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EVALUATION OF GLUTATHIONE PEROXIDASE (GPx) AND SUPEROXIDE DISMUTASE (SOD) SALIVARY LEVELS IN ORAL LICHEN PLANUS PATIENTS, DIABETICS, AND DIABETICS WITH ORAL LICHEN PLANUS

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ABSTRACT

Background: Oral lichen planus (OLP) is an autoimmune chronic muco-cutaneous disease that greatly affects the quality of life of LP patients with a progressive rate of malignant transformation. Diabetes Mellitus (DM) is assumed to be a globally affecting metabolic disorder that is exceedingly rising with a disturbing rate. Studies focusing on the association between LP and DM are still inconsistent. Objectives: This study aimed to analyze and compare the salivary levels of antioxidants GPx & SOD in the four groups of OLP, DM, OLP with DM and the healthy control, and to establish a correlation between the association of oral lichen planus with diabetes.

Materials and method: 44 subjects were enrolled in this prospective cohort study. The patients were divided into four groups, 11 in each group: healthy control patients (group A), OLP (group B), DM (group C) and OLP with DM (group D). The salivary levels of antioxidants GPx & SOD were analyzed and compared to establish a correlation between the association of OLP with DM.

Results: The mean GPx levels were highest in group A ,then, group C followed by group B, while group D had the lowest mean GPx level. The mean SOD levels were highest in group A followed by group C then group B while group D had the lowest mean SOD levels with insignificant difference between them.

Conclusion: Antioxidants play a vital role in human body, with a relationship existing between oxidative stress, hyperglycemia and cellular dysfunction.

KEYWORDS: Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD), Oral Lichen Planus (OLP), Diabetes, Diabetics with Oral Lichen Planus.

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INTRODUCTION

Oral lichen planus (OLP) is an inflammatory mucocutaneous disease in a chronic form which hits the scalp, nails, skin and mucous membranes along with oral and genital mucosae **(Katta et al., 2000)**, that occurs in 0.5% - 2% of the general population **(Alrashdan, Cirillo, & McCullough, 2016)**. The worldwide incidence of OLP shows higher incidence in African (1.43%) and South American (3.18%) populations **(Li et al., 2020)**.

Diabetes mellitus (DM) is a world health distress that imposes pathological impacts that are implicated in vasculature, inducing micro- and macro-vasculature complications. Definitely, DM correspondingly has been unswervingly bracketed together with oral lesions **(Fouani et al. 2021)**. DM levels are rising increasingly. It is considered a universal health issue where its prevalence is sky rocketing according to a study by **(Danaei et al., 2011, Ogurtsova et al., 2017)**, who narrated that diabetic patients with ages ranging from 20 to 79 have been around 415 million ones, with the death toll of 5 million suffering from the consequences of the disease, with global numbers of patients affected might reach 642 million in 2040 according to some international organizations **(Ramos-Garcia et al., 2021)**. Moreover, there were other studies revealing an association of DM with several types of malignancies as breast, pancreatic, endometrial, bladder and colorectal cancer **(Huxley et al., 2005; Larsson et al., 2005; Friberg et al., 2007; Larsson et al., 2007).**

One of the locations in the human body suffering from the consequences of DM is the oral mucosa. Patients might have OLP, gingivitis, halitosis and periodontitis, which are present frequently in patients suffering from diabetes in comparison with the general population **(Al- Maskari, et al., 2011)**. DM prevalence amongst patients with OLP fluctuates between 1.6% and 37.7% **(Otero Rey et al., 2019)**, and its incidence rises with age, especially after 40 years of age **(González-Moles et al., 2020)**. OLP is linked to low life quality, stress and unease **(Daume et al., 2020)**.

