Original Article

Salivary and serum estrogen level assessment in oral lichen planus patients and its correlative analysis with OLP and stress

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Abstract

Background: Lichen planus is a chronic inflammatory disease of the skin and mucous membrane with higher predilection seen in the female population. Oral lichen planus (OLP) has been associated with various etiological factors, such as stress, hormonal imbalance, and immunological variation. The purpose of this study was to assess serum and salivary estrogen (E2) levels in OLP patients and correlate them with stress levels. **Objectives:** This study aimed to evaluate serum and salivary estrogen levels in female patients with OLP, along with the assessment of stress and its correlation with estrogen levels. **Methods:** A total of 78 females, 39 clinically diagnosed with OLP and 39 healthy females, were included in the study as the case and control groups, respectively. 2 ml each of salivary and serum samples was obtained from each participant to measure the estrogen levels. Stress levels in the study group patients were assessed using the Depression Anxiety Stress Scale (DASS-21) and the Perceived Stress Scale (PSS). The nonparametric Mann–Whitney test was used for intergroup comparisons. **Results:** Significantly higher serum estrogen levels with higher DASS-21 and PSS scores were noted in patients with OLP. Overall, significant positive correlations were observed between salivary E2 and serum E2 ($r = 0.361$, $P = 0.001$). There was a positive correlation between salivary and serum E2 and DASS score ($r = 0.410$, $P < 0.001$, and $r = 0.768$, $P < 0.001$, respectively), serum/salivary E2 and PSS score ($r = 0.745$, $P < 0.001$, and $r = 0.410$, $P < 0.001$, respectively), and DASS score and PSS score ($r = 0.878$, $P < 0.001$). **Conclusion:** Estrogen can be used as a useful biomarker for OLP in the future. Salivary samples can prove to be an accurate and feasible alternative to serum estrogen level determination. We also suggest that OLP patients must be given supportive psychological treatment for improved life quality and disease management.

Keywords: Oral lichen planus, salivary estrogen, serum estrogen, stress

Introduction

Lichen planus is a chronic inflammatory disease of the skin and mucous membrane. The mucosal analog of cutaneous lichen planus, oral lichen planus (OLP), manifests often in the fourth decade, affecting females in a ratio of 1.4:1. The fraction of the population affected by the disease is approximately 1–2%.[1] Its prevalence is evaluated at 2.6% among the Indian population.[2]

Patients often complain of a burning sensation along with difficulty tolerating certain food items and toothpaste, resulting in a compromised quality of life.[3]

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The definite etiology of OLP is still not known. Stress is seen as an important contributing factor, as patients frequently report stressful experiences a few months before the clinical symptoms.[2] Psychological stressors are reported to have a significant relationship with the development and progression of chronic diseases, with disruption of the immune system by altering Th1/Th2 cytokine balance, being one of the proposed mechanisms.[4]

Various scales have been used to assess the psychological profile such as Depression Anxiety Stress Scale-21 (DASS-21) and Perceived Stress Scale (PSS). DASS-21 is used for assessing the emotional state of depression, anxiety, and stress and contains three self-report scale set.[5] PSS measures how much one perceives their current circumstances as stressful. PSS includes questions regarding emotions and thoughts during the last month.[6] Using both of these scales together, one can measure chronic or acute stress precisely.

It has traditionally been said that there are both specific and nonspecific pathways hypothesized to be involved in OLP pathogenesis. Specific pathways emphasize the crucial role of cytotoxic and helper T lymphocytes, whereas mast cells, chemokines, epithelial basement membrane, and matrix metalloproteinases are believed to mediate nonspecific pathways.[7] Specific mechanism includes stimulation of cluster of differentiation (CD) 8 + cytotoxic and CD4 + helper T lymphocytes after antigen presentation by Langerhans cells and keratinocytes. Interferon-γ (IFN-γ) and interleukin-2 (IL-2) are secreted by activated T helper cells, which further activate and promote the proliferation of cytotoxic T lymphocytes. The activated cytotoxic T cells promote apoptosis, leading to liquefaction degeneration of epithelial cells of the basal layer, a typical feature of OLP lesions.[8] It is unknown what the lichen planus antigen is; however, it could be a self-peptide.[9]

