EVALUATION OF OXIDATIVE STRESS BIOMARKER AND PSYCHOMETRIC ANALYSIS IN ORAL LICHEN PLANUS: A CASE-CONTROL STUDY

Dissertation Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In Partial Fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH IX

ORAL MEDICINE AND RADIOLOGY

2017-2020

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation titled **"EVALUATION OF OXIDATIVE STRESS BIOMARKER AND PSYCHOMETRIC ANALYSIS IN ORAL LICHEN PLANUS: A CASE-CONTROL STUDY"** is a bonafide work done **DR.NANITHA LAKSHMI.K,** Postgraduate student, during the course of the study for the degree of **MASTER OF DENTAL SURGERY** in the specialty of **DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY,** Vivekanandha Dental College for Women, Tiruchengode, during the period of 2017-2020.

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This is to certify that **DR. NANITHA LAKSHMI.K,** Post Graduate student (2017-2020) in the **DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY,** Vivekanandha Dental College for Women, has done this dissertation titled **"EVALUATION OF OXIDATIVE STRESS BIOMARKER AND PSYCHOMETRIC ANALYSIS IN ORAL LICHEN PLANUS: A CASE-CONTROL STUDY"** under our guidance and supervision in partial fulfillment of the regulations laid down by the Tamilnadu Dr.M.G.R.Medical University, Chennai-600032 for M.D.S **BRANCH-IX**

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DECLARATION

TITLE OF DISSERTATION	"Evaluation of oxidative stress biomarker and psychometric analysis in oral lichen planus: a case-control study"
PLACE OF STUDY	Vivekanandha Dental college, Elayampalayam, Tiruchengode, Namakkal district
DURATION OF THE COURSE	3 Years (2017-2020)
NAME OF THE GUIDE	Dr. N.Balan, MDS
HEAD OF THE DEPARTMENT	Dr. N.Balan, MDS

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission of the Principal, Vivekanandha Dental College for Women, Tiruchengode. In addition, I declare that no part of this work will be published either in print or electric without the guide who has been actively involved in the dissertation. The author has the right to reserve publishing of work solely with prior permission of the Principal, Vivekanandha Dental College for Women, Tiruchengode.

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PLAGARISM CERTIFICATE

This is to certify that this dissertation work titled **"EVALUATION OF OXIDATIVE STRESS BIOMARKER AND PSYCHOMETRIC ANALYSIS IN ORAL LICHEN PLANUS: A CASE-CONTROL STUDY"** of the candidate **DR. NANITHA LAKSHMI.K,** _____ for the award of degree **MASTER OF DENTAL SURGERY** in the branch of **ORAL MEDICINE AND RADIOLOGY**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **1%** of plagiarism in the dissertation.

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ACKNOWLEDGEMENT

"No one who achieves success does so without acknowledging the help of others".

-Alfred North Whitehead

I dedicate this dissertation with great gratitude and all the respect to the Almighty God without whose kind support and generous blessings this work of mine would not have been completed.

*My sincere thanks and deep sense of gratitude to **Capt. Dr.S.GOKULNATHAN, B.Sc, M.D.S.,** Dean, Vivekanandha Dental College for Women, for permitting me to pursue this study.*

*With submissive ambition, I aspire to register my gratitude to my respected HOD, Department of Oral Medicine and Radiology and the Principal of Vivekananda Dental College for Women, Prof. **Dr.N.BALAN M.D.S.,** for his inspiring guidance, invaluable counsel and encouragement throughout the course of the study. This work would not have seen the light of the day without his affectionate and compassionate counselling, which reposed by confidence in myself to undertake the challenges in the study.*

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Urkund Analysis Result

Sources included in the report:

https://scientonline.org/open-access/anxiety-and-depression-as-risk-factor-for-the-development-of-oral-lichen-planus-and-its-association-with-blood-antioxidant-level.pdf

Instances where selected sources appear:

8

# AIMS AND OBJECTIVES

AIM

To evaluate the Oxidative stress biomarker in saliva & serum & assessment of anxiety and depression in individuals with OLP

OBJECTIVE

- To measure the levels of superoxide dismutase in saliva & serum

- To compare salivary & serum SOD levels among case & controls

- To evaluate psychological status of individuals with OLP using Hospital Anxiety & Depression (HAD) Scale

REVIEW OF LITERATURE

Oral lichen planus (OLP) is one of the most common dermatological diseases to manifest itself in the oral cavity. It is considered as a premalignant lesion because of potential for the malignant transformation. It is reported that oxidative stress may play a role in oral LP. Stress and anxiety have frequently been mentioned as possible factors related to the development of oral lichen planus (OLP), although this association remains controversial[11].
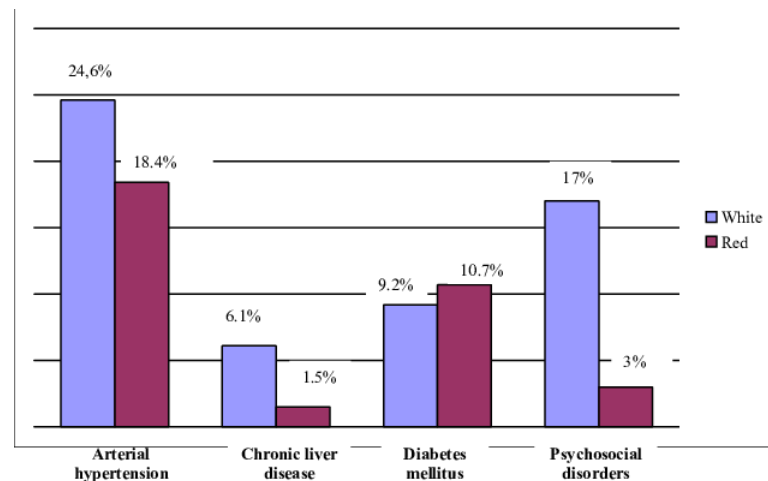
Graph 1: Relationship between the clinical presentation of oral lichen planus and the existence of systemic diseases[12].
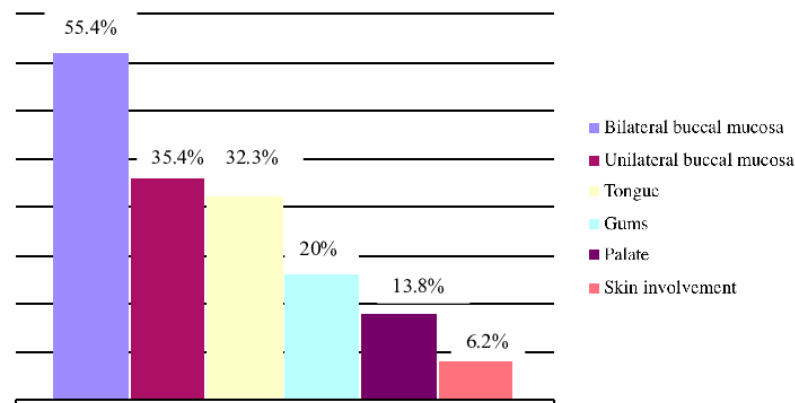
Graph 2: Anatomical location of the oral lichen planus lesions[13].
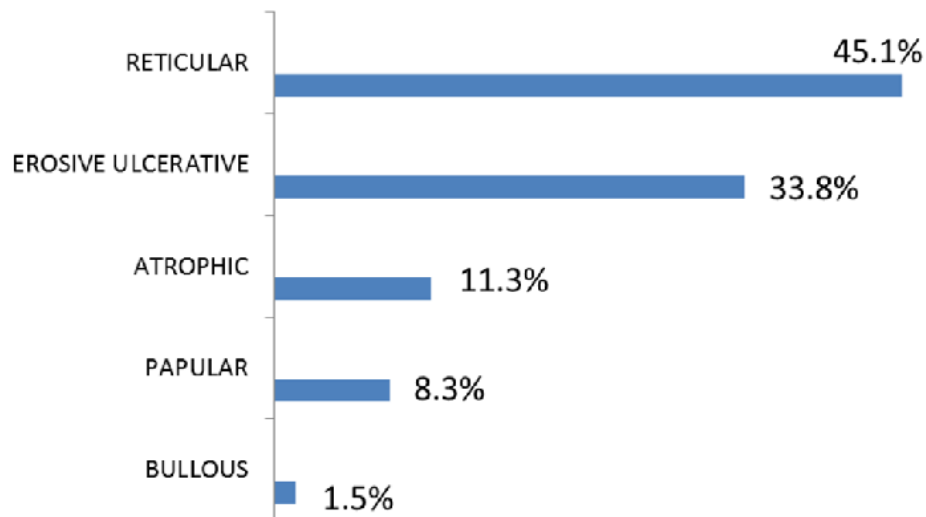
Graph 3: Lichen planus – distribution of clinical forms[13].

