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Altered Tissue Factor Activity and Disrupted Oxidant-Antioxidant Status in Saliva of Patients with Oral Lichen Planus

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ABSTRACT

Objective: The aim of our study was to investigate salivary tissue factor (TF) activity and oxidant-antioxidant status, which is an important defense system, in saliva samples collected from patients with oral lichen planus (OLP), and to determine the relationship between the antioxidant system and TF activity in OLP.

Materials and Methods: Saliva samples were collected from patients with OLP (n=20) and healthy subjects (n=13). TF activity, lipid peroxidation (LPO), nitric oxide (NO), glutathione (GSH) levels, glutathione S-transferase (GST), and superoxide dismutase (SOD) activities were measured.

Results: NO levels and GST activities were increased, whereas GSH levels and SOD activities were decreased, when compared with healthy subjects in the saliva samples collected from patients with OLP. In addition, TF activity was increased in the OLP group compared with the control group

Conclusion: The results revealed that the salivary oxidant-antioxidant balance was disrupted in parallel with the increase in TF activity in patients with OLP.

Keywords: Oral lichen planus, tissue factor, oxidative stress, saliva

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INTRODUCTION

Lichen planus (LP) is a chronic inflammatory disease commonly observed in the skin and mucosa, the cause of which is unknown. Oral lichen planus (OLP), which is most commonly seen in the buccal mucosa, dorsum of the tongue, and less commonly on the gingivae, can occur either alone or with skin lesions, and is usually bilateral and symmetrical (1, 2). The mouth and saliva reflect physiological or pathological changes in the organism (3). Saliva, with its rich content, can be used to diagnose various diseases or monitor the body's response to treatment. Saliva biomarkers are important for determination of these changes in the body. These biomarkers play a potential role in the diagnosis of various oral and systemic diseases. In addition, analysis with saliva fluid provides an advantage because it is an non-invasive method (3, 4).

Tissue factor (TF, Factor III, Thromboplastin) is an important activator of the coagulation cascade, and is the main regulator of haemostasis and thrombosis (5). Body fluids such as saliva, bile, semen, amniotic fluid, and various tissues have TF activity (6-9). The clotting function of saliva is due to TF present in saliva, and TF activity may be triggered by many different stimuli, including surface expression, inflammatory cytokines, endotoxins, and hypoxia (6, 7, 10).

In the presence of oxygen under normal cell conditions, reactive oxygen species (ROS) can be produced. The increase in ROS levels compared with antioxidant agents causes the oxidant-antioxidant balance to deteriorate. This imbalance causes various diseases because of lipid peroxidation (LPO) and cell damage (5, 6).

Oxidant-antioxidant imbalance has a potential effect on the development of oral pathologies. Many diseases are based on oxidative stress, which can also explain why the systemic and oral disease consequences can be so severe. Antioxidants, which are effective in the defense mechanism of the organism, are also included in the saliva content (4-6). LPO and salivary antioxidants can be utilized to measure the oral oxidant-antioxidant condition (5, 6, 11-13).

Free radicals produced by exogenous or endogenous sources in the organism react with lipids and cause LPO, which can have a very harmful effect on the organism. LPO causes the formation of toxic products that show harmful effects in areas far from where they are produced (12). Malondialdehyde (MDA) is the major end product of LPO and is recognized as an important marker of oxidative stress and damage mediated by free radicals (13). The antioxidant system, which is effective as a defense mechanism in the organism, consists of enzymatic and non-enzymatic agents. Glutathione (GSH), which has a tripeptide structure, is a non-enzymatic antioxidant. GSH, which has an antioxidant effect, is a reducing agent. GSH serves as a cofactor for enzymes such as glutathione peroxidase and glutathione S-transferase (GST). In addition, GSH plays a role in the regeneration of vitamin E for the detoxification of lipid peroxyl radicals. GST are enzymes involved in the catalysis of the reaction between the reducing agent GSH and xenobiotics. The key role of the GST enzyme in xenobiotic metabolism is to prevent cell damage by combining the reactive species produced by the enzymes in Phase I with glutathione (14).