Autoimmunity and neuroendocrines also have an imperative role in the pathogenesis of OLP. A higher incidence of OLP in females highlights prominent role of sex hormones in its incidence. Estrogen fluctuations are common throughout the lifespan of a female. Estrogen levels not only regulate female sexual functions but also play many important roles in different systems as well. It is closely related to the biobehavioral aspects of women. The biobehavioral pathways are linked to stress and illness. In primary care centers, various females report different types of oral and systemic illnesses due to this fluctuating estrogen, so it is important to evaluate the hormonal status of a female.

Estrogen has been seen to modulate major immune cells.[4] It has been observed that estrogen inhibits particular subsets of thymocytes and inhibits a number of cell-mediated immune responses.[10] Few studies associate OLP with decreased estrogen levels, but it has not been confirmed yet.[11] Contrary to this, few studies suggest that an increased level of estrogen modulates autoimmunity. Inflammatory cytokines, such as IL-1, IL-6, and TNF-α, stimulate the aromatase enzyme in the peripheral tissues, which causes the conversion of androgens to estrogen, resulting in increased levels of the hormone in autoimmune diseases. Thus, we can presume that the role of estrogen presents an intriguing dichotomy.[4]

Because sex hormones are lipid-soluble and weakly linked to serum proteins, they can diffuse across cell membranes. Therefore, it is anticipated that salivary steroid concentrations will reflect unbound serum steroidal levels, making them a more accurate indicator of the exposure of target cells to steroid hormones than serum concentrations.[12]

Considering the modulating role of estrogen in OLP, we conducted the study to assess estrogen levels in OLP patients, taking both serum and salivary samples. As stress can be seen as a confounding variable affecting both the occurrence of OLP and fluctuating estrogen levels, we also took stress levels into account in our study.

Aims and Objectives

1. To assess the salivary and serum estrogen levels in the control and case groups
2. To correlate the levels of salivary and serum estrogen in both groups
3. Assess stress levels in both groups
4. To correlate levels of salivary and serum estrogen with stress.

Materials and Methods

This study was conducted from January 2021 to June 2022 in the Department of Oral Medicine and Radiology in collaboration with the Department of Pathology.

Study Design: An observational case–control design was used for the study.

Participants

After obtaining appropriate approval from the institutional ethical committee (reference code: VI-PGTSC-IIA/P32), along with written approval consent from the subjects, the study comprised the following two groups:

1. **Group I:** The case group consisted of 39 females clinically diagnosed with OLP.
2. **Group II:** The control group consisted of 39 age- and gender-matched healthy females.

Clinical diagnostic criteria

The modified World Health Organization (WHO) diagnostic criteria for OLP (2003) (clinical criteria) were used to diagnose OLP. It is as follows:

1. Presence of gray-white lines in a lace-like network that are slightly elevated (reticular pattern) [Figures 1].

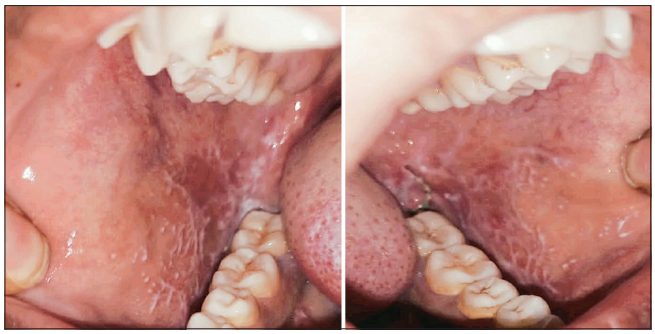


Figure 1: Reticular-type OLP lesion

2. Presence of bilateral lesions that are somewhat symmetrical.
3. Plaque-like, atrophic, erosive, and bullous lesions are accepted only as a subtype in the presence of reticular lesions elsewhere in the oral mucosa.