Oral Lichen planus

Oral lichen planus is a chronic inflammatory disease characterized by bilateral white striations or plaques on the buccal mucosa, tongue or gingival. It is found commonly in adults (50-55 years of age) and predominantly affects women usually by a 1.4:1 ratio over men. OLP has varied clinical presentations, with the reticular, erosive, and atrophic types being the most commonly reported. In the last few years, significant advances have been made in understanding the mechanisms involved in

the pathogenesis of the disease. OLP has been reported to be associated with different medical conditions such as diabetes, hepatitis C infection, and liver disease.Although, the condition is often referred to as stress associated ulcerations of the oral mucosa and research to date hints on a psychosomatic component in the etiology and progression of OLP, very little documentation has been presented to substantiate this widely held assumption[14].

<p style="text-align:center">Role of stress in oral lichen planus</p>

Stress can be defined as the biological reaction to any adverse internal/external stimulus, physical, mental or emotional, that tends to disturb the organisms homeostasis. Inadequate compensating reactions may lead to disorder. In the recent years, the injurious effects of stress have received attention. Stress has shown to manifest as fatigue, gastrointestinal symptoms, tachycardia anxiety and cynicism. The literature has documented the existence of mental disorders in patients with OLP, though the reported incidences vary considerably – from 10% to 22% and even 49%. In the series of Hampf et al., 21.4, 5.4 and 25% of the patients suffered mild, moderate and severe mental alterations, respectively, and 21% required psychiatric care. It has been suggested that certain psychosomatic situations could be factors underlying OL. The pathogenic link between stress and OLP has been based. upon experimental studies in animals. In fact, animals respond to stress by reducing their mononuclear cell counts (especially T lymphocytes) in the spleen and in peripheral blood, with a decrease in the T helper/T suppressor cell ratio and an increase in the presence of natural killer cells. At present, the relationship between this diminished lymphocyte proliferation response and depressive states is the subject of ongoing debate. In the past, a number of authors has drawn attention to the fact that a history

of psychological trauma is often present in patients with OLP. The prevalence rate of psychic stress is between 51.4 and 86.6%.Puchalski and Szlendak reported such an association in 86.6% of a series of 30 patients with cutaneous lichen planus. In a series of patients with age and sex characteristics very similar to our own, Burkhart et al. observed the causes of stress present in 51.4% of the patients. The psychological trauma recorded by these authors included e.g. severe systemic illness or death of a relative or unemployment. Such traumatic events have all been considered as psychosocial factors in the development of anxiety disorders. In the general population, anxiety is a relatively common disorder. The prevalence of pathological or severe anxiety in a World Health Organization (WHO) survey conducted in 11 countries was found to be 10%[15].

Superoxide dismutase as an oxidative stress biomarker

Superoxide dismutase (SOD) is considered the first line defense against ROS, converting the superoxide anion (O2 −) into H2 O2. Glutathione peroxidase (GPx) catalyzes the reduction of hydrogen peroxide and lipid hydro peroxides. GPx in combination with catalase and SOD function to protect the cell from damage due to ROS. Malondialdehyde (MDA) is used as an indicator of lipid peroxidation. UA is the most important antioxidant molecule in saliva. Saliva has been used in the past few decades as a new diagnostic fluid. The use of saliva as a diagnostic tool presents many advantages: it is easy to collect, by a noninvasive technique; no special equipment is needed for collection. Collection of saliva is associated with fewer compliance problems compared with blood collection, and salivary levels correlate well with serum levels[16].

Anshumalee et al. and Sezer et al. reported that oxidative stress and ROS may be involved in the pathogenesis of the LP, In Sezer et al study on Lipid peroxidation and antioxidant status in lichen planus, A total of 40 patients with LP (23 men, 17 women; mean ± SD age 43.27 ± 1.96 years) and 40 control subjects, matched for age and gender, were enrolled in this prospective study. Serum SOD levels (18.19 ± 3.71 U∕mL) in patients with LP were also higher than in healthy controls (P ¼ 0.002). The findings of this study suggest that increased oxidative stress, increased lipid peroxidation and an imbalance in the antioxidant defence system may be involved in the pathogenesis of LP[17].

In a study by Hassan et al. in 2013, SOD activity was estimated, and the mean value of plasma SOD in cases was 5.32 ± 0.57 U/ml, while in controls, the mean value was 4.07 ± 0.99 U/ml. This difference was statistically significant (P < 0.0001)[18]

Aly and Shahin in 2010 included 45 Egyptian LP patients and 45 healthy volunteers as controls and conducted a study in which serum levels of SOD were higher in LP patients with mean ± SD of 17.33 ± 2.05 when compared to controls (P = 0.009) leading to an imbalance in the antioxidant defense system. This study showed a positive correlation between nitric oxide NO, MDA and SOD and the duration of LP. No relation between SOD and the clinical types of LP was noted, The association between local oral diseases and systemic effects is surprising, but has been confirmed repeatedly and points towards the issue of causality direction. Serum levels of malondialdehyde and superoxide dismutase were found to be lower in patients with lichen planus[19].

Serum SOD levels were found significantly lower in oral LP patients in a study by Jingyan et al., 2001 in 42 OLP patients before the treatment than those in healthy controls (P = 0.001), while after treatment, the SOD levels increased and LPO levels decreased significantly in OLP patients, and no significant difference were found as compared with healthy controls (P = 0.05)

It has been evident from recent studies that oxidative stress plays an important role in the pathogenesis of several inflammatory and autoimmune diseases. ROS (superoxides, hydroxyl radicals) can cause damage to the cellular components via protein peroxidation of nucleic acids, free amino acids and lipoproteins. These radicals can also induce gene mutation and post transitional modification of cancer-related proteins, which in turn are said to disrupt cellular processes such as DNA repair and apoptosis. It has been found that ROS produced by keratinocytes, fibroblasts and various inflammatory cells could result in disequilibrium between the pro-oxidants and antioxidants[20].
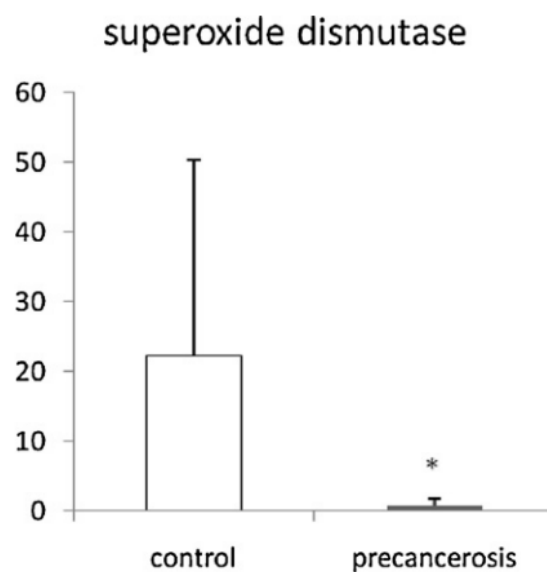
Graph 4: Salivary RNA analysis using real time PCR. Superoxide dismutase mRNA was lower in patients with oral premalignant lesions (precancerosis) in comparison to age-matched healthy probands (control)[21].

A pathophysiological process leads to down-regulation of antioxidant genes leading to lower antioxidant status and higher levels of lipid peroxidation. It is only a speculation that hypoxia might be the mysterious process. Hypoxia decreases the expression of antioxidant enzymes that are not needed during hypoxia, including superoxide dismutase. In addition, total antioxidant activity was found to be lower in the serum of patients vs. controls indicating that the local oral disease might be associated with systemic antioxidant status.

<div align="center">

Psychological morbidity in oral lichen planus

</div>

A complex multidirectional communication pathway involving the endocrine, immune and central nervous systems the skin maintains internal homeostasis. Brazzini et al. proposed the "an active neuro-immuno-endocrine interface" or neuroendocrine organ, exhibiting multidirectional and local communication, which is made possible by the production in the skin of cytokines, hormones and neurotransmitters, and anatomical links between the central nervous system (CNS) and skin. In addition, circulating immune cells, recruited in the skin, express receptors for a variety of neuropeptides, cytokines, neurotransmitters and hormones, identical to those expressed centrally, allowing the CNS to communicate with the skin. This means that systemic signals affecting the skin initiate a flow of information between this and other organs, leading to modulation of local immune activity, vascular functions, sensory reception, thermoregulation, exocrine secretion and maintenance of skin barrier integrity[22].
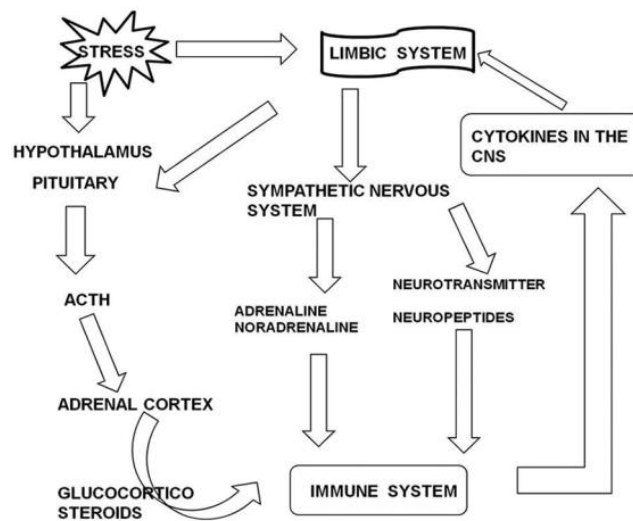
Figure 1: Simplified overview of the channels of communication between the immune, endocrine and central nervous (CNS) systems in the stress response.