Superoxide dismutase (SOD) catalyzes the conversion of superoxide radicals, a by-product of oxygen metabolism, into molecular oxygen and hydrogen peroxide. The primary antioxidant enzyme systems SOD and glutathione peroxidase can destroy free radicals. OLP develops as a result of increased oxidative stress (13). Because salivary fluid naturally contains various antioxidants, many studies have been conducted using salivary fluid, and these studies have revealed that the total antioxidant status in salivary fluid collected from erosive OLP patients is lower than that in healthy controls (14, 15). Nitric oxide (NO), which is synthesized from arginine, play roles as a neurotransmitter, immunomodulator, vasodilator, and antiplatelet. The neuronal nitric oxide synthase (nNOS) enzyme, which catalyzes NO synthesis, is located in the salivary gland. Therefore, it is thought that NO may play a potential role in salivary gland secretion and blood flow. In addition, because NO has antibacterial properties, it can protect the oral mucosa at low concentrations in saliva (16).

ROS may interfere with platelet activation and increase the expression levels of TF in endothelial cells, monocytes, and vascular smooth muscle cells, all of which can promote coagulation (17). In view of the ability of ROS to induce oxidative structural as well as functional alterations in key proteins involved in the coagulation cascade, the aim of our study was to investigate salivary TF activity and oxidant-antioxidant parameters to evaluate the relationship between TF activity and oxidant-antioxidant status in patients with OLP.

MATERIALS AND METHODS

Saliva Collection and Storage

The Istanbul Medipol University Non-invasive Clinical Research Ethics Committee approved the study protocol (ethic no: E-10840098-202.3.02-986). All oral clinical examinations were performed at the Outpatient Clinic of the Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Marmara University.

Patients with OLP whose disease was confirmed by clinical examination by two specialists with more than 20 years of experience based on clinical manifestations (reticular lesions alone or in association with erosive/ulcerative lesions) or combined with histopathological examination were included in the current study.

After the selection of participants, unstimulated saliva samples were collected from 20 OLP patients (aged 30-55 years), and 13 healthy controls using the spitting method. Participants were instructed to stop eating, drinking, and brushing their teeth 90 min before saliva samples were collected. The patient

must sit in a comfortable position while the saliva sample was being collected. Leaning slightly forward, the patient drained the saliva from the tube by spitting 1-2 times every 1 min for 5 min. After saliva collection, the test tube was sealed with a plastic cap and transported to the biochemistry laboratory. Whole saliva samples were used to assess tissue factor activity. For biochemical parameters, saliva samples were centrifuged at 825xg for 4-5 minutes and the supernatant was separated and used in the analysis. The analyses were performed in a blinded manner.

Saliva Analysis

Assay of TF Activity

Quick's one-step method was used to measure TF activity in saliva samples (18). This was accomplished by addition of 100 µL of 0.02 M $CaCl_2$ to 100 µL of saliva and 100 µL of plasma to initiate the coagulation reaction. Activity was measured after the temperatures of all solutions used in the experiment were brought to 37°C. Because clotting time is inversely proportional to TF activity, prolonged clotting time is an indicator of decreased TF activity.

Assay of the Total Protein

Protein levels were measured to present biochemical data as values per mg of protein. Proteins react with Cu^{2+} ions in an alkaline environment. It is then reduced with the foline reagent. The intensity of the resulting color was evaluated spectrophotometrically. The density of the colored (blue-violet) solution formed is proportional to the protein concentration (19).

Assay of Lipid Peroxidation

LPO determination was made by spectrophotometric evaluation at 532 nm of the density of the coloured solution resulting from the reaction between the LPO product MDA and thiobarbituric acid (20).

Assay of NO

The determination of NO was performed by spectrophotometric measurement at 540 nm of the colored product resulting from the formation of a complex diazonium compound by the reaction of nitritesulfanilamide with N-(1-Naphthyl) ethylenediaminedihydrochloride in an acidic environment (21).

Assay of the GST Activity

The determination of GST activity was evaluated spectrophotometrically by measuring the absorbance of the product formed after the conjugation of GSH with 1-chloro-2,4-dinitro-benzene at 340 nm (22).

Assay of the SOD Activity

SOD activity is measured as the ability to increase the photooxidation rate of riboflavin- sensitized o-dianisidine. The superoxide radical formed by riboflavin under the influence of fluorescence light turns into hydrogen peroxide under the influence of SOD in the environment. Hydrogen peroxide reacts with o-dianisidine to form a colored product. The absorbance of the resulting colored product was evaluated spectrophotometrically at 460 nm (23).

Assay of GSH

GSH determination was made spectrophotometrically by the colored product resulting from the reaction of Elmann's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and sulfhydryl groups (24).