Sampling

Two groups of 39 subjects each were included as healthy control and OLP case groups. The sample size calculation was calculated based on the prevalence of OLP, with 2.6% in the Indian population (Gupta *et al.*, 2017),[13] and 95% level of confidence and error rate, usually set at the 0.05 level, is 4.

$$n = Z^2P\ (1\text{-}P)/d^2$$

where

- n = sample size.
- Z = Z-statistic for a level of confidence; for the level of confidence of 95%, which is conventional, the Z-value is 1.96.
- P = expected proportion or prevalence (in proportion of 1; if 2.6%, $P = 0.026$).
- d = precision (in proportion of 1; if 5%, d = 0.05).

$\mathbf{n} = 1.96 \times 1.96 \times 0.026 \times 0.974/0.05^2$.

n = 39.

Inclusion criteria

1. Female subjects of reproductive and postmenopausal age (16–55 years) with clinically diagnosed OLP.
2. Age- and gender-matched healthy control group

Exclusion criteria

1. Patients taking any medications for the last few months
2. Pregnant patients
3. Patients on oral contraceptive pills
4. Patients with any other skin disorder and systemic disorder
5. Patients not willing to participate in this study

Collection of saliva

2 ml of whole unstimulated saliva was collected in the morning between 8 and 10 am with a simple spit method. The subjects were instructed to rinse their mouths with tap water and subsequently asked to spit into sterile containers. After collection, samples were stored at -20°C until analyzed.

Collection of serum

2 ml of blood was withdrawn from the antecubital vein. The samples were then centrifuged at 3000 rpm for 10 mins. The supernatants were drawn off and stored in tube-like containers at -80°C until analyzed.

Stress analysis

Stress levels in the study group patients were assessed using the DASS-21 and PSS.

Statistical analysis

The nonparametric Mann–Whitney test was used for intergroup comparisons.

Depending on the type of variable, the result measures for various variables were summed up as mean ± standard deviation (SD) and proportions and percentages. Correlation and regression analyses were performed to find various relationships. Statistical Package for Social Sciences, version 23 (SPSS Inc., Chicago, IL), and MS Excel were used to analyze the data. For the comparisons of proportions, the Chi-square test was used.

P value < 0.05 was taken to be a significant level.

Observation and Results

Comparison of salivary E2 levels between the case and control groups

Statistically, there was no significant difference in the mean salivary E2 level in both groups, though the levels were slightly higher in the case group (9.15 ± 5.08) than in the control group (7.68 ± 5.05) [Table 1 and Graph 1].

Comparison of serum E2 levels between the case and control groups

The mean serum E2 level in the control group was significantly low (161.73 ± 37.13) as compared to the case group (237.08 ± 84.43) [Table 2 and Graph 2].

Comparison of DASS scores between the case and control groups

There was a significantly high ($P < 0.001$) score of DASS-21 in the case group. The mean DASS score in the control group was 11.44 ± 5.03, whereas in the case group the mean score was 23.13 ± 7.35 [Table 3 and Graph 3].

Comparison of PSS scores between the case and control groups

In the control group, the mean PSS score was 7.10 ± 4.07, whereas in the case group the mean score was 18.13 ± 7.61. The difference in mean PSS score was found to be significant between both groups ($P < 0.001$) [Table 4 and Graph 4].

Correlations between study parameters among all the subjects

Overall, significant positive correlations were observed between salivary E2 and serum E2 (r = 0.361, P = 0.001), salivary E2 and DASS score (r = 0.410, P < 0.001), salivary E2 and PSS score (r = 0.410, P < 0.001), serum E2 and DASS score (r = 0.768, P < 0.001), serum E2 and PSS score (r = 0.745, P < 0.001), and DASS score and PSS score (r = 0.878, P < 0.001) [Table 5 and Graph 5].

