 ± 0.58	5.52 ± 1.14	0.0001
GSH (mg/dL)	0.70 ± 0.19	0.23 ± 0.09	0.0001
Total thiol (μmol/ml)	0.13 ± 0.04	0.06 ± 0.02	0.0001

OLP, oral lichen planus; MDA, malondialdehyde; GSH, glutathione.
Values are expressed as mean ± SD.

3.2. Salivary MDA, GSH and total thiol concentrations

Saliva MDA concentration was significantly higher in the OLP group than that in the control group (Table 2). In the OLP group, saliva GSH and total thiol concentrations were significantly lower than in the control group (Table 2).

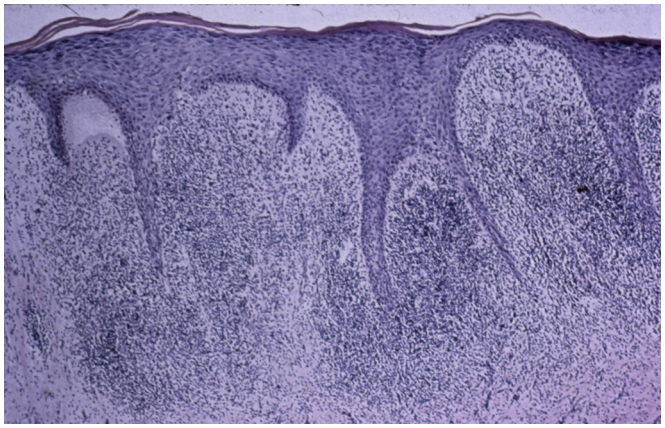

Fig. 2. Lichen planus biopsy specimen showing hyperkeratosis, saw-toothed rete ridges and a band-like infiltrate of lymphocytes immediately subjacent to the epithelium.

3.3. Salivary TF activity and concentration

The shortening of the clotting time is an indication of increased TF activity. In this study, salivary TF activity and TF concentration significantly increased in the OLP group as compared to those in the control group (Fig. 3).

3.4. Salivary TOC, TAC and OSI

TOC and OSI significantly increased in the OLP group compared to those in the control group. TAC significantly decreased in the OLP group compared to that in the control group (Fig. 4).

4. Discussion

OLP presents in various clinical forms, and previous studies have indicated that the erythematous/ulcerative subtype seemed to cause more serious symptoms such as pain and bleeding than the reticular subtype and was considered the more severe subtype [22,23]. Dan et al. also observed that serum and saliva IL-10 levels were increased in the erythematous/ulcerative group [22]. In our another study, we evaluated the serum levels of Th1 and Th2 cytokines according to the clinical forms, and we observed that the serum levels of IL-10 tended to be higher in both the reticular and erosive groups, but the serum levels of IL-2 tended to be lower in the same groups [24]. In the present study, most of our patients (70%) had the reticular type of OLP.

An imbalance between oxidative stress and antioxidant defence system is involved in the pathogenesis of OLP [25,26]. Among the

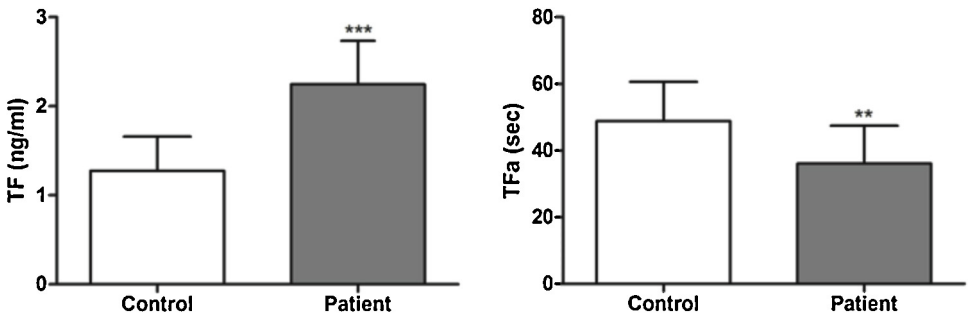

Fig. 3. Salivary tissue factor concentration and activity. TF, tissue factor; TFa, tissue factor activity, *** $p < 0.0001$, ** $p < 0.001$ versus the control group.