Statistical Analyses

GraphPad Prism 9.0 was used for statistical analysis, and the mean and standard deviation (SD) was presented as the result. An unpaired samples t-test was performed to compare the control and OLP groups.

Results

When we examined TF activity to measure the coagulative function of saliva and compared it with the control group, increased salivary TF activity was determined in patients with OLP (Figure 1).

In the LPO analysis, where we measured MDA levels, there was no statistically significant difference in MDA levels (Figure 2A), and NO levels were raised in the patients with OLP compared with the control group (Figure 2B).

While GST activity was increased statistically in patients with OLP compared with the control (Figure 3A), SOD activity (Figure 3B), and GSH levels (Figure 3C) were decreased.

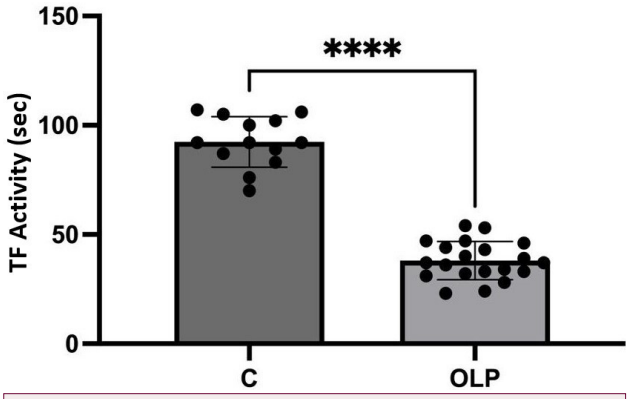

Figure 1. Tissue Factor (TF) activities of the groups. Clotting time is inversely proportional to TF activity. The longer clotting time in seconds is a manifestation of lower TF activity. Data are given as mean ± SD, **** p<0.0001, SD: Standart deviation, C: Control group; OLP: Oral Lichen Planus

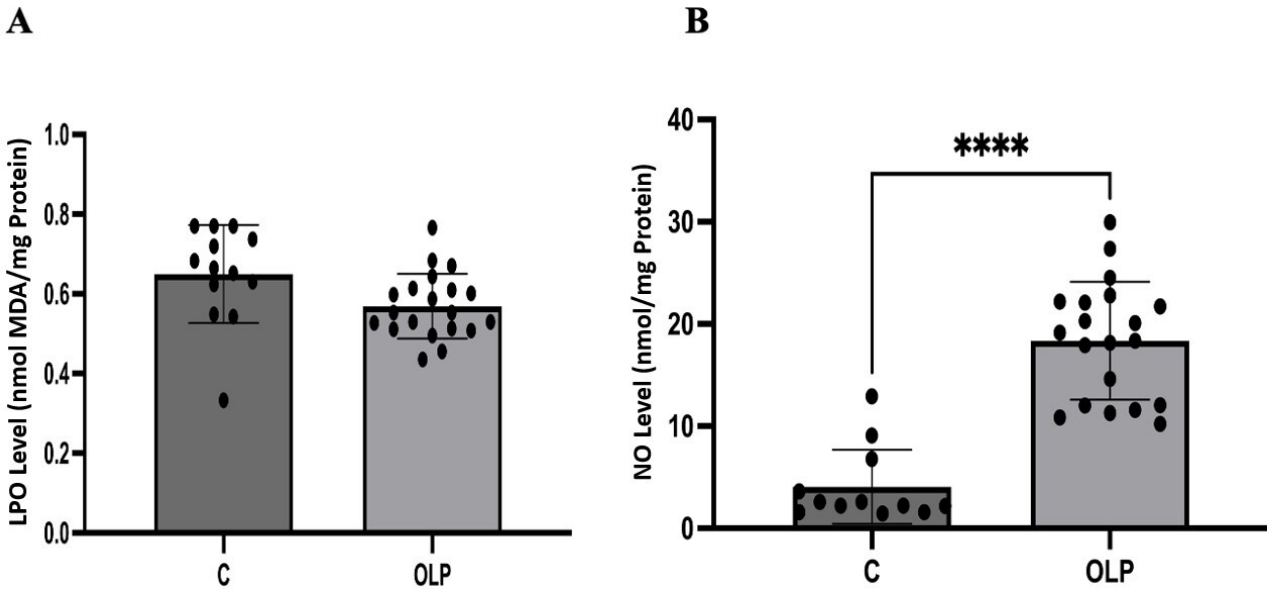

Figure 2 A. MDA levels serve as an index of lipid peroxidation (LPO). **B**. Nitric Oxide (NO) levels. Data are given as mean ± SD, **** p<0.0001, SD: Standart deviation, C: Control group; OLP: Oral Lichen Planus

DISCUSSION

The body has specific mechanisms called antioxidant defense systems to deal with free radical damage. Increased cellular damage is caused by oxidative stress or a failure of the antioxidant defense system (25-29). Various antioxidant parameters (such as GST and SOD activity) are analyzed to determine the level of oxidative stress.