Different studies have been done for the evaluation of the relationship between OLP and psychiatric disorders. In 1961, Altman and Perry conducted a study on of 197 patients with LP, which revealed that "10% were aware of a precipitating stressful incident at the onset of their LP." Andreasen pointed out in 1968 that patient with LP were found to be in conditions of stress, anxiety, and emotional changes.

Dermatology has a distinct relation with psychosomatics as the skin has strong psychological implications. The skin is a complex system made up of glands, blood vessels, nerves and muscle elements, many of which are controlled by the autonomic nervous system and can be influenced by psychological stimuli. These have the capacity to cause autonomic arousal and capability of affecting the skin and the development of various skin disorders. Clinical studies have shown that psychological stress can cause suppression of killer T cells and macrophages, both of which play important roles in skin-related immune reactions. Field described the skin as the

"shock organ" for emotional stress, manifesting in the form of several skin diseases. Clinical observations have identified psychological stress as either precipitating, aggravating or prolonging many skin diseases and the psychosomatic aspects of many disorders. Therefore, any factor that can influence the cell-mediated immune response can have a role in the development of the disease. Factors such as stress and psychological problems, especially depression and anxiety, have been mentioned as etiologic factors in LP but there is still controversy concerning the role of stress as a major or minor etiologic factor in the pathogenicity of LP[23].

Exacerbations of OLP have been linked to the periods of psychological stress and anxiety. Ivanovski et al. proposed that prolonged emotive stress in OLP Patients has been proposed to lead to psychosomatization which in turn may contribute to the initiation and clinical expression of OLP and also suggested that psychosocial and emotional stress is one possible factor that may precipitate reticular OLP to transform to the erosive form.The progression from the reticular to the atrophic to the more severe erosive/bullous forms of OLP has been proposed to be psycho-somatically determined. Clinical immunological assessments of peripheral blood CD3+ cells obtained from OLP patients reveal enhanced major histocompatibility complex (MHC)-restricted cytotoxicity toward oral mucosal epithelial cells.Study findings by Chiappelli et al, reveal human CD4+ cell activation. Steroid significant associations between systemic alterations in certain subpopulations of T cells in OLP patients with non-erosive or with erosive lesions and psychological mood states, and suggest a possible psycho-neuroendocrine- cavity and lymphocyte migration: relevance for alcohol abusers. Immune model of pathology, in which psychological mood states could impact upon the neuroendocrine system (e.g., cortisol levels), which then could influence the migratory properties of T cell subpopulations (e.g., CD4+CD45RA+) to

the site of the oral mucosa, thus leading to an increased propensity to develop the more aggressive form of the OLP pathology. If this hypothetical model of OLP disease is confirmed, then new avenues could open for the prevention and treatment of the clinical manifestations that afflict patients with OLP, including the more painful erosive lesions[24].

Rojo-Moreno et al. in a controlled study on 100 patients using different psychometric tests found greater anxiety and depression in OLP patients than the controls[25].

HADS Scale and it's psychometric properties

The HADS was used for identifying and quantifying the two most common forms of psychological disturbances in patients, namely anxiety and depression. HADS has been used extensively, and we identified 747 papers that referred to HADS in Medline, ISI and PsycINFO indexed journals by May 2000. Based on approximately 200 papers on HADS in approximately 35,000 individuals in various patient populations, Herrmann concluded in 1996 that ''HADS is a reliable and valid instrument for assessing anxiety and depression in medical patients.''

Using the highest score of either HADS-A or HADS-D as an indicator of psychiatric morbidity, Morriss and Wearden, found that a cut-off score for caseness of 10+ resulted in sensitivity 0.92 and specificity 0.71 in a sample of chronic fatigue syndrome patients (n = 136)[26].

The sensitivity and specificity of HADS-A and HADS-D with a threshold of 8+ were most often found to be in the range of 0.70 to 0.90. The variation in both optimal cut-off values and sensitivity and specificity might be due to differences in diagnostic systems, 'gold standard' instruments, HADS translations used. These

results indicate excellent case finding abilities of HADS in unselected samples of patients seeking a general practitioner. HADS was tested in three studies of primary care populations. Wilkinson and Barczak (n = 100) found an excellent ability of HADS to detect DSM-III-defined psychiatric morbidity, and the ROC curves showed that a score of 8+ was the optimal threshold[27].

Twenty-one studies reported the Pearson correlation coefficient between HADS-A and HADS-D (mean .56). In seven studies of non-patient samples the correlations varied between .49 and .74 (mean .59)[28,29].

MATERIALS AND METHODS

Source of data

The present study is a case-control study done to evaluate and compare the salivary and serum oxidative stress biomarker- superoxide dismutase and assess psychometric properties in oral lichen planus and healthy controls.

Ethical Clearance

A detailed protocol about the aim and procedures of the present research was approved by the Institution Ethical Committee, Vivekanandha Dental College for Women. The study was carried out after obtaining ethical clearance

Sample size

The sample size was estimated using A Priori comparison test. The Software used to calculate sample size was G Power version 3.1.9.2. It was done to estimate the number of subjects required for the study. The required sample size with 90% power was estimated as 30 per group with a total of 60 subjects. The details of the research were explained to all participants and written consent was obtained from all of them.

The present study included a total of 60 subjects. The subjects were categorised into 2 groups

Table 1: Inclusion criteria for case and control group

Group	No of cases	Criteria for Inclusion
Group A	30	• Cases of OLP with typical clinical presentation & histopathological confirmation according to modified WHO diagnostic criteria (2003) • Age ranging above 18 years.
Group B	30	• Patients with no other co-existing mucosal lesions such as aphthous ulcer, leukoplakia, OSMF. • Patients who are not under drug therapy for anxiety, depression, sleep disorders. • Patients with underlying significant systemic illness, Patients with tobacco, alcohol related habits.

Inclusion criteria

Cases with typical clinical presentation of OLP with histopathological confirmation according to Modified WHO diagnostic criteria (2003)

- Bilateral more or less symmetrical lesions

- Presence of lace-like network of slightly raised grayish-white lines (reticular pattern)

- Erosive, atrophic, bullous, plaque-type lesions are accepted only as sub-types in presence of reticular lesions elsewhere in mucosa

- In all other lesions that resemble OLP that do not complete the aforemented criteria the term "clinically compatible with" will be used

- Age ranging above 18 years

- Routine investigation for Orthodontic and trans alveolar extractions

Exclusion criteria

- Patients with co-existing oral mucosal lesions such as aphthous ulcer, Leukoplakia, OSMF

- Patients under treatment modalities like topical/systemic corticosteroids for OLP

- Patients under drug therapy for anxiety, depression, sleep disorders

- Patients with underlying significant systemic illness

- Patients with tobacco, alcohol related habits were excluded

Figure 2: Erosive oral lichen planus in relation to right buccal mucosa

Figure 3: Ulcerative lichen planus lesions in relation to hard palate region
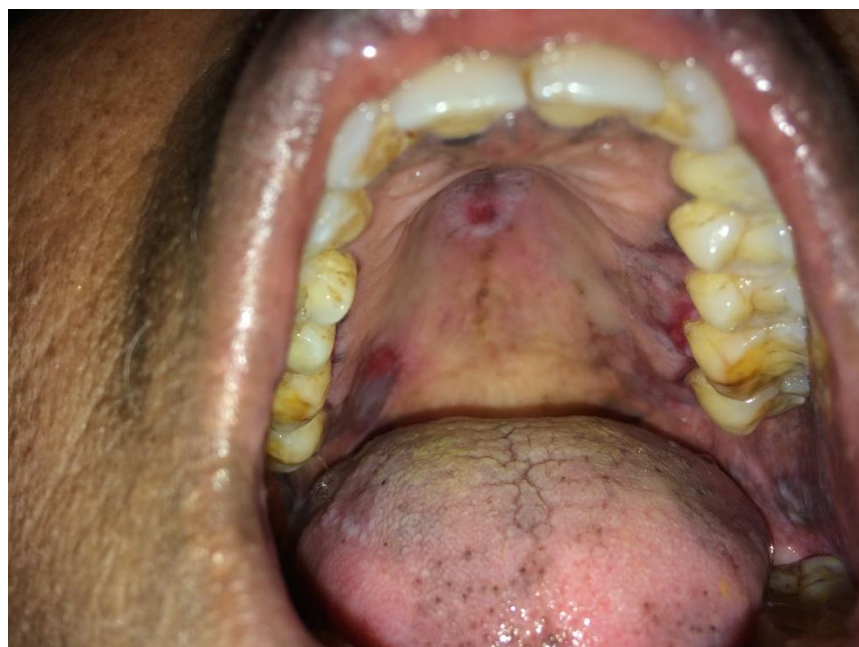
Figure 4: Sutures placed following incisional biopsy in relation to right buccal mucosa