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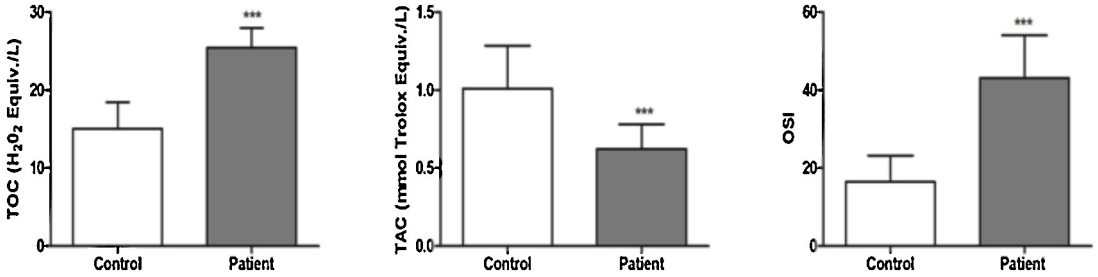

Fig. 4. Salivary total oxidant capacity, total antioxidant capacity and oxidative stress index. TOC, total oxidant capacity; TAC, total antioxidant capacity; OSI, oxidative stress index. *** $p < 0.0001$ versus the control group.

different potent mediators released in saliva in OLP, we focused our attention on salivary TF activity as oxidants were shown to induce the expression of TF by endothelial cells [9]. TF (Thromboplastin, FIII, CD 142) is an inducible 47-kDa transmembrane cell-surface glycoprotein. It is the primary initiator of the extrinsic coagulation cascade [27]. As the relationship between OLP and salivary TF expression has not been previously mentioned, this study shows the facts underlying this relationship. TF also functions in inflammation, angiogenesis and tumour growth [11]. Histamine, serotonin, IL1-β, lipopolysaccharides, tumour necrosis factorα and vascular endothelial growth factor may induce TF expression [28]. In the present study, in addition to the increased oxidative stress and decreased antioxidant capacity, salivary TF concentration and TF activity increased in the patients with OLP. This increased salivary TF concentration and TF activity represent the increased TF expression by the cells found in saliva. As TF is a labile protein and its activity can be changed by the disturbances in membrane composition, heating and pH changes, the increased salivary TF activity in OLP shows active TF. The findings of this study reveal that the increased and active TF in OLP may protect tissues from bleeding, but it may help the progression of oral carcinogenesis as it plays a role in angiogenesis. Different disease states might determine the behaviour of tissues in terms of TF. Ferro et al. suggested that oxidative stress may represent a common pathway for the activation of the clotting system in different clinical settings characterized by chronic inflammation [29]. In this study, salivary TOC; OSI; and MDA, an indicator of lipid peroxidation, were increased in patients with OLP. This increase in oxidative stress during OLP was accompanied by a significant increase in TF in this study, suggesting that increase in TF activity might impair haemostatic balance and possibly contribute to the occurrence of angiogenesis and carcinogenesis [30–32]. In the present study, total antioxidant capacity (TAC), GSH, and total thiol concentrations were lower in patients with OLP. These findings are in accordance with the studies that report an increased oxidative stress in lichen planus [27,33]. Because a significant reduction of thrombin generation in vivo was related to the increased antioxidant level [34], the impaired oxidant-antioxidant balance in OLP could be related to the increased TF-based thrombin generation.

5. Conclusion

According to the findings of this study, impaired oxidant-antioxidant balance and inflammatory features of OLP might cause an increase in the salivary TF concentration and TF activity. Because TF plays a critical role in inflammation progression, the use of antioxidants in OLP may decrease the salivary TF concentration by decreasing oxidative stress.

Salivary TF concentration and/or TF activity may also serve as a complementary diagnostic marker and as a new parameter to assess the disease progression or treatment follow-up of OLP as it can serve as a painless biopsy measure complementary to clinical monitoring.

Conflict of interest statement

There is no conflict of interest related with this manuscript.

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