Studies focusing on the association between OLP and DM are inconsistent, some of them suggested a direct relation and risk ration between OLP and DM, with a great deal of them showed an association with a considerable variance **(Arduino et al., 2017)**. Though DM is a chronic metabolic disease presenting hyperglycemia clinically, is linked to general immune dysfunction **(Berbudi et al., 2019)**. The alliance of DM with OLP was first described by Grinspan **(Grinspan et al., 1966)**, with DM being part of the triad in in Grinspan Syndrome. Researches have proved that reactive oxygen species ROS are the culprit in both DM and oral diseases, with the free radicals resulting from oxidation of glucose and non-enzymatic glycation of plasma proteins resulting in damage to the cells rendering them susceptible to higher risk of infectious and inflammatory diseases of the oral cavity **(Maritim et al., 2003)**.

In the same context, a number of studies have shown the link between those free radicals and the pathogenesis of OLP. Where an imbalance occurs between the creation of these free radicals and the antioxidant defense system, leading to a condition known as oxidative stress (**Shirzad et al., 2014)**. Oxidative stress expresses a state where oxidants production surpasses the antioxidant capacity of the cell **(Handya and Loscalzo, 2022)**. The antioxidant mechanisms that take accountability for achieving balance between oxidation and reduction. Such a balance when broken through, leads to ROS being produced hugely and subsequent cell damage. Such an upsurge in the ROS and lipid peroxides were involved in the OLP pathogenesis **(Hassan et al., 2013)**.

From the different antioxidant defense systems contributing to protection of cells against free radicals is the glutathione system, a chief cellular,

water soluble antioxidant, encompasses certain and various group of enzymes responsible for cellular homeostasis versus oxidative stress. It is involved in multiple processes that aim for protection against additional reactive oxygen (ROS) and nitrogen (RNS) species **(Anderson and Stopper, 2021)**. There are exogenous and endogenous antioxidants, the endogenous ones consist of non-enzymatic antioxidants as proteins, glutathione and low molecular weight scavengers, while the enzymatic ones include glutathione peroxidase, catalase and superoxide dismutase. The exogenous ones comprise vitamin A, E, C and other compounds **(Pisoschi and Pop, 2015).**

Such enzymes as glutathione peroxidase (GPx) and superoxide dismutase (SOD), could possess a crucial protective role for cells against ROS **(Rekha et al., 2017)**. Glutathione shares in the cellular protection via glutathione peroxidase and radical chain termination. GPx acts on lipids and hydrogen peroxides producing non-toxic lipid alcohols and water respectively **(Anderson and Stopper, 2021)**. GPx is widely expressed in most tissues and could be detected in mitochondria and the cytosol. It is one of several cellular antioxidant enzymes, together with peroxiredoxins, and catalases, that reduce hydrogen peroxide **(Lubos et al., 2011)**. GPx, at a cellular level, oxidizes cellular glutathione (GSH) and reduces cellular hydrogen peroxide, thus influencing the thiol redox state and preserves balance among essential and damaging cellular oxidants levels **(Fourquet et al., 2010)**. SOD attains its key role as an antioxidant against ROS **(Noureen and Khan, 2021)**. Estimation and monitoring of the antioxidant defense system elements such as the GPx and SOD in saliva represents a non-invasive substitute for the surgical option approached for biopsy and histopathology **(Darczuk et al., 2019)**.

OLP has definitely been linked to specific systemic diseases as DM along with other diseases **(Lauritano et al., 2016)**, yet the relationship between OLP and these systemic conditions remains controversial **(Dave et al., 2021)**. A recent study by **Sun et al. (2024)** concluded that DM is linked to OLP, with higher prevalence in diabetic patients than non-diabetic ones.

Our study aimed to analyze and compare the salivary levels of antioxidants GPx &SOD in OLP, DM, OLP with DM sample groups and the healthy control ones, and establish a correlation between the association of oral lichen planus with diabetes.

PATIENTS AND METHODS

Study design: This study is a prospective cohort study.

Setting: Participants were recruited among patients referred to the department of oral medicine, faculty of dentistry, Cairo university.

Inclusion criteria:

- Patients who were diagnosed with any form of oral lichen planus.
- Patients with type 2 diabetes mellitus (whether controlled or uncontrolled).
- Male or female patient with age range between 20-75 years old.