Correlations between study parameters among subjects in the control group

In the control group, correlations between salivary E2 and PSS score (r = 0.564, P < 0.001), serum E2 and DASS score (r = 0.416, P = 0.008), and DASS score and PSS score (r = 0.776, P < 0.001) were significantly positive [Table 6 and Graph 6].

Correlations between study parameters among subjects in the case group

In the case group, correlations between salivary E2 and serum E2 (r = 0.384, P = 0.016), salivary E2 and DASS score (r = 0.523, P = 0.001), salivary E2 and PSS score (r = 0.384, P = 0.016), serum E2 and DASS score (r = 0.762, P < 0.001), serum E2 and PSS score (r = 0.719, P < 0.001), and DASS score and PSS score (r = 0.779, P < 0.001) were found to be significantly positive [Table 7 and Graph 7].

Logistic regression analysis showing the relationship of disease with the E2 marker and DASS and PSS scores

The logistic regression analysis showing the relationship of disease with E2 marker and DASS and PSS scores [Table 8] revealed the following equation of prediction of disease:

D = -3.674 – 0.126(Salivary E2) +0.002(Serum E2) +0.082 (DASS score) +0.256(PSS score)

The disease will be predicted if D > 0.

The accuracy of the above model is 84.60%.

Receiver operating characteristic (ROC) curve analysis for finding the optimum cutoff for the disease by serum E2 marker

The ROC analysis for finding optimum cutoff for the disease by serum E2 marker [Table 9 and Graph 8] revealed the optimum cutoff as

Serum E2 level > 221.15

The area under the ROC (AUROC) curve of the above ROC curve was 0.773, which showed a good model of predictivity. Furthermore, the sensitivity of the cutoff is 58.97%, the true positivity for disease finding, which was relatively lower.

Table 1: Comparison of salivary E2 levels between the case and control groups

Group	Salivary E2		Mann–Whitney test	
	Mean	SD	z-value	*P*
Control	7.68	5.05	-1.83	0.067
Case	9.15	5.08		

Table 2: Comparison of serum E2 levels between the case and control groups

	Serum E2		Mann–Whitney test	
	Mean	SD	z-value	*P*
Control	161.73	37.13	-4.14	<0.001
Case	237.08	84.43		

Table 3: Comparison of DASS scores between the case and control groups

Group	DASS score		Mann–Whitney test	
	Mean	SD	z-value	*P*
Control	11.44	5.03	-5.99	<0.001
Case	23.13	7.35		

Table 4: Comparison of PSS scores between the case and control groups

Group	PSS score		Mann–Whitney test	
	Mean	SD	z-value	*P*
Control	7.10	4.07	-6.06	<0.001
Case	18.13	7.61		

Table 5: Correlations between study parameters among all the subjects

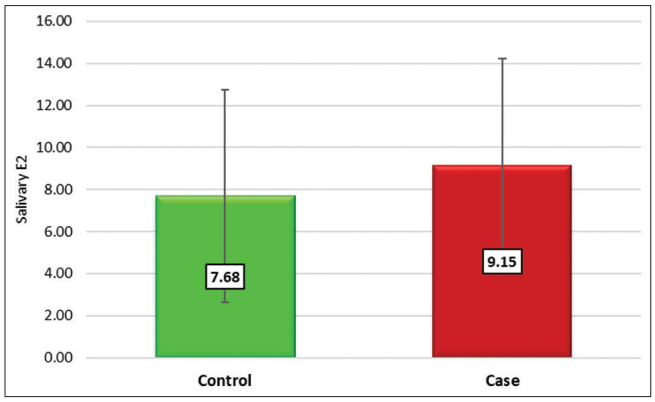
Overall	Salivary E2		Serum E2		DASS score	
	r-value	*P*	r-value	*P*	r-value	*P*
Serum E2	0.361	0.001	-	-	-	-
DASS score	0.410	<0.001	0.768	<0.001	-	-
PSS score	0.410	<0.001	0.745	<0.001	0.878	<0.001