Figure 5: Histopathological confirmation following biopsy revealing sub-epithelial band of lymphocytic inflammatory infiltrate with drop-shaped rete pegs
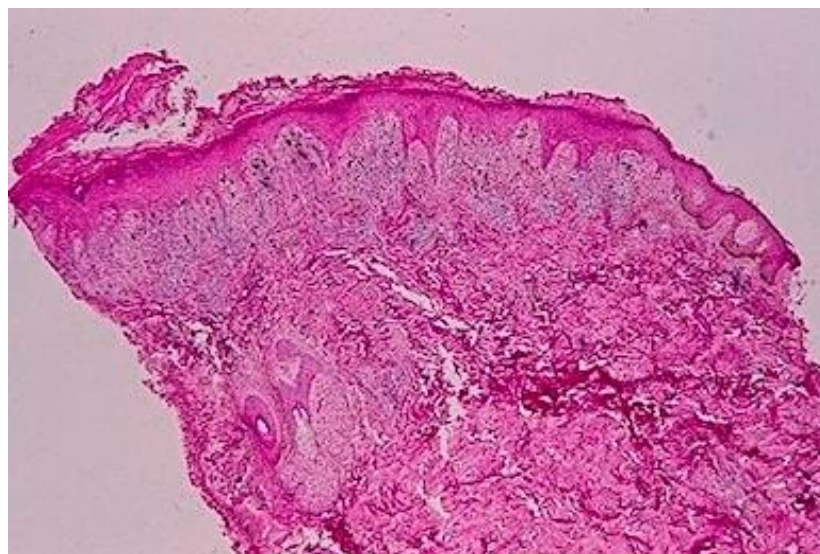
Materials used

Examination of subjects

1. Dental chair with good lighting attachment was used

2. Disposable gloves and mask

3. Stainless steel kidney tray

4. Disposable paper cups with water

5. Sterilised diagnostic instruments

- Mouth mirror

- Straight probe

- Explorer

- Tweezers

- Cotton rolls

- Gauze pads

Biopsy instruments

1. 3ml sterile disposable syringe with 26-gauge needle

2. 2% lignocaine hydrochloride with 1:100,000 adrenaline

3. Biopsy tray

4. BP handle no.4

5. Disposable No.15 BP blade

6. 1 small surgical curved scissors

7. Artery forceps

8. Needle holder

9. Allis tissue forceps

10. A 3-0 black braided silk suture

11. Curved suture needle (half circle)- 2 numbers

12. Sterile gauze and cotton

13. 10% neutral buffer formalin

Figure 6: Sterlised set of diagnostic instruments

Figure 7: Sterilised set of biopsy instruments

METHODOLOGY

Collection of data

- ✓ The participants included in the study were thoroughly examined in the dental chair. Thorough clinical history was taken and written consent was obtained.

- ✓ Patients were selected based on clinical criteria for oral lichen planus (WHO 2013 criteria)

- ✓ Hospital anxiety and depression scale (HAD) was assessed in all individuals participating in the study

- ✓ The patients who were clinically diagnosed with oral lichen planus underwent routine haematological investigations required for minor surgery.

- ✓ Biopsy was done under strict aseptic conditions and submitted for pathological diagnosis. On histopathological confirmation, saliva and blood samples were collected.

- ✓ Collected samples were centrifuged and the saliva supernatant and serum was separated and stored in deep freezer until further steps

- ✓ Processing was carried out in laboratory using ELISA method

COLLECTION OF SALIVA SAMPLES

- Unstimulated saliva is to be obtained using spitting method into dry plastic vials with patients sitting in relaxed position,

- Centrifugation is done immediately at 900rpm for 10 min, eliminating the supernatant, remaining sample containing cells will be stored -80 ˚C SOD biomarker is analysed by ELISA.

Figure 8: Disposable plastic container and disposable pipette to store saliva

Figure 9: Collection of saliva by drooling method

COLLECTION OF BLOOD SAMPLE

- Under Aseptic conditions, 2ml of venous blood will be drawn from the ante-cubeital vein using a 5ml disposable syringe,

- Blood samples will be centrifuged at 3000rpm for 5min at 4˚C, serum obtained from Erythrocyte suspension will be stored at -40˚C,SOD is analyzed by ELISA.

Figure 10: Tourniquet, disposable syringe with needle and blood collection tubes for collection of blood samples

Figure 11: Collection of venous blood from ante-cubital fossa

Figure 12: ELISA kit used to process the samples
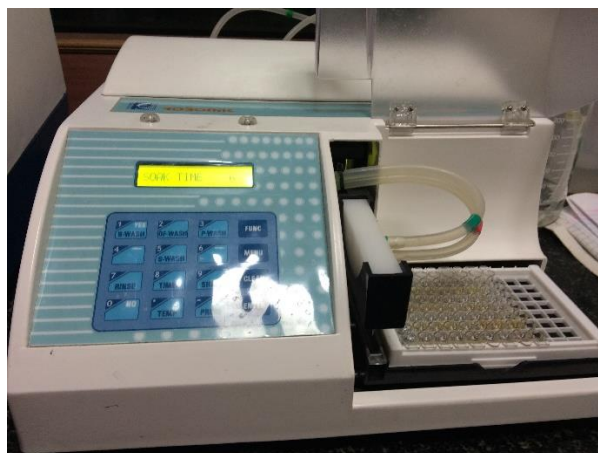

Figure 13: Microplate reader in the process

Figure 14: Colorimetric changes following completion of the process
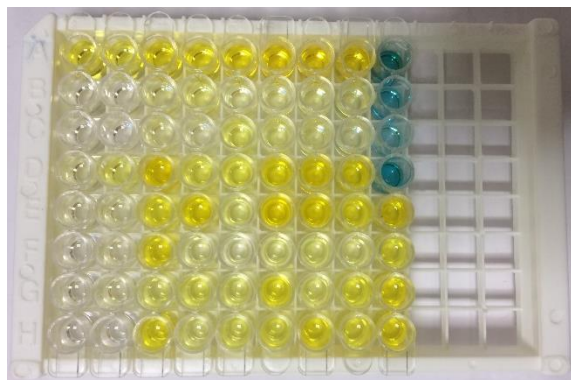
RESULTS

Table 2- Distribution of subjects in study groups

	Male		Female		Total	Chi square	p
	N	%	N	%			
Control	9	30	21	70	30	2.50	0.114
Case	15	50	15	50	30		
Total	24	40	36	60	60		

The table 2 shows the distribution of subjects in each group, total number of male population in controls is 9 (30%), and female is 21 (70%), and total number of male population in cases is 15 (50%), and female is 15 (50%),

Table 3- Distribution of age of subjects in study groups

	Age in years					Mann-Whitney U	Z	p
	N	Min	Max	Mean	SD			
Control	30	18	49	23.07	6.95	32.00	6.19	0.001**
Case	30	32	60	45.87	7.48			

The table 3 shows the distribution of age in years of individuals in each study group. In control group, the minimum age is 18 years and maximum is 49; and the minimum age among individuals in the case group is 32 years and maximum age range is 60 years. Hence, the mean age prevalence of lichen planus is in middle age individuals as verified by the present result.

Table 4- Mean anxiety levels among study groups

	Anxiety					Mann-Whitney U	Z	p
	N	Min	Max	Mean	SD			
Control	30	0	10	3.97	2.48	13.00	6.49	0.001**
Case	30	5	16	11.83	2.18			

The table 4 shows mean levels of anxiety among control group, in which minimum score is 0 and the maximum score is 10. These values prove that the control groups have no evidence to borderline anxiety. The mean and standard deviation is 3.97 and 2.8 respectively. The mean values of anxiety in the case group reveals that the minimum value is 5 and the maximum value is 16; where the inference is patients in case group have borderline to abnormal values of anxiety as measured using HAD scale. The mean and standard deviation is 11.83 and 2.18. Mann- Whitney U test score is 13.00, with Z- value 6.49, hence the p value is 0.001 suggestive of high statistical significance.

Table 5- Mean depression levels among study groups

		Depression				Mann-Whitney U	Z	p
	N	Min	Max	Mean	SD			
Control	30	1	2	1.50	.504	119.500	4.906	.000***
Case	30	0	19	8.92	4.350			

The table 5 shows mean depression among control group, in which minimum score is 0 and the maximum score is 1. These values prove that the control groups exhibit no abnormality of depression. The mean and standard deviation is 1.50 and .504 respectively. The mean values of depression in the case group reveals that the minimum value is 0 and the maximum value is 19; where the inference is patients in case group have no abnormality to abnormal values of depression as measured using HAD scale. The mean and standard deviation is 8.92 and 4.350. Mann- Whitney U test score is 119.00, with Z value 4.906, hence the p value is 0.000 suggestive of high statistical significance.