Exclusion criteria:

- Smokers.
- Pregnancy.
- The use of antioxidants within four weeks prior to enrolment in the study.
- Active liver diseases
- Active cancer.

Ethical consideration: After confirmation of the diagnosis, patients who met the inclusion criteria were enrolled in the study after signing an Arabic approval consent form by the willing participant in both groups. In which treatment plan, patient's education with all the data needed and

complications that could be met were discussed. Those meeting all the inclusion criteria and none of the exclusion criteria were included in this study, the study protocol and consent form was approved by the Research Ethics Committee, Faculty of Dentistry, Cairo University (IEC/ 30-9-23). After explanation of all aspects of the study and the available alternative treatments, a signed consent form was obtained from all patients.

Sample Size: Sample size calculated depending on a previous study **(Shirzaiy et al., 2022)** as reference. According to this study, the minimally accepted sample size was 11 per group, when the response within each subject group was normally distributed with Mean ± standard deviation of GPx of healthy group was 1224.69 ± 1020.70, while the mean ± standard deviation of OLP group was 261.64±270.96, with 1.28 effect size when the power was 80% & type I error probability was 0.05. Sample size was calculated by using Independent t test which was performed by using G. Power 3.1.7.9. A total sample size of 44 participants were included and divided equally into 4 groups; A, B, C& D, in which each group included 11 participants. They were divided as follows:

Group A, the control group: This group included only healthy participants who were medically free and not suffering from neither oral lichen planus nor diabetes for comparison with the other groups.

Group B, Oral lichen planus patients: This group included patients who were clinically diagnosed with any form of oral lichen planus, where symptomatic form were treated as per the protocol of treatment followed in the oral medicine department.

Group C, Diabetic patients: This group included only diabetic patients whether controlled or uncontrolled. Glycosylated hemoglobin's level test was required from all diabetic patients to evaluate whether the patients are controlled or not.

Group D, Diabetic Patients with oral lichen planus: This group included patients who were suffering from the presence of both oral lichen planus and diabetes. Symptomatic form of oral lichen planus was treated as per the protocol of treatment followed in the oral medicine department.

Clinical examination: Intraoral examination was performed for all participants using visual and tactile examination technique to examine lips, tongue, gingiva, hard palate, soft palate, labial mucosa and buccal mucosa. Altogether with, intraoral photographs were taken for any patient with oral lichen planus.

Pain and OLP clinical score recording for OLP patients: Pain was be recorded and graded by numerical rating scale (NRS) which consists of a 10-cm horizontal line between extremities with (0) indicating no pain and (10) for unbearable pain. Moreover, the size of the lesions was estimated by Thongprasom scale (TS) **(Thongprasom et al., 1992)**.

Salivary sample collection: Unstimulated salivary samples were collected from all participants. Saliva was collected in clear plastic Eppendorfs and was divided equally for evaluation of both GPx & SOD levels. The samples were stored and processed at_the biochemistry department at the Faculty of Medicine and were disposed there_per protocol followed at the department labs.

After collecting the saliva, the sample was transmitted to biochemistry laboratory as soon as possible. The salivary concentration of superoxide dismutase and glutathione peroxidase was examined in these individuals using the spectrophotometry and coulometric technique. In the laboratory, saliva samples were centrifuged for 10 minutes at 2000 Xg to separate cell debris and were kept at -80 °C. Based on the protocol presented by the kit manufacturer's company, spectrophotometric and coulometric methods were used to determine the types of salivary antioxidants. Available commercial kits

for superoxide dismutase (SOD; Ransod; Randox Laboratories Ltd, UK); glutathione peroxidase (Ransod; Randox Laboratories Ltd, UK were used.

Estimation of SOD:

Fifty microliters (50 µl) of saliva samples were added to a test tube containing 3 ml of the reaction mixture (50 mM potassium phosphate buffer [7.8], 45 µM methionine, 5.3 mM riboflavin, 84 µM nitroblue tetrazolium [NBT] and 20 µM potassium ferric cyanide). SOD activity was analyzed by the reduction of NBT by superoxide, which formed formazan and detected spectrometrically at 560 nm using ultraviolet spectrophotometer and expressed in terms of U/ml.