Inflammatory cellular infiltrates caused by $CD4^+$ lymphocytes are a potential source of reactive oxygen species. Increased oxidative stress has previously been reported in patients with OLP, a chronic inflammatory disease (25-29). Consistent with these studies, in our study the oxidant-antioxidant balance was disturbed, characterized by decreased antioxidant enzyme activities; however, we did not find any statistical difference between the control group and the OLP patient group.

In our study, changes in oxidant-antioxidant parameters and TF activity were analyzed in saliva samples collected from patients with OLP. Consistent with our results, Tunali-Akbay et al. showed that TF activity was increased in saliva samples of patients with OLP (29). Our results showed that the oxidant-antioxidant balance was disrupted and salivary TF activity was increased in patients with OLP. Over the past few years, oxidative stress has become increasingly important in controlling endothelial dysfunction and thrombus development. ROS may hinder the coagulation process through various intricately linked pathways. ROS, mostly produced by NADPH oxidase (NOX) enzymes, can directly induce the coagulation cascade by upregulating TF expression in various cells such as monocytes and endothelial cells. By oxidatively altering proteins implicated in the coagulation process, ROS can also induce a procoagulant situation (17).

TF is the activator of the extrinsic pathway of the coagulation cascade, and its activity is sensitive to changes in membrane content, temperature, and pH (29). The increase in TF activity is related to the activation of the coagulative functions of tissues. In our study, we suggest that this situation is triggered by increased oxidative stress in the OLP. The increase in TF activity appears to be significant in OLP, where inflammation is important in pathogenesis. An autoimmune reaction is often suggested to be the cause of OLP, but some experts argue that microbial infection initiates the autoimmune response (30). Salivary cells are responsible for approximately 78% of the TF activity in saliva (9). Emekli-Alturfan et al. (6) demonstrated an inverse relationship between salivary TF activity and bacterial cell count, and suggested that the storage time of saliva is the reason for this negative association.

In our study, although no difference in LPO levels was found in the saliva samples of OLP patients, the increase in NO as a level of oxidative stress was notable. NO is an important free radical, and has proinflammatory and cytotoxic effects on human skin (25). Our result is compatible with the findings of Mehdipour et al. (31) who revealed increased NO levels in serum samples collected from patients with OLP, and suggested the activation of lymphocytes and the cellular immune system as a consequence. By increasing oxidant production and lowering antioxidant protection, the elevated NO in saliva may cause oxidative stress on reactive nitrogen species, which would tangentially relate to OLP. In addition, elevated NO causes tissue and cell damage, and studies have shown that elevated NO can damage oral epithelial cells, keratinocyte, and fibroblasts (32).