Table 6- Saliva SOD levels in case and controls

	Group	N	Mean	SD	SE	t	p
Saliva SOD	Control	30	0.62	0.73	0.13	6.70	0.001**
	Case	30	1.75	0.58	0.11		

Table 6 explains the mean value of salivary superoxide dismutase levels among the controls is 0.62 and level of standard deviation is 0.73. The mean value of salivary superoxide dismutase levels among the cases is 1.75 and level of standard deviation is 0.58. superoxide dismutase levels in saliva of cases is greater when compared to the controls. Student's t-test gives p-value of 0.001, suggestive of high statistical significance.

Table 7- Serum SOD levels in case and controls

	Group	N	Mean	SD	SE	t	p
Serum SOD	Control	30	0.99	0.87	0.16	2.99	0.004**
	Case	30	1.60	0.71	0.13		

Table 7 gives the mean value of serum superoxide dismutase levels among the controls is 0.99 and level of standard deviation is 0.87. The mean value of serum superoxide dismutase is elevated in levels among the cases with a value of 1.60 and level of standard deviation of 0.58. Student's t-test gives p-value of 0.001, suggestive of higher statistical significance.

Table 8- One- sample Kolmogorov-Sminorv Normality test

One-Sample Kolmogorov-Smirnov Normality Test						
		Age in years	Anxiety	Depression	Saliva SOD	Serum SOD
N		60	60	60	60	60
Normal Parameters(a,b)	Mean	34.467	7.900	8.917	1.185	1.297
	SD	13.541	4.594	4.350	0.870	0.848
Kolmogorov-Smirnov Z		1.689	1.421	0.909	1.185	1.004
p		0.007**	0.035*	0.381	0.120	0.266

Table 8 shows that Kolmogorov-Smirnov Normality Test was done to assess the normality among the parameters compared between the cases and controls, and the results are suggestive of significant p values of 0.007 and 0.035 in age group of the individuals and anxiety respectively.

Table 9- Pearson's correlation of intra-group parameters in control group

| Correlations – Control group						
		Age in years	**Anxiety**	**Depression**	**Saliva SOD**	**Serum SOD**
Age in years	r	**1.000**	-0.324	0.247	0.103	0.257
	p	.	0.081	0.187	0.589	0.170
Anxiety	r		**1.000**	0.581	-0.277	-0.332
	p		.	0.001**	0.138	0.073
Depression	r			**1.000**	0.001**	-0.115
	p			.	1.000	0.546
Saliva SOD	r				**1.000**	0.424
	p				.	0.020*
	N	30	30	30	30	30

Pearson's correlation among control group reveal positive association between saliva and serum superoxide dismutase levels, with p-value of 0.020, also a positive correlation between anxiety and depression with p-value of 0.001, a positive correlation between depression and saliva SOD with p- value 0.001.

## Table 10- Pearson's correlation of intra-group parameters in case group

Correlations – Case		Age in years	Anxiety	Depression	Saliva SOD	Serum SOD
Age in years	r	**1.000**	0.159	0.061	0.043	-0.091
	p	.	0.401	0.750	0.821	0.632
Anxiety	r		**1.000**	0.648	0.309	0.168
	p		.	0.001**	0.097	0.374
Depression	r			**1.000**	0.479	0.200
	p			.	0.007**	0.290
Saliva SOD	r				**1.000**	0.676
	p				.	0.001**
	N	30	30	30	30	30

Table 10 describes Pearson's correlation among case reveals positive association between anxiety and depression with positive p-value of 0.001, p- value of 0.007 suggestive of statistically significant correlation between depression and levels of SOD in saliva, serum SOD and saliva SOD is also highly statistically significant with p-value of 0.001, the results are suggestive of SOD levels capable of being exposed similarly in both saliva and serum.

GRAPH 5- DISTRIBUTION OF AGE AMONG STUDY GROUPS

Age in years
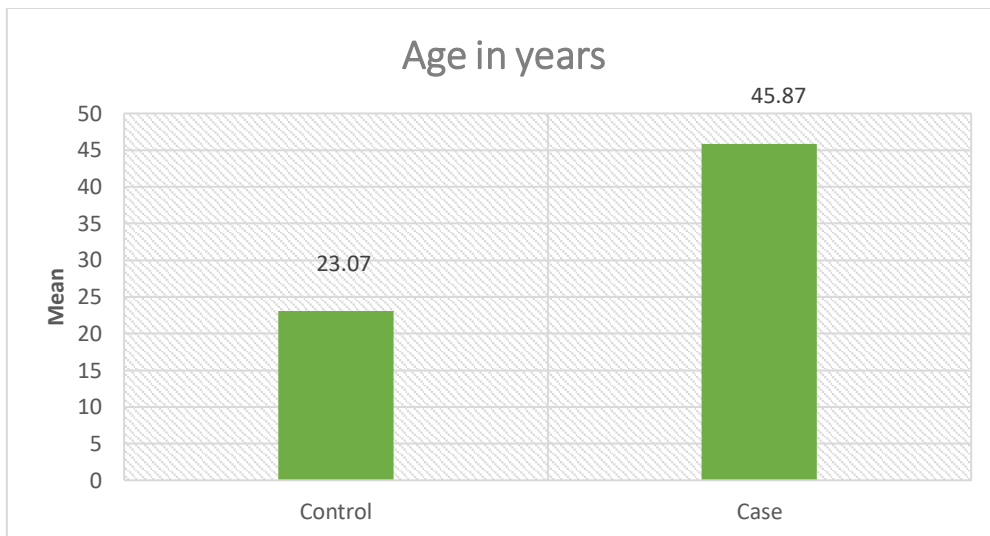
GRAPH 6- DISTRIBUTION OF GENDER AMONG STUDY GROUPS
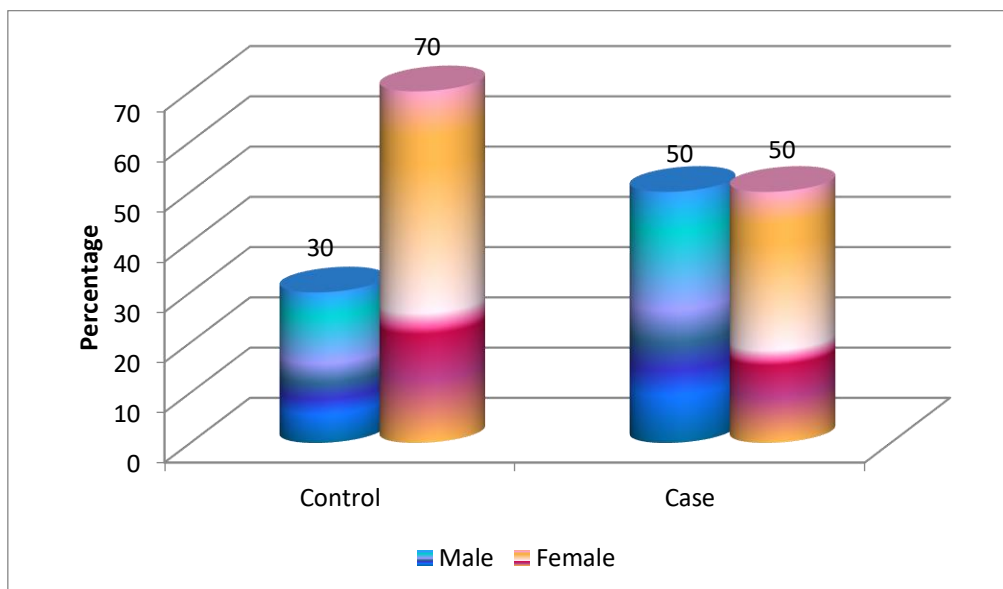
GRAPH 7- MEAN VALUE OF ANXIETY AMONG STUDY GROUPS

Anxiety

3.97, 25%

11.83, 75%

■ Control ■ Case
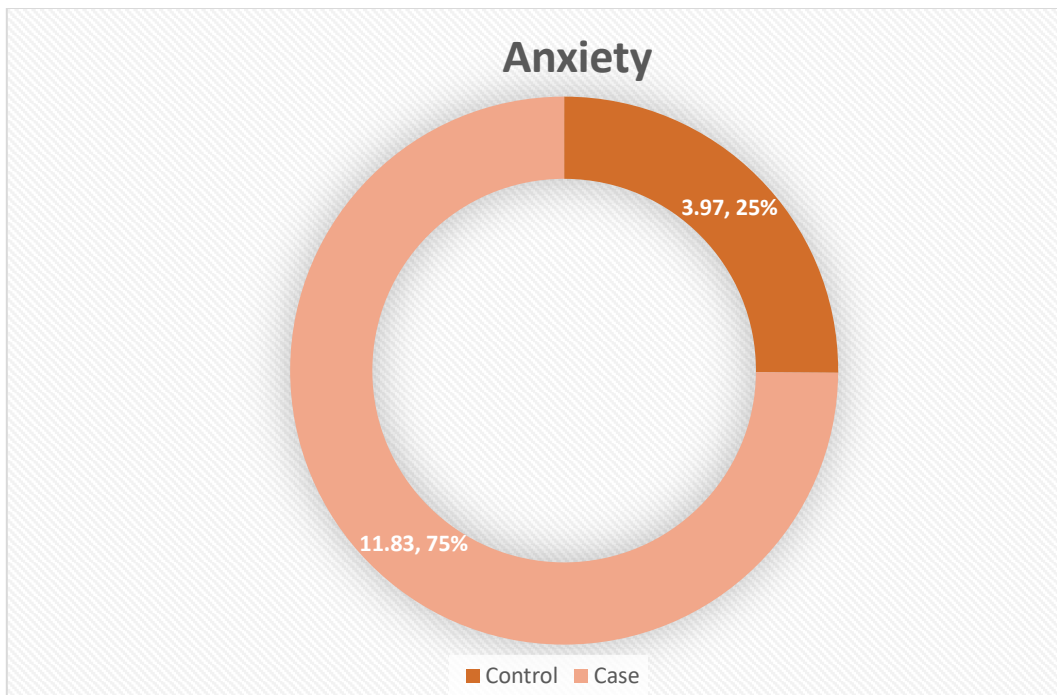
GRAPH 8- MEAN VALUE OF DEPRESSION AMONG STUDY GROUPS

