Clinical Evaluation, Oxidative Status and Apoptotic Activities Biomarkers in Serum and Saliva of Patients with Oral Lichen Planus

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in Oral Medicine

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بِسمِ اللهِ الرَّحمَنِ الرَّحِيمِ

قَالُوا سُبحانَكَ لا عِلمَ لَنَا إلا مَا عَلَّمتَنَا إنَّكَ أنتَ العَلِيمُ الحَكِيمُ

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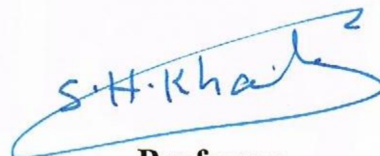

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To My Beloved Family

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ABSTRACT

Background:

Oral lichen planus is a T-cell-mediated chronic inflammatory disease in which the apoptosis of the basal cells of the oral epithelium triggered by cytotoxic $CD8^{+}$ T cells. In this regard, it has been proposed that a mechanism may be established that allows T lymphocytes to escape apoptosis, explaining the persistence of the infiltrates.

Oxidative stress caused by oxidant-antioxidant imbalance leads to damage of biological macromolecules and dysregulation of normal metabolism and physiology which may play a key role in the onset and development of several inflammatory oral pathologies.

Aims of the study:

The aim of this study was to investigate the diagnostic ability of saliva in comparison to serum regarding apoptotic activities biomarkers through the study of (soluble Fas, soluble Fas ligand and Bcl-2) and oxidative stress markers (Malondialdehyde and Nitric Oxide) and antioxidants (Ceruloplasmin, Uric Acid and Oxidized Glutathione) in reticular and erosive forms of oral lichen planus patients compared to apparently healthy individuals and to investigate if there is a relation between the clinical findings and the laboratory investigations under study.

Methods:

Forty eight (48) patients with histologically confirmed oral lichen planus were included in this study. The patients group was split up in to two groups according to the clinical presentation of the lesions, 21 patients with reticular form and 27 patients with erosive form all together compared with 32 healthy looking volunteers that were age-matched with the patients.

Serum and saliva samples had been taken from each subject for analysis. Severity of oral lichen planus has been calculated using the disease severity scoring sheet.

Results:

Most oral lichen planus patients had single-site involvement and the buccal mucosa was the most affected site (91.7%). Erosive lesions showed painful symptoms varied from mucosal sensitivity to continuous debilitating pain with a significantly severe lesion extension.

The present study showed that no statistically significant difference ($p>0.05$) has been found in the mean of serum apoptotic markers (soluble Fas, soluble Fas ligand and Bcl-2) in patients with oral lichen planus compared with control group. Also the mean of saliva soluble Fas in patients with oral lichen planus showed no statistically significant difference ($p>0.05$) compared with control group. Saliva soluble Fas ligand and saliva Bcl-2 were undetectable.

Regarding oxidative stress markers, the mean of serum (4.725 µmol/l) and saliva (0.972 µmol/l) Malondialdehyde in oral lichen planus patients group was significantly higher ($p<0.001$ and $p<0.05$) than that of control group, respectively (1.626 µmol/l and 0.732 µmol/l), whereas the mean of serum and saliva Nitric Oxide in patients with oral lichen planus showed no statistically significant difference ($p>0.05$) compared with controls.

With respect to antioxidant activities, the present study revealed that the mean of serum (0.408 g/l) and saliva (0.014 g/l) Ceruloplasmin in patients with oral lichen planus was significantly higher ($p<0.001$ and $p<0.01$) than that of control group, respectively (0.311 g/l and 0.009 g/l), while the mean of serum and saliva Uric Acid and Oxidized Glutathione in

patients with oral lichen planus showed no statistically significant difference ($p>0.05$) compared with controls.

The study showed that in oral lichen planus patients group there was no statistically significant correlation between serum and saliva measurements of all parameters except with Nitric Oxide which showed a positive highly significant linear correlation ($r=0.449$, $p<0.001$).

The present study found that there was no statistically significant differences were found in serum and saliva apoptotic and oxidative status biomarkers, when compared between reticular and erosive forms, with the exception of soluble Fas and Nitric Oxide in saliva which is significantly higher in erosive form, respectively ($p<0.01$ and $p<0.05$).

There was no statistically significant difference in serum and saliva parameters in relation to severity scores of reticular and erosive oral lichen planus patients, with the exception of serum Ceruloplasmin which was significantly higher in reticular oral lichen planus patients ($p<0.05$).

Conclusions:

Oxidative status play a role in the pathogenesis of oral lichen planus represented by increased serum and saliva Malondialdehyde and Ceruloplasmin levels. Apoptotic biomarkers (soluble Fas, soluble Fas ligand and Bcl-2) in serum and saliva are unreliable indicator of apoptosis in oral lichen planus patients. Saliva is not always a reliable indicator of the internal environment of the body. Salivary Nitric Oxide and sFas can be used as markers of disease activity. Number of affected site, extension of the lesion and intensity of pain did not exert a meaningful influence on the serum and saliva levels of apoptotic and most of the oxidative status parameters.