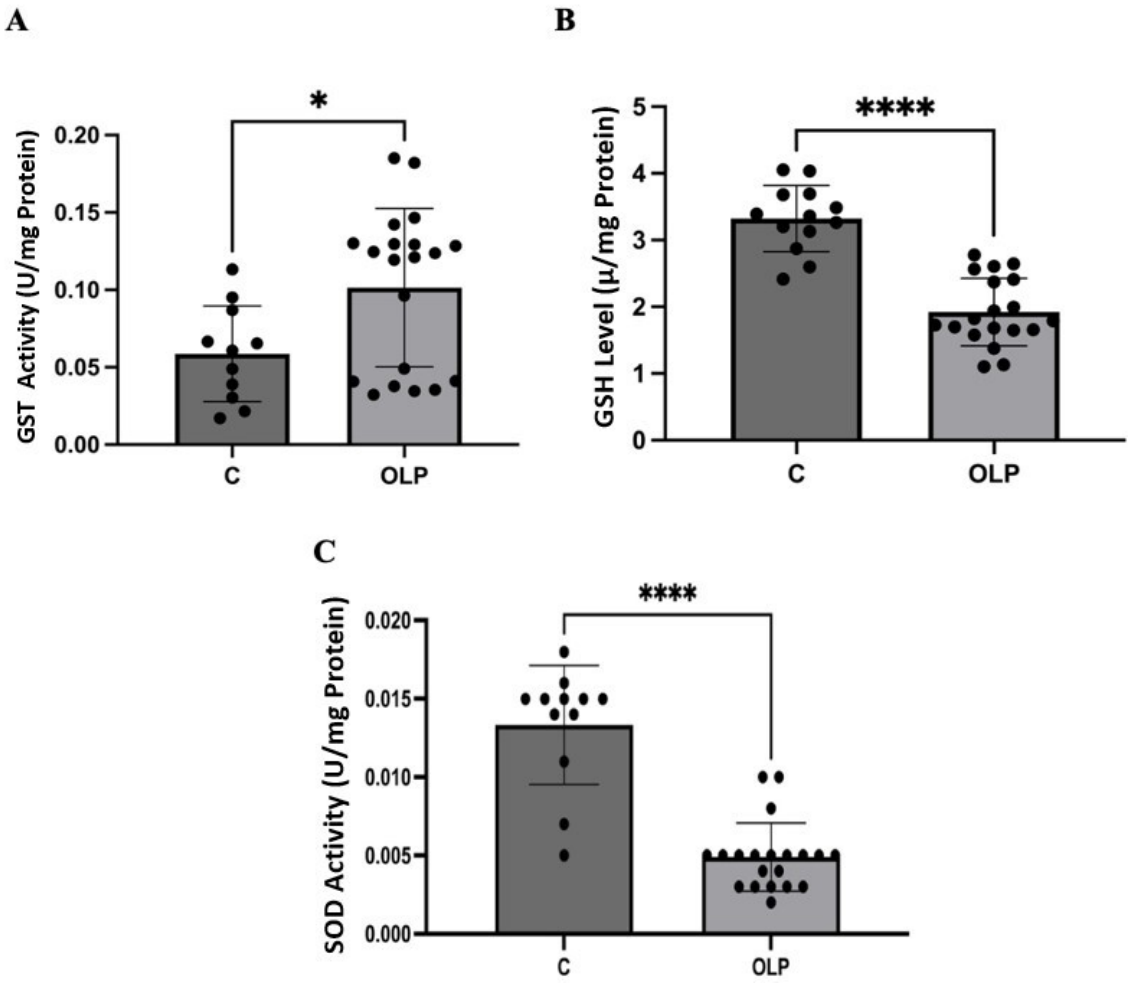

Figure 3 A. Glutathione S-transferase activities of the groups **B**. Superoxide dismutase (SOD) activities of the groups **C**. Glutathione (GSH) levels in the groups. Data are given as mean ± SD, * p<0.05; **** p<0.0001, SD: Standart deviation, C: Control group; OLP: Oral Lichen Planus

Increased oxidative stress in patients with OLP seriously compromises the integrity of cells by interfering with membrane fluidity, organization, crosslinking, and functioning and may influence the interactions between Bcl-2 and Bax, which in turn affects signal transmission and apoptosis (1, 33). The apoptotic cascade triggered by mitochondrial apoptotic signaling and oxidative stress begins with a decrease in cellular GSH content (34). In our study, the decreased GSH levels in the saliva samples of patients with OLP may be related to the triggering of apoptotic processes in these patients. Similar to our results, Hassan et al. revealed a decline in GSH levels in patients with OLP (25). Decreased GSH levels may cause a boost in hydrogen peroxide levels, which may damage the basal cell layer (25).

An array of electrophilic compounds can be conjugated with GSH through the action of GST enzymes, whose most well-known function is that of cell housekeepers involved in xenobiotic detoxification. It has recently been shown that GSTs also function as regulators of pathways of signaling that regulate the growth and death of cells. GST provides a protective effect against various toxic chemicals in cells (14). In contrast to the increased GST activity in our study, Jana et al. (28) reported decreased GST activity in OLP patients and suggested that the erosive LP is more under stress than the reticular form. The reason for the difference in the results of these two studies may be the low number of severe (erosive/ulcerative and atrophic) cases in our study. This may also be due to the triggering of the GST defense mechanism against increased oxidative damage.