Depression

6.2, 35%

11.63, 65%
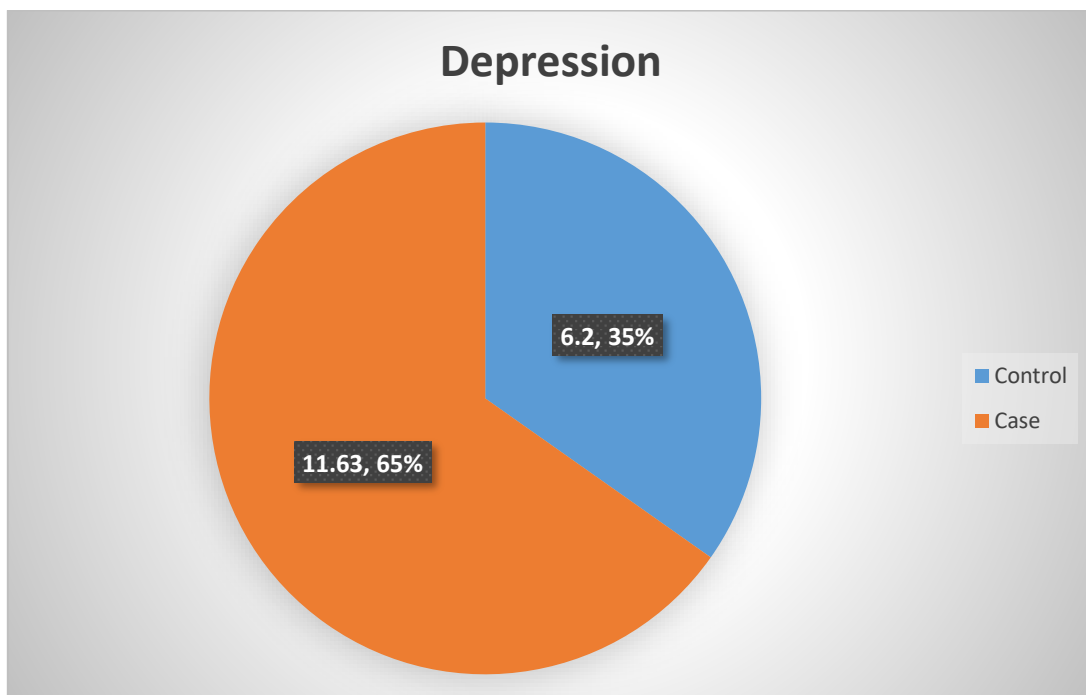
■ Control
■ Case

GRAPH 9- MEAN VALUE OF SALIVARY SUPEROXIDE DISMUTASE
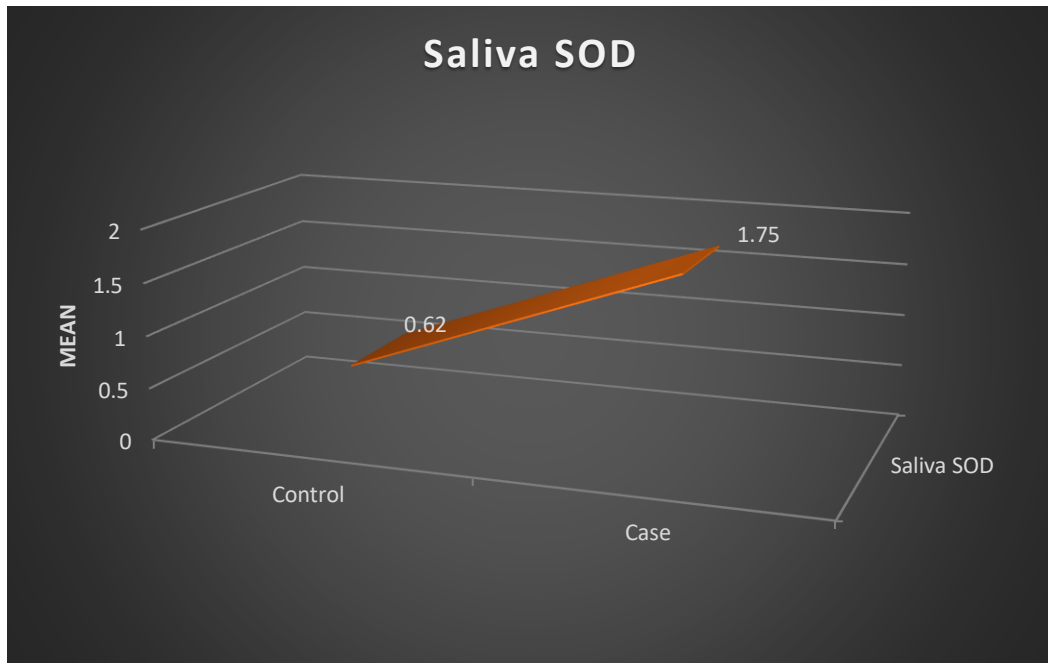
AMONG STUDY GROUPS

GRAPH 10- MEAN VALUE OF SERUM SUPEROXIDE DISMUTASE AMONG

STUDY GROUPS
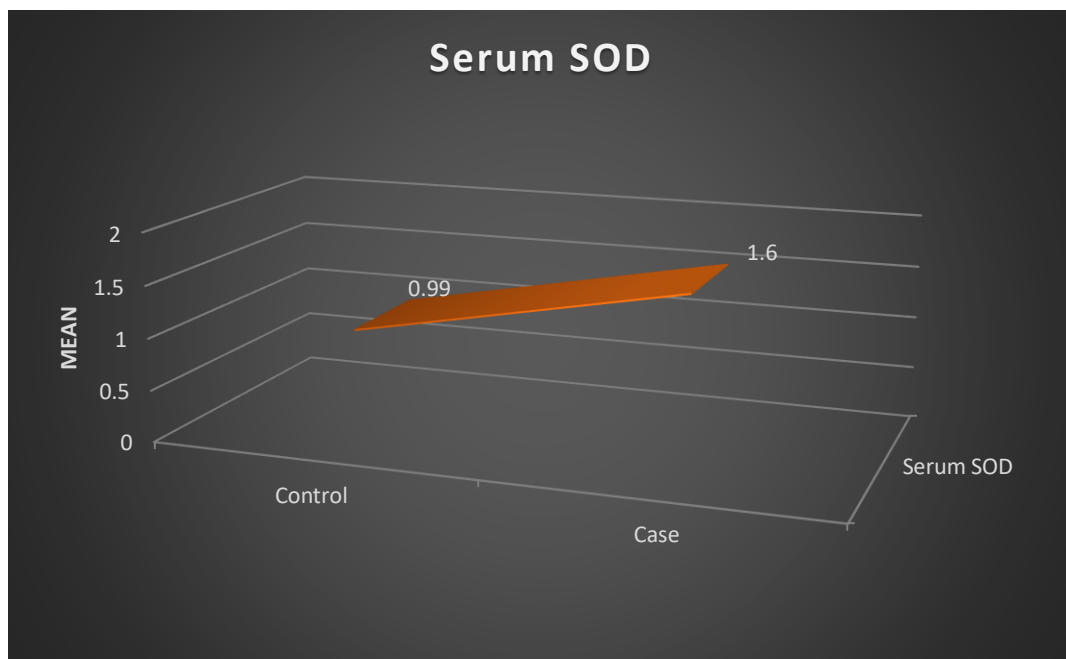
GRAPH 11- SCATTER DIAGRAM COMPARISON OF SALIVARY AND SERUM SOD LEVELS IN CONTROLS