Table 6: Correlations between study parameters among subjects in the control group

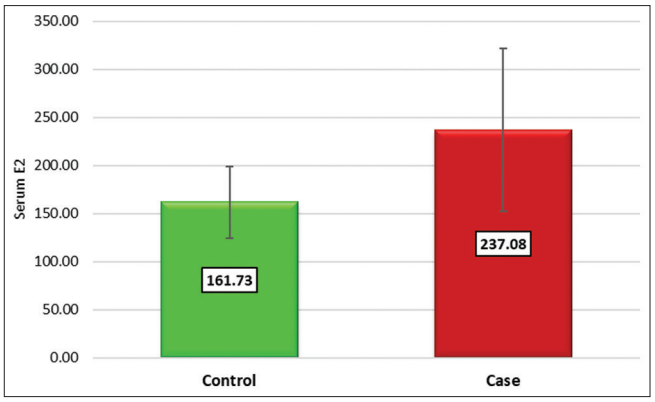
Control	Salivary E2		Serum E2		DASS score	
	r-value	*P*	r-value	*P*	r-value	*P*
Serum E2	0.305	0.059	-	-	-	-
DASS score	0.314	0.052	0.416	0.008	-	-
PSS score	0.564	<0.001	0.287	0.076	0.776	<0.001

Table 7: Correlations between study parameters among subjects in the case group

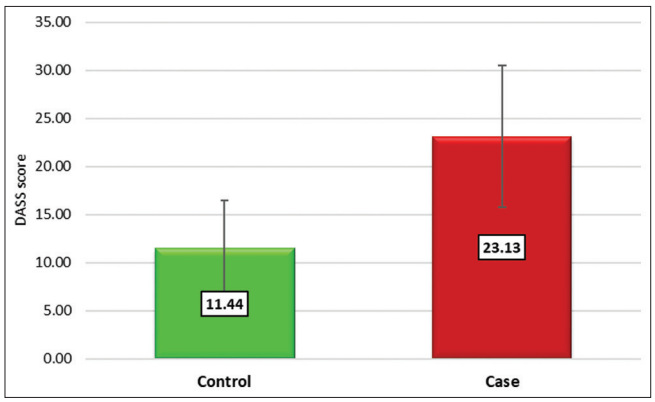
Case	Salivary E2		Serum E2		DASS score	
	r-value	*P*	r-value	*P*	r-value	*P*
Serum E2	0.384	0.016	-	-	-	-
DASS score	0.523	0.001	0.762	<0.001	-	-
PSS score	0.384	0.016	0.719	<0.001	0.779	<0.001



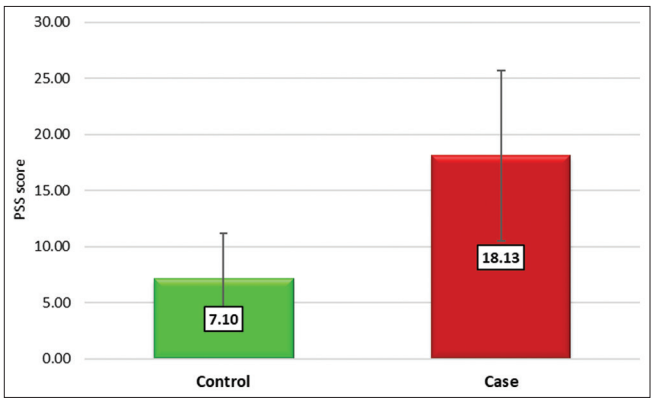
Graph 1: Comparison of salivary E2 levels between the control and case groups