ROS triggers pro-inflammatory and oxidative stress-associated conditions, as well as DNA damage, which further activates signaling pathways leading to cell death and apoptosis, creating a vicious cycle of damage (33). The SOD enzyme catalyzes the formation of hydrogen peroxide and molecular oxygen from superoxide radicals, making them less harmful. We found a decrease in SOD activity, which is the first defense

enzyme in the antioxidant system against oxidative stress, in patients with OLP. Similar to our findings, Shirzaiy et al. (35) reported decreased SOD activity in patients with OLP. However, Hassan et al. showed an increase in SOD activity in patients with OLP (25). It is useful to note that the decrease in LPO levels in our study may have been prevented by the SOD enzyme, leading to its consumption and decrease.

We propose that the alteration in TF activity may be related to the disturbed oxidant-antioxidant balance based on the data obtained in this study. Although our study supports the diagnostic importance of saliva in OLP disease, we believe that further studies involving the investigation of the hemostatic system and coagulation factors in the immune, ROS, inflammatory, and apoptosis-related pathways of OLP will clarify this issue.

The limited sample size and the absence of analysis of inflammatory cytokines in saliva are among the limitations of our study. In future studies, determination of the inflammatory cytokines and oxidative stress markers in saliva at both protein and gene levels and comparing them with the clinical findings of patients will contribute to elucidating the molecular mechanism of OLP.

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Author Contributions: Conception/Design of Study- D.C., S.E.Y., B.G., E.E.A.; Data Acquisition: M.B., I.U., G.E., Z.M., S.I., E.I.A.; Data Analysis/Interpretation: D.C., S.E.Y., B.G., E.E.A.; Drafting Manuscript- M.B., I.U., G.E., Z.M., S.I., E.I.A.; Critical Revision of Manuscript- D.C., S.E.Y., B.G., E.E.A.; Final Approval and Accountability- D.C., S.E.Y., B.G., E.E.A., M.B., I.U., G.E., Z.M., S.I., E.I.A.

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REFERENCES

1. Erçalık Ş, Gümrü-Tarçın B. Oral liken planus ve güncel tedavi yaklaşımları: Bölüm 8. Sağlık Bilimlerinde Güncel Araştırmalar Cilt 2. Eds. Cem Evereklioğlu C, Erten M. Gece Kitaplığı. 2022.p. 97-112.
2. Gumru B. A retrospective study of 370 patients with oral lichen planus in Turkey. Med Oral Patol Oral Cir Bucal 2013; 18(3): e427-32.
3. Streckfus CF, Bigler LR. Saliva as a diagnostic fluid. Oral Dis 2002; 8(2): 69-76.
4. Lee JM, Garon E, Wong DT. Salivary diagnostics. Orthod Craniofac Res 2009; 12(3): 206-11.
5. Alturfan AA, Alturfan EE, Dariyerli N, Zengin E, Aytac E, Yigit G, et al. Investigation of tissue factor and other hemostatic profiles in experimental hypothyroidism. Endocrine 2006; 30: 63–7.
6. Emekli-Alturfan E, Kasikci E, Alturfan AA, Pisiriciler R, Yarat A. Effect of sample storage on stability of salivary glutathione, lipid peroxidation levels, and tissue factor activity. J Clin Lab Anal 2009; 23: 93-8.
7. Emekli-Alturfan E, Basar I, Malali E, Elemek E, Oktay S, Ayan F, et al. Plasma tissue factor levels and salivary tissue factor activities of periodontitis patients with and without cardiovascular disease. Pathophysiol Haemost Thromb 2010; 37(2-4): 77-81.
8. Yarat A, Tunali T, Pisiriciler R, Akyuz S, Ipbuker A, Emekli N. Salivary thromboplastic activity in diabetics and healthy controls. Clin Oral Invest 2004; 8: 36–9.
9. Zacharski LR, Rosenstein R. Reduction of salivary tissue factor (Thromboplastin) activity by warfarin therapy. Blood 1979; 53: 366–74.
10. Emekli-Alturfan E, Demir G, Kasikci E, Tunali-Akbay T, Pisiriciler R, Caliskan E, et al. Altered biochemical parameters in the saliva of patients with breast cancer. Tohoku J Exp Med 2008; 214: 89-96.
11. Rekha VR, Sunil S, Rathy R. Evaluation of oxidative stress markers in oral lichen planus. J Oral Maxillofac Pathol 2017; 21: 387–93.
12. Darczuk D, Krzysciak W, Vyhouskaya P, Kesek B, Galecka-Wanatowicz D, Lipska W, et al. Salivary oxidative status in patients with oral lichen planus. J Physiol Pharmacol 2016; 67: 885-94.
13. Bonola-Gallardo I, Irigoyen-Camacho ME, Vera-Robles L, Campero A, Gómez-Quiroz L. Enzymatic activity of glutathione S-transferase and dental fluorosis among children receiving two different levels of naturally fluoridated water. Biol Trace Elem Res 2017; 176(1): 40-7.
14. Agha-Hosseini F, Mirzaii-Dizgah I, Farmanbar N, Abdollahi M. Oxidative stress status and DNA damage in saliva of human subjects with oral lichen planus and oral squamous cell carcinoma. J Oral Pathol Med 2012; 41: 736-40.
15. Mansourian A, Agha-Hosseini F, Kazemi HH, Mortazavi N, Moosavi MS, Beytollahi J et al. Salivary oxidative stress in oral lichen planus treated with triamcinolone mouthrinse. Dent Res J 2017; 14: 104-10.
16. Humberto JSM, Pavanin JV, Rocha MJAD, Motta ACF. Cytokines, cortisol, and nitric oxide as salivary biomarkers in oral lichen planus: a systematic review. Braz Oral Res 2018; 32: e82.
17. Bettiol A, Galora S, Argento FR, Fini E, Emmi G, Mattioli I, et al. Erythrocyte oxidative stress and thrombosis. Expert Rev Mol Med 2022; 24: e31
18. Ingram GI, Hills M. Reference method for the one-stage prothrombin time test on human blood. Thromb Haemostas 1976; 36: 237–8
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-75.
20. Yagi K. Assay for blood plasma or serum. Methods Enzymol 1981; 105: 328-37.
21. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001; 5(1): 62-71.
22. Habig WH, Pabst MJ, & Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249(22): 7130–9.
23. Mylorie AA, Collins H, Umbles C, Kyle J. Erythrocyte SOD activity and other parameters of copper status in rats ingesting lead acetate. Toxicol Appl Pharmacol 1986; 82: 512-20.