Saliva SOD

GRAPH 12- SCATTER DIAGRAM COMPARISON OF SALIVARY AND SERUM SOD LEVELS IN CASES

Saliva SOD

DISCUSSION

Oxidative stress which is considered as an imbalance of the body's ability to scavenge free radical species, (both reactive oxygen species [ROS] and reactive nitrogen species [RNS]) was found to be associated with cancer by several studies. Moreover, the development of malignant process occurs through several steps involving initiation, promotion and progression. Premalignant lesions are the first clinically identified lesions of cancer. In t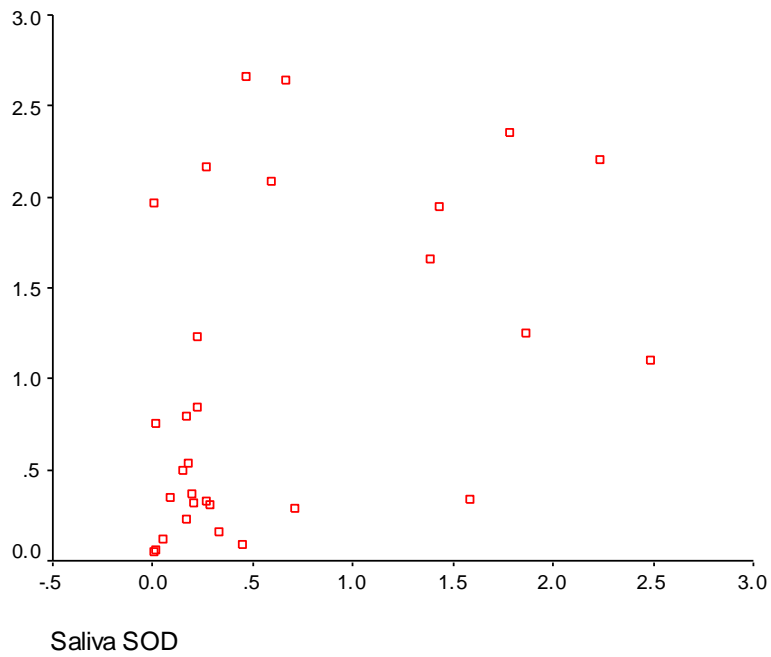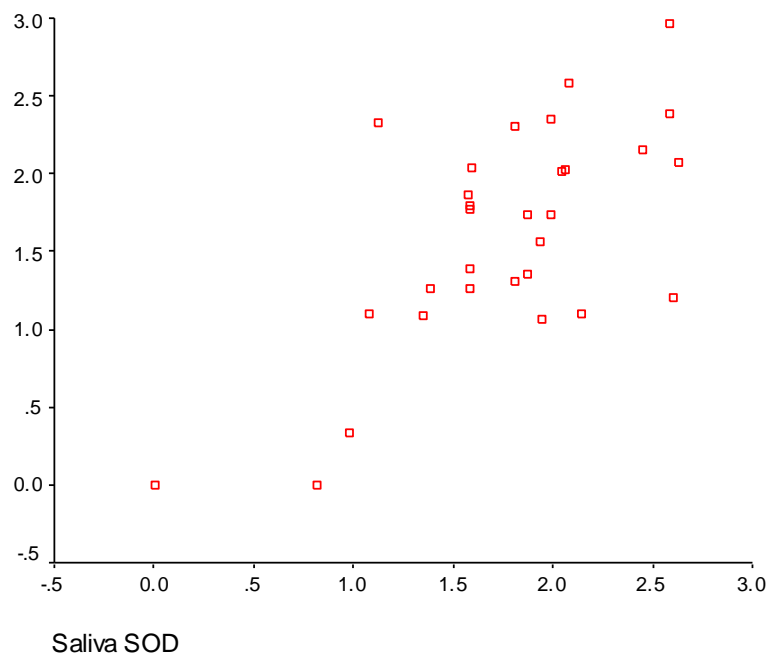he backdrop of increased prevalence of cancer, identification and management of premalignant stage seem to be important steps to prevent the development of cancer. Antioxidants (AOs) are known to neutralize ROS and RNS by several mechanisms.[30,31]

Psychological stress, associated with OLP has been linked with depressive and anxiety disorders (Andreasen 1968) reported that 12% of OLP patients (erosive form) suffer from borderline or morbid depression, while 50% of these patients have border or morbid anxiety. Although Allen et al. (1986) were not able to confirm a connection between anxiety level and OLP, they concluded that such a connection cannot be definitely denied[32,33].

Oxidative stress is greater in men than in women. It is found a significantly high increase in the serum levels of MDA and a highly significant decrease in the serum levels of erythrocyte CAT levels in the male patients when compared to the females. However, NO and SOD showed an insignificant difference between the sexes[34].

The mechanism by which females are thought to be more protected from the damaging effects of oxidative stress may be related to the antioxidant properties of

estrogens. Moreover, estradiol has been documented as having antioxidant effects. In some studies, a negative correlation was reported between SOD activity and the duration of other diseases for which ROS is thought to be involved in the pathogenesis[35].

The demographic variables in a study by Neena S. Sawant [36] revealed the mean age to be 45.15 ± 12.72 respectively, and the present study, the average is 45.87 ± 7.48 years, this proves that mean age of occurrence of oral lichen planus is 4th decade of life.

In a research by VR Rekha [37], The mean value of salivary SOD in the case group was 1.23 ± 0.34 and in the control group was 0.54 ± 0.26., in the present study the salivary SOD levels in case group is 1.75 ± 0.58 and controls is 0.62 ± 0.73, the mean values of salivary SOD were elevated in the patients with oral lichen planus in comparison to controls

The mean value of serum superoxide dismutase is elevated in levels among the cases with a value of 1.60 ± 0.58 in the present study, whereas in study by Sunita Tiwari et al, serum SOD levels is 0.83 ±0.01, the values were lower[38], which proves that Studies show that depending on gender, anxiety, stress or depression can elevate inflammation in your body and subsequently put you at higher risk for inflammatory related illnesses.

Serum SOD levels (18.19 ± 3.71 U∕mL) in patients with LP were also higher than in healthy controls (P ¼ 0.002) in a research conducted by Sezer et al.[39] and In a study conducted by D. G. Aly and R. S. Shahin [40], Serum levels of SOD was higher in LP patients with means ± SD of 17.33 ± 2.05, respectively, when compared to controls 15.44 ± 2.8,results of the present study revealed serum SOD levels of 1.60 ±

0.71 U ∕ mL in patients with lichen planus and the values in healthy controls was much decreased with values of 0.99 ± 0.87 U ∕ mL. These findings suggest that suggest that excessive ROS leading to lipid peroxidation may also be generated from inflammatory infiltrate composed of lymphocytes or histiocytes in LP upon immunological or non-immunological stimulation.[40]

The psychological characteristics of the study group with lichen planus in a study by Małgorzata Radwan-Oczko[41], the HADS Anxiety Score is 7.62 ± 4.08, HADS Depression Score HADS Depression Score is 5.05 ± 3.92, in the present study the HADS Anxiety score is 11.83 ± 2.18, HADS Depression score is 8.92 ± 4.930. Therefore, psychological profile and the psychopathological consequences of OLP in patients may be determined due to individual's perception of stress

In a study conducted on 30 patients with lichen planus by Abhishek Ranjan Pati et al [42] and Hampf et al.,[43], there was a higher mean HAD in the case group with oral lichen planus when compared to the control group. These results are consistent with results in the present study.

The mean HAD anxiety score was 8.0 (SD 4.14) and the mean depression score was 3.5 (SD 3.27) in a study on Psychological factors associated with oral lichen planus [46], in the present study, the mean HAD anxiety score is 11.83 (SD 2.18) and the mean depression score is 8.92 (SD 4.35)[44].

Hospital Anxiety and Depression Scale (HADS) were used to evaluate psychosocial stressors in a study by S Chaudhary[45], Mann Whitney U test of significance for two independent variables suggested a highly significant difference in depression level between the two control groups (Z=4.841; p <0.05, significant), and

the Z-value in the present study is Z= 4.906; suggests that psychological stressors play an important role in the causation of OLP.

Recent articles have confirmed that patients with OLP effectively suffer more anxiety and depression than control subjects and that anxiety may in turn aggravate the clinical manifestations of the disease[46].

SOD is an enzyme that removes the superoxide (O2 -) radical, repairs cells and helps to reduce the damage done to them by superoxide, which is the most common free radical in the body. This antioxidant is found in both the dermis and the epidermis, and is the key to the production of healthy fibroblasts which are the skin-building cells. SOD catalyzes the reduction of superoxide anions to hydrogen peroxide which can also be destroyed by catalase or GPx[47].

SOD plays a critical role in the defence mechanism of cells against the toxic effects of oxygen radicals. It competes with nitric oxide (NO) for superoxide anion, which inactivates NO to form peroxynitrite. Therefore, by scavenging superoxide anions, SOD promotes the activity of NO. Superoxide dismutase (SOD) is considered the first line defense against ROS, converting the superoxide anion (O2 −) into H2 O2. Glutathione peroxidase (GPx) catalyzes the reduction of hydrogen peroxide and lipid hydro peroxides. GPx in combination with catalase and SOD function to protect the cell from damage due to ROS[48].