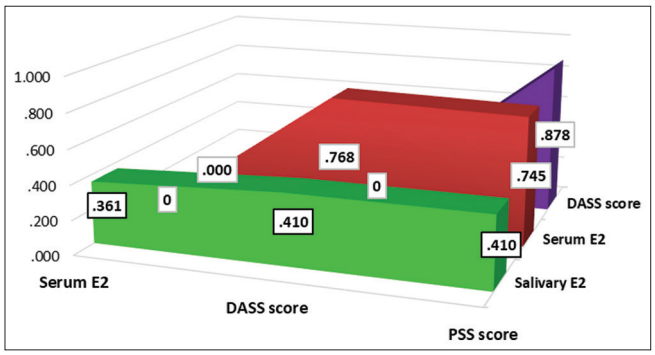
Graph 2: Comparison of serum E2 levels between the case and control groups



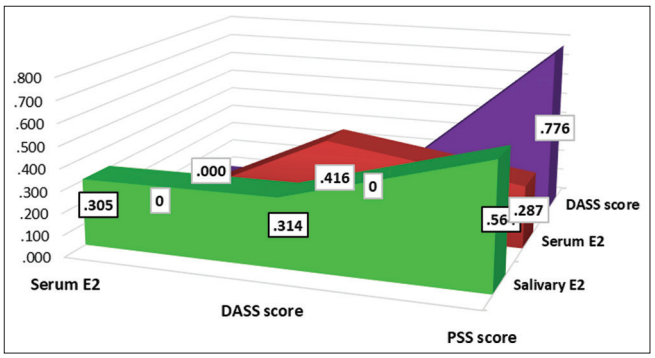
Graph 3: Comparison of DASS scores between the case and control groups



Graph 4: Comparison of PSS scores between the case and control groups



Graph 5: Correlations between study parameters among all the subjects



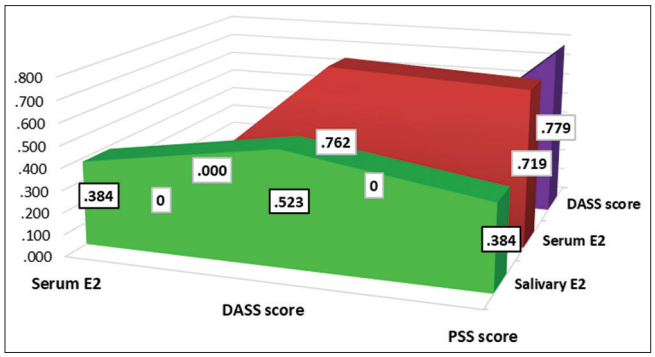
Graph 6: Correlations between study parameters among subjects in the control group

However, the specificity was 94.87%, a higher level of true negativity.

Discussion

OLP is a chronic inflammatory disease involving the oral mucosa, which is mediated by T cells. The etiology of the disease is multifactorial. It is considered to be an autoimmune disorder prevalent in females aged between 30 and 60 years.[8] Predilection in females points toward the possibility that sex hormones may play a significant role in the initiation or progression of the disease, but so far, no strong connection has been established. Thus, we designed the study undertaking female subjects to analyze the possible role of the sex hormone, estrogen, by measuring its concentration in serum and saliva in OLP patients. Considering the age of prevalence according to Chiang CP *et al.* (2018),[8] the age group of our study subjects was between 16 and 55 years.

Previous literature indicates that OLP is a localized autoimmune disease induced by T-cell dysfunction.[8] Also, the role of estrogen has been noted in modulating major immune cells. Grossman



Graph 7: Correlations between study parameters among subjects in the case group

Table 8: Logistic regression analysis showing the relationship of disease with the E2 marker and DASS and PSS scores

Variable	B	S.E.	*P*	Exp (B)	95% CI for EXP (B)		Accuracy
					Lower	Upper	
sSalivaryE2	-0.126	0.08	0.113	0.88	0.76	1.03	84.60%
SerumE2	0.002	0.01	0.817	1.00	0.99	1.02	
DASSscore	0.082	0.11	0.457	1.09	0.87	1.35	
PSSscore	0.256	0.13	0.049	1.29	1.00	1.66	
Constant	-3.674	1.27	0.004	0.03			