Estimation of glutathione peroxidase

Fifty microliters of saliva samples were added to a test tube containing 3 ml of reaction mixture (1 mM of β-NADPH+1 mM sodium azide solution, 200 mM reduced glutathione). Mixed by inversion and equilibrated to 25°C and monitored the absorbance at 340 nm until constant. The tube containing 3 ml reaction mixture and 50 µl of phosphate buffer (pH 7) was taken as blank. 50 µl of 0.042% of hydrogen peroxide was added to these tubes. Immediately mixed by inversion and recorded the decrease in absorbance at 340 nm for approximately 5 min.

The enzyme GPx catalyzes the oxidation of reduced GSH to oxidized form, which reacts with NADPH and gets converted to oxidized form of NADP and two molecules of reduced glutathione and is measured spectrophotometrically at 340 nm.

Statistical Analysis

Numerical data were explored for normality by checking the data distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data were presented as mean & standard deviation. Data were collected, tabulated, and statistically analyzed using Microsoft Excel ® 2016, Statistical Package for Social Science (SPSS)® Ver. 24. and Minitab ® statistical software Ver. 16.

RESULTS

The present study aimed to analyze and compare the salivary levels of antioxidants GPx & SOD in the four groups of OLP, DM, OLP with DM and the healthy control, and to establish a correlation between the association of oral lichen planus with diabetes.

The levels of GPx and SOD were measured in saliva of the designated patients. Data was collected from 11 patients with OLP, 11 patients with diabetes, 11 with both OLP and diabetes and 11 healthy individuals, including a total of 20 males and 24 females. Statistical analysis was performed with SPSS 16 ® (Statistical Package for Scientific Studies), GraphPad prism & Microsoft Office and presented in 5 tables. Data of all groups were presented as mean &standard deviation. Exploration of the given data was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality which revealed that all data originated from normal distribution. Accordingly, comparison between different groups was performed by using One Way ANOVA test followed by Tukey`s Post Hoc test for multiple comparisons. Comparison between two groups was performed by using Independent t test and Pearson's correlation coefficient was used in all correlation.

Concerning the age of patients, there was an insignificant difference between groups as P=0.052, as the age in group A was 44.82 ± 12.99 years, while it was higher for group B with 57.45 ± 10.85 years and group C with 56.4 ± 12.13 years. Group D had a mean age of 46.73 ± 14.62 years.

The gender distribution was similar across groups A, B, and C, with approximately 54.5% males and 45.5% females in each group. However, group D had a higher proportion of females (81.8%) compared to males (18.2%), with insignificant difference between groups as P= 0.22.

As for diabetes control: For group C and group D, the table presents the medical history of diabetes control. In both groups, approximately half of the participants had controlled diabetes (54.5% in group C and 45.5% in group D), while the remaining half had uncontrolled diabetes (45.5% in group C and 54.5% in group D), in both groups. There was insignificant difference between them a P=0.81. Table 1 presents demographic data for the four different groups: group A, group B, group C, and group D.

GPx and SOD in all groups:

Table 2 presents the mean and standard deviation values of GPx and SOD in all groups:

GPx: The mean GPx levels were highest in group A with 1378.18 ± 126.48, significantly different from all other groups. Then, group C with 451.5 ± 205.2 (superscript 'c'), followed by group B with 280.64 ± 22.79, while group D had the lowest mean GPx level of 168.82 ± 25.66. The p-value of 0.0001 indicates a statistically significant difference in GPx levels among the groups.

SOD: The mean SOD levels were highest in group A with 172.45 ± 36.37, followed by group C with 145.6 ± 46., while groups B 126.64 ± 29.66 and D 117.27 ± 37.17 had lower mean SOD levels with insignificant difference between them. The p-value of 0.009 suggests a statistically significant difference in SOD levels among the groups.