24. Beutler E. Gluthatione: Red cell metabolism a manual biochemical methods, 2nd ed. New York: Grune and Stratton, 1975. p.112–4.
25. Hassan I, Keen A, Majid S, Hassan T. Evaluation of the antioxidant status in patients of lichen planus in Kashmir valley–A hospital based study. J Saudi Soc Dermatol Dermatol Surg 2013; 17: 13–6.
26. Vlková B, Stanko P, Minárik G, Tóthová L, Szemes T, Baňasová L, et al. Salivary markers of oxidative stress in patients with oral premalignant lesions. Arch Oral Biol 2012; 57: 1651–6.
27. Ergun S, Troşala SC, Warnakulasuriya S, Ozel S, Onal AE, Ofluoglu D, et al. Evaluation of oxidative stress and antioxidant profile in patients with oral lichen planus. J Oral Pathol Med 2011; 40: 286–93.
28. Jana A, Thomas J, Ghosh P. Erosive oral lichen planus inflicts higher cellular stress than reticular type. J Oral Maxillofac Pathol 2021; 25(2): 279-85.
29. Tunali-Akbay T, Solmaz Z, Namdar Pekiner F, İpekci H. Salivary tissue factor concentration and activity in patients with oral lichen planus. Oral Sci Int 2017; 14(1): 13-7.
30. Villa TG, Sánchez-Pérez Á, Sieiro C. Oral lichen planus: a microbiologist point of view. Int Microbiol 2021; 24(3): 275-89.
31. Mehdipour M, Taghavi Zenouz A, Bahramian A, Gholizadeh N, Boorghani M. Evaluation of serum nitric oxide level in patients with oral lichen planus. J Dent (Shiraz) 2014; 15(2): 48-51.
32. Alamir AWH, Arakeri G, Patil S, Awan KH, Kujan O, Aljabab A, et al. Association of nitric oxide with oral lichen planus. J Oral Pathol Med 2019; 48(5): 345-50
33. Li X, Wang Z. Mechanisms of reactive oxygen species in oral lichen planus: A literature review. Europ J Inflam 2022; 20:1-9
34. Circu ML, Aw TY. Glutathione and apoptosis. Free Radic Res 2008; 42(8): 689-706.
35. Shirzaiy M, Salehian MA, Dalirsani Z. Salivary antioxidants levels in patients with oral lichen planus. Indian J Dermatol 2022; 67(6): 651-6.