Table 9: ROC analysis for finding the optimum cutoff for the disease by serum E2 marker

Optimum cutoff	Serum E2 >221.15
AUROC	0.773
Sensitivity	58.97%
Specificity	94.87%

et al. (1991)[10] stated the notion that estrogen inhibited particular subsets of thymocytes, inhibiting a number of cell-mediated immune responses. Cutolo *et al.* (2006)[10] again revisited the subject and found that estrogen enhanced humoral immunity. He reported that in some autoimmune diseases there was increased peripheral conversion of androgens to estrogen. Moulton *et al.* (2018)[10] emphasized that estrogen was immune-stimulatory and consequently pathogenic in autoimmune disorders. Additionally, estrogen has an impact on physiological processes and autoimmune diseases by affecting CD4 + T-cell activation, production, and differentiation of cytokines.[10] Gabriela Recalde *et al.* (2018)[14] showed that estrogen has mostly stimulatory action on the immune system, boosts its activation, and promotes the induction of autoimmunity. Thus, we can presume that the role of estrogen in affecting immune responses presents an intriguing paradox[10] and may significantly contribute to the pathophysiology of OLP through immunological regulation.

OLP patients often report stressful events a few months before the clinical findings, which are considered to be an important factor in initiating the inflammatory cascade.[2] Therefore, we evaluated stress in our study as well.

For estrogen hormone analysis, regular serum collection is invasive, inconvenient, and requires trained personnel to collect samples. Therefore, we assessed the efficiency of whole saliva for monitoring estrogen in our study.

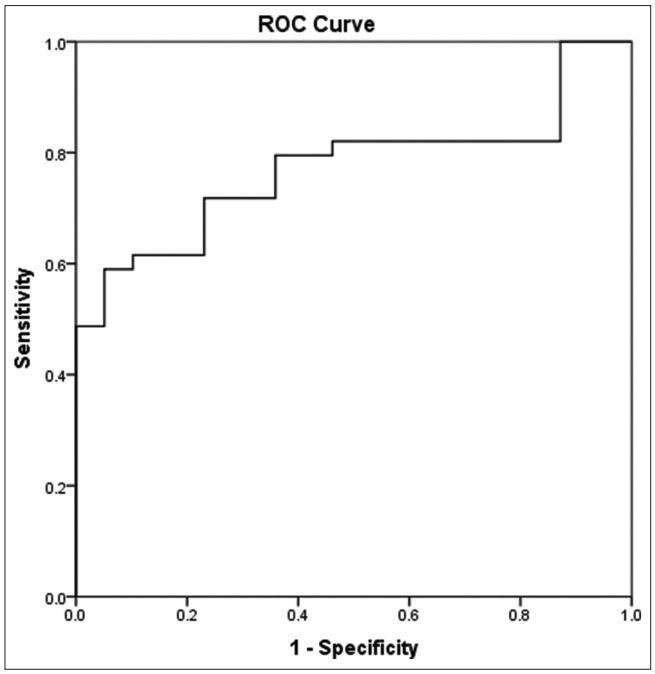
In the present study, the case group showed a higher mean salivary E2 value (9.15 ± 5.05) than the control group (7.68 ± 5.08), but no significant difference was noted ($P = 0.067$). Serum E2 levels, however, were significantly higher ($P < 0.001$) in the case group (237.08 ± 37.13). The increased serum E2 levels can be corroborated by the study of Gholizadeh N *et al.* (2021),[15] in which he measured serum estrogen levels in Iranian female OLP patients and found significantly higher hormone levels in patients than healthy females. He gave the hypothesis that estrogen is involved in the onset of disease by enhancing humoral immunity, promoting angiogenesis, and reducing immune cell apoptosis during the premenopausal period and stimulating cellular immunity after menopause.

However, we could not find any study in the literature assessing the salivary estrogen levels in OLP patients to compare our results.