Pain and OLP clinical scores in groups B and D:

Table 3 compares the mean pain scores and OLP clinical scores between group B and group D.

TABLE (1) Demographic data of all groups and comparison between them:

		Group A (control)	Group B (Oral lichen planus)	Group C (Diabetes)	Group D (Oral lichen planus + diabetes)	P value
Age M ± SD		44.82 ± 12.99	57.45 ± 10.85	56.4 ± 12.17	46.73 ± 14.62	0.052
Gender	Male N (%)	6 (54.5%)	6 (54.5%)	6 (54.5%)	2 (18.2%)	0.22
	Female N (%)	5 (45.5%)	5 (45.5%)	5 (45.5%)	9 (81.8%)	
Med Hx.	Controlled diabetes N (%)	-----	-----	6 (54.5%)	5 (45.5%)	0.81
	Uncontrolled diabetes N (%)	-----	------	5 (45.5%)	6 (54.5%)	

TABLE (2) Mean and standard deviation of GPX and SOD in all groups and comparison between them:

	Group A (control)		Group B (Oral lichen planus)		Group C (Diabetes)		Group D (Oral lichen planus + diabetes)		P value
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	
GPx	1378.18 [a]	126.48	280.64 [b]	22.79	451.55 [c]	205.22	168.82 [b]	25.66	0.0001*
SOD (U/ml)	172.45 [a]	36.37	126.64 [b]	29.66	145.68 [ab]	46.96	117.27 [b]	37.17	0.008*

Significant difference as P<0.05.

Means with different superscript letters were significantly different as P<0.05.

Means with the same superscript letters were insignificantly different as P>0.05.

Pain Score: group B (6.91 ± 1.58) was insignificantly lower than group D (7.36 ± 1.12) with mean difference (0.45), The 95% confidence interval for the mean difference ranged from -1.67 to 0.76 with p-value = 0.45.

OLP clinical score: group B (3.91 ± 0.94) was insignificantly higher than group D (3.64 ± 0.81) with mean difference (0.27). The 95% confidence interval for the mean difference ranged from -0.51 to 1.05, with p-value = 0.48.

Correlation between GPx, SOD and pain score, OLP clinical score in groups B and D:

Table 4 presents the correlations between the levels of GPx and SOD with pain and OLP clinical scores in group B and group D.

In group B: There was a significant negative correlation between GPx levels and pain scores (r = -0.679, p = 0.021), indicating that higher GPx levels were associated with lower pain scores. However, there was no significant correlation between GPx levels and OLP clinical scores (r = -0.062, p = 0.856). Also, there was no significant correlation between SOD levels and pain scores (r = 0.281, p = 0.402) or OLP clinical scores (r = 0.006, p = 0.986).

In group D: There was insignificant negative correlation between GPx levels and pain scores (r=-0.589, p = 0.057) and OLP clinical scores (r =-0.591, p =0.055). There was insignificant negative correlation between SOD levels and pain scores (r = -0.250, p = 0.458) or OLP clinical scores (r =-0.103, p=0.764).

GPx and SOD in controlled and uncontrolled diabetes in groups C and D:

Table 5 compares the mean and standard deviation of GPx and SOD levels between controlled and uncontrolled diabetes patients in group C and group D.

Group C: In patients with controlled diabetes, the GPx level (620.8±89.03) was significantly higher than in patients with uncontrolled diabetes (248.4±28.4) as p=0.0001). Similarly, the mean SOD level was higher in controlled diabetes patients (167.9±32.7) compared to uncontrolled diabetes patients (119±50.4), and this difference was statistically significant (p = 0.01).