The use of saliva as a "diagnostic tool" is an upcoming area of research. It offers the advantage over serum as the collection process of saliva is noninvasive. It can be performed easily and cost effectively in a number of clinically challenging situations such as obtaining samples from children, disabled or anxious patients, etc., in whom blood sampling could be a difficult act to perform. The results of our study

helped us to arrive at the conclusion that saliva can indeed be used as an excellent medium for biochemical analysis. Further studies on larger samples with more sensitive devices for more accurate detection of biochemical changes in saliva would probably elevate saliva as a diagnostic tool that is in par with the gold standard serum. If that becomes true, only saliva needs to be investigated for estimating the various biochemical parameters that indicate the development and progression of many systemic diseases[49,50].

SUMMARY

A study titled "Evaluation of Oxidative Stress Biomarker and Psychometric analysis in Oral Lichen Planus: A case-control study" was conducted in Department of Oral Medicine and Radiology at Vivekanandha Dental College for Women between 2017-2019. It is a case-control study with 30 individuals of oral lichen planus in the case group and 30 healthy individuals in the control group. Oral lichen planus cases were clinically diagnosed and histopathologically confirmed before inclusion in the study.

Blood was withdrawn under strict aseptic conditions, and whole unstimulated saliva was collected and centrifuged immediately. Salivary supernatant and serum was subjected to biochemical analysis using ELISA and colorimetric analysis was carried out. The serum and salivary superoxide dismutase levels were estimated for all the samples.

The following inferences were made in the present study:

- The mean anxiety levels were higher among cases when compared to controls, the difference between the groups is highly significant (p value = 0.001)

- The mean depression levels were higher among cases when compared to controls, the difference between the groups is highly significant (p value = 0.000)

- The mean salivary SOD levels were higher among cases when compared to controls, the difference between the groups is highly significant (p value = 0.001)

- The mean serum levels were higher among cases when compared to controls, the difference between the groups is highly significant (p value = 0.004)

In the future, researches should be done by considering larger sample size, follow-up of patients was not done in this study, Further research by including the follow-up of salivary SOD status can yield better understanding on the salivary biomarker level in premalignant and malignant states.

<u>CONCLUSION</u>

This study was carried out to assess the validity of salivary biomarker, Superoxide Dismutase for the detection of oxidative stress in oral lichen planus

The results suggest that there is a significant difference in mean salivary SOD values among the 2 groups. The salivary SOD is significantly expressed in the individuals with oral lichen planus. Therefore, salivary SOD can be used as a biomarker for the detection of oral lichen planus.

The detection of this enzyme can serve a potent diagnostic aid for the early detection of oxidative stress occurring in the oral cavity. Further studies are required to validate the results and use the test in the future

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<u>INFORMATION SHEET AND CONSENT FORM</u>

This informed consent is for the patients who attend the Vivekanandha Dental College for Women and who are willing to participate in research titled "Evaluation of Oxidative Stress Biomarker and Psychometric analysis in Oral Lichen Planus: A case-control study"

We are doing a research on Oxidative Stress Biomarker and Psychometric analysis in Oral Lichen Planus. I am going to give you information and invite you to be a part of this study. This involves collection of 2 ml of venous blood and 5 ml of unstimulated saliva and it's further analysis. Tissue biopsy will be done to confirm provisional diagnosis in case of pathology.

Informed consent form for patients

I consent to voluntarily to participate as a participant in this research.

Signature of Participant / Thumb Impression   **Date:**

PATIENT PROFORMA

Title of the study - Evaluation of Oxidative Stress Biomarker and Psychometric analysis in Oral Lichen Planus: case-control study

Date-

Patient identification

Name-

Age-

Gender-

Address-

Phone number-

Chief complaint

History of presenting illness-

Past Medical history-

Past Dental history-

Family history

Social history

Tobacco/alcohol consumption

Review of system-

INTRAORAL EXAMINATION

Inclusion criteria-

- Soft tissue examination (positive clinical characteristics according to modified WHO criteria 2003)

- Age ranging above 18 years.

Exclusion criteria-

- Patients with other co-exisiting mucosal lesions such as apthous ulcer, leukoplakia, OSMF.

- Patients under treatment modalities like topical/ systemic corticosteroids for OLP.

- Patients under drug therapy for anxiety, depression, sleep disorders.

- Patients with underlying significant systemic illness, Patients with tobacco, alcohol related habits.

Provisional Diagnosis-

Diagnostic Methodology

- ✓ Chair side questionnaire- Hospital Anxiety and Depression Scale
- ✓ Biopsy of tissue sample
- ✓ Saliva collection- unstimulated saliva of 5ml
- ✓ Serum collection-Venous blood of 2ml

Results of diagnostic information

Chair side questionnaire

Hospital Anxiety and Depression scale inference

Clinical laboratory studies

SOD levels in Saliva-

SOD levels in Serum-

Microscopic examination of tissue samples-

MASTER CHART FOR STUDY GROUP A- ORAL LICHEN PLANUS GROUP

S.no	Patient name	Age	Sex	Anxiety	Depression	Salivary SOD	Serum SOD
1.	Usharani.R	51	Female	11	6	0.821	0.001
2.	Gopal	43	Male	5	5	0.001	0.001
3.	Amaravathi	47	Female	13	17	1.386	1.265
4.	Mohana	44	Female	12	8	1.122	2.33
5.	Krishnaveni	47	Female	11	8	1.596	2,038
6.	Ananthi	40	Female	12	13	2.483	2.963
7.	Rangasamy	32	Male	11	9	1.586	1.793
8.	Sneha	35	Female	11	14	2.083	2.586
9.	Barkavi	43	Female	13	15	2.602	1.203
10.	Revathi	38	Female	12	11	1.583	1.389
11.	Kumarasamy	56	Male	11	13	2.586	2.381
12.	Duraisamy	45	Male	11	9	1.583	1.768
13.	Marimuthu	55	Male	12	11	1.573	1.866
14.	Sathya	46	Female	13	11	1.806	2.301
15.	Aravindh	45	Male	9	9	2.453	2.158
16.	Ravi	42	Male	14	14	1.986	1.735
17.	Hari	39	Male	12	13	1.876	1.358
18.	Velammal	50	Female	16	19	1.345	1.083
19.	Rangan	45	Male	11	11	1.948	1.063
20.	Ram	36	Male	8	12	1.807	1.305
21.	Rahul	56	Male	10	11	1.938	1.568
22.	Guruprasath	60	Male	12	14	2.631	2.073
23.	Mary	45	Male	11	11	1.987	2.346
24.	Jacob	40	Male	15	16	2.146	1.096
25.	Sadhasivam	57	Male	13	12	0.978	0.333
26.	Kamali	35	Female	11	11	1.076	1.105
27.	Lakshmi	50	Female	13	13	2.058	2.031
28.	Sivasankari	48	Female	15	11	1.874	1.735
29.	Valarmathi	46	Female	15	16	2.043	2.011
30.	Jaya	60	Female	12	6	1.584	1.257

MASTER CHART FOR STUDY GROUP B- HEALTHY CONTROL GROUP

S.no	Patient name	Age	Sex	Anxiety	Depression	Salivary SOD	Serum SOD
1.	Priyadharshini	20	Female	5	3	0.169	0.79
2.	Arshiya Fathima	21	Female	5	11	0.013	0.061
3.	Meghnaa Mithra	21	Female	6	6	0.018	0.759
4.	Kiruthiga.R	22	Female	8	9	0.006	0.051
5.	Kiruthiga.B	23	Female	7	11	0.446	0.093
6.	Placida Jency	23	Female	4	13	0.054	0.12
7.	Gowtham	19	Male	6	6	0.001	1.964
8.	Hari Manickam	18	Male	4	3	0.17	0.23
9.	Mouleeswaran	23	Male	1	3	2.489	1.101
10.	Vaishnavi	18	Male	3	7	1.384	1.654
11.	Varsha	19	Female	5	7	1.58	0.334
12.	Thamitha shree	19	Female	4	4	0.333	0.155
13.	Geethanjali	20	Female	3	3	0.594	0.288
14.	Jeeva Sneha	21	Female	4	3	0.15	0.497
15.	Shruthi Keerthana	21	Female	2	4	0.267	2.166
16.	Valarmathi	23	Female	3	3	0.176	0.54
17.	Ayesha	19	Female	5	5	0.705	0.284
18.	Shilpa	21	Female	10	8	0.089	0.343
19.	Hema Nivetha	20	Female	4	10	0.461	2.66
20.	Rabia	20	Female	2	5	0.191	0.37
21.	Sadhana	22	Female	3	4	1.778	2.353
22.	Mamthashree	22	Female	6	10	0.22	1.228
23.	Vijaya	49	Female	0	2	0.664	2.644
24.	Madhan	20	Male	4	7	0.201	0.313
25.	Sathya	27	Female	3	6	2.234	2.206
26.	Balaji	23	Male	0	0	0.263	0.33
27.	Mohanraj	18	Female	2	12	1.43	1.948
28.	Kowshik Boopathi	24	Male	8	10	0.284	0.311
29.	Anto .A	33	male	6	10	1.865	1.253
30.	Chinnasamy	43	Male	0	1	0.221	0.845