Our results showed overall significant positive correlations between salivary and serum E2 levels. This positive correlation was also seen as significant in the case group individually. In the control group, the difference between serum and salivary E2 levels was almost statistically significant ($P = 0.059$), showing a potential for an association. Thus, we can assume that the salivary E2 assay is analogous with the serum E2 for monitoring the fluctuations in hormone levels similar to the study of Yu-cai Lu *et al.* (1999)[12] and Beatrice K. Gandara *et al.* (2007).[16] Therefore, whole salivary samples may prove to be a practical and noninvasive way to assess the levels of estrogen in health and disease.

Various psychoneuroimmunological research studies have proven a relevant clinical relationship between the development and progression of chronic diseases and psychological stressors. Firdaus S. Dhabhar *et al.* (1998)[17] studied the role of stress in inducing the enhancement of cell-mediated immunity. OLP patients have been found to experience greater levels of stress or anxiety than the normal population.[18] In our study, stress levels were assessed using the DASS-21 and PSS. The case group had significantly higher mean scores on both scales, which were 23.13 ± 7.35 and 18.13 ± 7.61, respectively. There is an increased risk of various chronic illnesses associated with stress in daily life. Stressors in daily life may lead to chronic mental and systemic health problems. Despite the high prevalence of these stress-associated problems, stress assessment and analysis are a rare practice in primary care settings. As in this study we found a positive association between OLP and stress, we must consider stress assessment as an important aspect of diagnosing and treating such patients in primary care centers.[19]

B Manczyk *et al.* (2019)[20] and Akanksha Gupta *et al.* (2017)[13] used DASS-21 for psychometric evaluation of OLP patients



Graph 8: ROC analysis for finding the optimum cutoff for the disease by serum E2 marker

and observed higher stress scores in the study group, similar to the findings of our study. The PSS scores in our study can be supported by the results of Wiriyakijja *P et al.* (2020).[21]

Therefore, we can suggest that there is a direct relation of stress with OLP, which is in agreement with the findings of S Chaudhary *et al.* (2004)[18], Mileno Soto Araya *et al.* (2004)[22], Kruna Valter *et al.* (2013)[23], Lidia Gavic *et al.* (2014)[24], Chaithra Kalkur *et al.* (2015)[25], and Honglin Liao *et al.* (2021).[26] This may lead us to the understanding that the psychological profile of the patient can play an imperative part in the onset or extension of the disease.

It has been observed that excess estrogen can affect a woman's body in many ways, including symptoms of mood swings, anxiety, and panic attacks, leading to stressful conditions.[27] The findings of our investigation, which demonstrate a positive relationship between stress and increased estrogen levels using both DASS-21 and PSS, lend weight to this assertion.

Based on the results, the inference of our study is that OLP is associated with higher levels of estrogen. Also, salivary samples can prove to be an easy and noninvasive substitute to assess estrogen levels. In addition to these findings, our study confirmed that stress can be considered an important factor closely associated with the occurrence or extension of the disease.

As serum or salivary estrogen levels fluctuate in females depending on the cyclic phases of menstruation, non-consideration of the phases while collecting samples could be considered a drawback of our study. The second shortcoming was the lack of racial variation as all the participants belonged to North India. Therefore, more multicentric studies, considering the phases of the menstrual cycle, are required to properly comprehend estrogen's role in the OLP.

Conclusion

OLP has a complicated etiopathogenesis influenced by the interaction of hereditary and environmental variables. In this study, the role of estrogen was assessed in patients with OLP, along with the measurement of stress levels. The results indicated the association of higher estrogen levels with the occurrence of disease. Thus, estrogen can be used as a useful biomarker for OLP in the future.

Our study also shows that salivary samples can prove to be an accurate and feasible alternative to serum estrogen level determination. Therefore, this salivary biomarker holds great potential for studying the hormone in health and disease.

Stress was seen to be in direct association with the disease and also showed a positive correlation with the higher estrogen levels. We also suggest that OLP patients must be given supportive psychological treatment for improved life quality and disease management.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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