TABLE (3) Mean and standard deviation of pain score and OLP clinical score in group B and group D:

	Group B (Oral lichen planus)		Group D (Oral lichen planus + diabetes)		Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		P value
	Mean	Standard Deviation	Mean	Standard Deviation			Lower	Upper	
Pain score	6.91	1.58	7.36	1.12	-0.45	0.58	-1.67	0.76	0.45
OLP clinical score	3.91	.94	3.64	.81	0.27	0.37	-0.51	1.05	0.48

TABLE (4) Correlation between GPx and SOD with Pain and OLP clinical scores in both groups B and D:

		Correlations			
		Pain score		OLP clinical score	
		Correlation	P value	Correlation	P value
Group B (Oral Lichen Planus)	GPx	-0.679*	0.021	-0.062	0.856
	SOD (U/ml)	0.281	0.402	0.006	0.986
Group D (Oral Lichen Planus + Diabetes)	GPx	-0.589	0.057	-0.591	0.055
	SOD (U/ml)	-0.250	0.458	-0.103	0.764

TABLE (5) Mean and standard deviation of GPx and SOD in controlled and uncontrolled diabetes in groups C and D:

	Group									
	Group C					Group D				
	Controlled		Uncontrolled		P value	Controlled		Uncontrolled		P value
	Mean	Standard Deviation	Mean	Standard Deviation		Mean	Standard Deviation	Mean	Standard Deviation	
GPx	620.83	89.03	248.40	28.44	0.0001*	180.8	28.61	158.83	19.97	0.58
SOD (U/ml)	167.92	32.73	119.00	50.42	0.01*	145	17.68	94.17	33.23	0.02*

Significant difference as P<0.05

Group D: In patients with controlled diabetes, the GPx level (180.8 ± 28.61) was slightly higher than in patients with uncontrolled diabetes (158.83±19.97), but this difference was not statistically significant (p = 0.58). However, the mean SOD level was significantly higher in controlled diabetes patients (145.0±17.68) compared to uncontrolled diabetes patients (94.17±33.23), with a p-value of 0.02.

DISCUSSION

Saliva is one of the diagnostic tools serving as a non-invasive diagnostic fluid **(Spielmann and Wong, 2011)**. It assists in the diagnosis of many diseases sparing the patients the difficulties of blood sample collection and it correlates well with serum levels **(Singh et al., 2014)**. Saliva is considered the primary defense line versus oxidative stress, the main culprit of many oral and systemic diseases. The oral free radicals and ROS are produced from periodontitis, tobacco and other oral illnesses. Thus, the presence of salivary antioxidants represents the antioxidant potential of saliva **(Miricescu et al., 2011)**.

Our search studied the saliva of OLP, diabetics and patients with both diseases. We aimed to investigate the levels of GPx and SOD and correlate the association of OLP with diabetes. Oxidative stress and ROS are indeed involved in the pathogenesis of OLP **(Anshumalee et al., 2007)**. Moreover, it is established that free radical oxidation caused by free radicals with elevated chemical activity is one of the chief pathogenetic mechanisms of diabetes mellitus development **(Cheprasova et al., 2022)**.

Our study showed an insignificant difference between the groups regarding age, and gender distribution, which is coherent with a previous study by **Rezazadeh et al. 2023** who declared no association between age and gender with antioxidants level in OLP patients. As for diabetes control, there was insignificant difference between them.

Concerning the levels of GPx in the studied groups, our study revealed that there was a highly statistically significant difference among all groups, with group D having the lowest mean GPx level, with groups C and B in between them respectively.

This is in accordance with **Tunali-Akbay et al. 2017** who showed that glutathione (GSH) and the total antioxidant capacity accumulation were minimal in OLP patients. **Miricescu et al. 2011** demonstrated that oxidative stress has decreased the level of antioxidants intraorally, especially OLP patients than in the control groups. **Hassan et al. 2013** revealed that plasma GPx had lower levels

in OLP patients than control. **Shirzaiy et al. 2022** declared that GPx levels were extremely reduced in OLP patients compared to control group. **Jia et al. 2020** mentioned in their systematic review that oxidative stress markers elevated and antioxidant levels got depleted in OLP patients, hence, **Wang et al. 2021** elucidated the crucial role of oxidative stress in the pathogenesis of OLP, however it is still obscure whether oxidative stress is an etiology or consequence of OLP.

This could be explained by the fact that there is an imbalance between pro- oxidation versus anti- oxidation substances via antioxidants in OLP patients, where it is already established that there is a definite link between OLP and oxidative stress. Thus, antioxidants could have the ability for neutralization of the harmful damage caused by oxidative stress and its related diseases. They can restore the deleterious effects caused by free radicals through inhibiting their production or scavenging them. **Wang et al. 2021** postulated that antioxidants have the potential of reducing the interaction between inflammatory factors and free radicals in patients with OLP, cutting down the ROS production, thus restoring the cellular damage and improving the clinical state of patients.

Rekha et al. mentioned in their study in **2017**, that in a state of oxidative stress, the ratio of reduced glutathione GSH/oxidized glutathione GSSG is altered, whilst GPx consumes GSH rapidly. That mechanism might not happen rapidly enough in the prolonged presence of higher concentrations of H2O2 due to low GSH levels plus the deleterious effects of free radicals on GPx and GSH, hindering and the diminishing GPx activity. Taken together, in their study, they attributed the lower levels of GPx in saliva to higher H2O2 concentrations in the lesions.

Studies by **Anshumalee et al. 2007** and **Aly and Shahin 2010** showed that cytokines and T- cells inflammatory infiltration in OLP patients induce the production of ROS, with their high toxic levels that can upregulate the expression of intercellular adhesion molecule (ICAM)-1 thus damaging endothelial cells, which subsequently promotes T lymphocytes recruitment in the inflammatory infiltration site, resulting in a reciprocal effect. Moreover, those free radicals have the ability to activate nuclear factor-ϰB that regulates the inflammatory factors TNF-α and IL-2 expression and transcribed receptor genes IL-2 and MHC-I, thus having a crucial role in OLP development and progression. In addition to TNF-α that can bring about the synthesis of superoxide anion (O2–) and hydrogen peroxide (H2O2) in epidermal keratinocytes. Taken together, those findings show that high levels of ROS can upregulate inflammation via immune mechanisms leading to development of OLP. Thus **Bao et al. 2022** concluded that treatment with antioxidants might be a valuable approach for OLP patients.

SOD is an antioxidant enzyme that promotes the dismutation of toxic superoxide radicals generated throughout the oxidative processes into molecular oxygen and hydrogen peroxide **(Koca et al., 2004)**. Our study showed mean SOD levels were highest in the control group, followed by the diabetic patients with OLP, while the OLP and diabetic patient groups were lower in their means with insignificant difference between them. This is in agreement with **Jingyan et al. 2001** who revealed that levels of serum SOD have been significantly lower in patients with OLP prior to treatment compared to healthy controls. **Jana et al. 2021** mentioned that salivary SOD among other enzymes showed depletion in the OLP study group.

However, this was in contrast with a study by **Aly and Shahin 2010** who declared that SOD levels were significantly higher in OLP patients compared to control group. Another study by **Sezer et al. 2007** showed that concentration of SOD increased in OLP patients in comparison with healthy controls, they added that in OLP patients, the up rise in the oxidative stress and the consequent imbalance in the antioxidant defense system can in

fact participate in OLP pathogenesis. **Vidhya et al. 2023** noted significantly higher SOD levels tissue samples in OLP patients compared to the control cases. They explained the higher SOD levels with the fact that SOD is the weapon against free radicals by conversion of $O2^-$ to H2O2, which accumulates causing vacuolization in the basal cells of OLP. With CAT being the principal enzyme built for removal of H2O2, which is produced by superoxide anion radicals through SOD. With an imbalance present in the antioxidant system, and accumulation of H2O2 resulting in degeneration of basal cells. This could be why SOD tissue levels were higher than healthy controls.

The different levels of antioxidants in the OLP groups, B and D, could be explained by the presence of different forms of OLP, with the erosive subtype having the lower levels. studies proved that erosive forms of OLP suffer from higher rates of oxidative stress than reticular forms. The oxidant-antioxidant control checked by estimating these parameters in saliva of OLP patients, might possess an important role in the disease prevention and progression. Thus, therapy by antioxidants is evolving with a new importance not just as a temporary anti-inflammatory but via its prolonged positive effects **(Darczuk et al., 2019)**. Moreover, group D which showed the least mean levels of antioxidants could be explained by the putative link between OLP and diabetes that originates from the existence of common histocompatibility antigens (HLA) particularly HLA28 in both illnesses. Moreover, high expressions of IL-8 in serum was observed in diabetes and OLP whether associated or independent, which enforces the hypothesis of the relationship amongst them **(Tavangar et al., 2016)**.

According to a study by **Sun et al. 2024**, DM is associated with OLP, since there has been more prevalence of OLP in type 2 diabetic patients than those non-diabetic ones. The diminished levels of GPx might be explicated by the lower percentage of GSH in patients with diabetes, as GSH supposed to be a cofactor and substrate of GPx **(Domingues et al., 1998)**. Inactivation of enzymes would add to the lower activity of GPx, which is a fairly stable enzyme that might be inactivated by critical oxidative stress, or, via glycation controlled by predominant concentration of glucose causing an effect on the amino acids next to the enzyme's active sites leading to structural and functional variations in the molecule. In addition to H2O2 accumulation which further lowers the activity of GPx leading eventually to an ongoing decline in SOD in advanced stages of diabetes **(Nobar et al., 1999)**.

With regards to pain score estimation in groups B and D, or study showed that the mean of group B was insignificantly lower than that of group D, while the mean OLP clinical score of group B turned out to be insignificantly higher than group D. Considering the correlation between the levels of GPx and SOD with pain and OLP clinical scores, group B showed a significant negative correlation between GPx levels and pain scores indicating that higher GPx levels were associated with lower pain scores, which show that antioxidants might decrease the pain associated with symptoms of OLP and improving the patients condition, suggesting that antioxidants might be a valuable remedy for OLP patients. However, we found insignificant negative correlation between SOD and pain score. Regarding OLP clinical score, there was insignificant correlation between GPx and SOD levels and OLP clinical scores. On the other hand, in group D, we found insignificant negative correlation between GPx levels and pain scores which implies also the beneficial effect of antioxidants on pain symptoms of OLP, and no significant correlation was found between SOD levels and pain scores or OLP clinical scores.

Studies proved the efficiency of oral antioxidants and antioxidant medicaments in suppressing high levels of oxidative stress, therefore aid in improving the clinical state of OLP patients **(Rivarola de Gutierrez et al., 2014)**. A systematic review and meta-analysis by **Bao et al. 2022** revealed that treatment with antioxidants might indeed decrease the pain and clinical scores and improving the

clinical state of patients with OLP. They added that the meta-analysis of adverse effects revealed that the difference in the antioxidant group against placebo group, and, conventional treatment group against conventional +antioxidant group was statistically non-significant. They concluded that antioxidants are considered safe and efficient in OLP treatment.

Concerning diabetes control, our study showed that patients with controlled diabetes in group C had significantly higher GPx level than uncontrolled ones, similarly SOD level was significantly higher in controlled diabetes patients compared to uncontrolled diabetes ones. In group D, GPx and SOD levels were higher in controlled diabetes patients than uncontrolled ones with the SOD mean level being statistically significant. This could be explained by the high oxidative stress levels and diminished antioxidant capacity that relates to the complications of type 2 diabetes patients. **Hisalkar et al. 2012** reported that the levels of plasma antioxidants in uncontrolled diabetics were significantly lower than controlled ones. Our study showed lower means of GPx and SOD in group D which might be attributed to the combined effect of higher levels of oxidative stress and hyperglycemia in diabetic patients with its subsequent effects.

CONCLUSION

The findings of our study indicate that saliva, being non-invasive substitute for serum, could be a potential tool for monitoring and treatment of OLP and other oxidative stress related diseases as diabetes. Antioxidants play a vital role in human body, with a relationship existing between oxidative stress, hyperglycemia and cellular dysfunction. However, we recommend using larger sample size and further researches for more confirmation of the presented results.

Conflicts of interest

The authors declare no conflict of interests.

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