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List of abbreviations:

AICD	activation-induced cell death
AIF	apoptosis-inducing factor
AP-1	activator protein 1
Apaf-1	apoptotic protease activation factor-1
CAT	catalase
CP	Ceruloplasmin
Cyto c	cytochrome c
dATP	deoxyadenosine triphosphate
DED	death effector domain
DISC	death-inducing signal complex
ELISA	(Enzyme-Linked Immunosorbent Assay)
FADD	Fas-associated death domain
FasL	Fas ligand
GPx	glutathione peroxidase
GSH	glutathione
GSSG	Oxidized glutathione
HRP	Horseradish Peroxidase
H_2O_2	hydrogen peroxide
HSP	heat-shock protein
id	idiotype peptides
LOOH	lipid peroxides
MDA	Malondialdehyde
MIF	Migration inhibitory factor
MMP	Matrix metalloproteinase
MPTPs	Mitochondrion permeability transition pores
NB-UVB	Narrow band ultraviolet B phototherapy
NF-kappa B	Nuclear factor kappa-light-chain-enhancer of activated B cells
$NO^{\bullet}$	Nitric oxide
$NO2^{-}$	nitrite
$NO3^{-}$	nitrate
NSAIDs	Nonsteroidal anti-inflammatory drugs
$O2^{\bullet}-$	superoxide anion radical
OD	Optical density
$^{\bullet}OH$	hydroxyl radical
OLP	Oral lichen planus
ONOO−	peroxynitrite anion
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSH	thiol compounds
SCC	squamous cell carcinoma
SOD	superoxide dismutase
TBA	thiobarbituric acid
TBARS	thiobarbituric acid–reacting substances
TCA	trichloroacetic acid
TCRs	T cell receptors

TMB	Tetramethylbenzidine
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor
UA	Uric acid
Vitamin C	ascorbic acid
Vitamin E	α-tocopherol
WHO	World Health Organization

Introduction:

Oral lichen planus (OLP) is a T-cell-mediated chronic inflammatory oral mucosal disease of unknown etiology (Roopashree *et al.,* 2010).

Essentially there are two forms of oral lesions; reticular and erosive (Neville *et al.,* 2002). The atrophic, ulcerative, and bullous forms of the disease are referred to as erosive lichen planus (Silverman and Bahl, 1997).

Reticular pattern is the most frequent clinical presentation and appears in the form of a network of connections and overlapping white lines (Scully and Carrozzo, 2008) combined with a few symptoms and reflecting a milder stage of the disease (Karatsaidis et al., 2003; Karatsaidis et al., 2007). Erosive/ulcerative OLP constitute the most destructive form and causes a great oral discomfort (Karatsaidis *et al.*, 2007; Scully and Carrozzo, 2008).

Although the precise cause of OLP is unknown, its pathogenesis has been linked to an autoimmune mechanism involving autocytotoxic $CD8^+$ T cells, which trigger apoptosis of basal keratinocytes (Porter *et al.*, 1997; Eisen, 1999).

The abnormal cell apoptosis plays an important role in the pathogenesis of OLP (Deng *et al.*, 2009). Accelerated apoptosis of the keratinocytes that confined within the basal or suprabasal cell layer instead of the superficial cell layer (Shen *et al.*, 2004) and inhibition of lymphocyte apoptosis may coexist and contribute to the formation and progression of OLP (Shi *et al.*, 2010).

From the other hand, oxidative stress resulting from the increased production of free radicals and reactive oxygen species and/or a decrease in antioxidant defense leads to damage of biologic macromolecules and dysregulation of normal metabolism and physiology (Knight, 1998; Toyokuni, 1999) and may play a key role in the onset and development of several inflammatory oral pathologies (Battino *et al*., 2002).

In the present study, several parameters of apoptotic activities and oxidative status had been discussed as biomarkers which may open a window to potential mechanisms of the disease pathogenesis and provide insight into disease progression.

Aims of the study:

1- To evaluate apoptotic activities in serum and saliva of reticular and erosive forms of oral lichen planus patients in comparison with apparently healthy individuals through the study of soluble Fas, soluble Fas ligand and Bcl-2.

2- To estimate the oxidative stress markers (Malondialdehyde and Nitric Oxide) and antioxidants (Ceruloplasmin, Uric Acid and Oxidized Glutathione) in serum and saliva of these groups.

3- To investigate whether there is relation between the clinical findings and the laboratory investigations under study.

Chapter One
Review of Literature

Review of literature:

1.1. Oral lichen planus:

1.1.1. Definition:

Oral lichen planus (OLP) is a common chronic inflammatory immune-mediated disease, with an etiopathogenesis associated with cell-mediated immunological dysfunction (Arirachakaran *et al*., 2013). Oral lichen planus varies in appearance from keratotic (reticular or plaque like) to erythematous and ulcerative (Greenberg *et al*., 2008).

It was first described on the skin by Erasmus Wilson in 1869 who also recorded oral lesions in several patients. The first independent oral lesions were reported by Audry in 1894 (Isaac *et al*., 2003).

1.1.2. Etiopathogenesis:

During recent years, it has become more evident that the immune system has a primary role in the development of the oral lichen planus (Ghaleyani *et al*., 2012). This supported by the histopathologic characteristics of a subepithelial band-formed infiltrate dominated by T lymphocytes and macrophages and the degeneration of basal cells known as liquefaction degeneration. These features can be interpreted as an expression of the cell mediated arm of the immune system being involved in the pathogenesis of the OLP through T-lymphocyte cytotoxicity directed against antigens expressed by the basal cell layer (Greenberg *et al*., 2008).

Lichen planus is a chronic autoimmune disease that involves a type IV hypersensitivity reaction to antigen variations observed in the mucosal lining and skin (Mollaoglu , 2000; Ismail *et al*., 2007; Anuradha *et al*., 2008).

It is complicated to identify a single etiologic factor behind OLP. Other factors, such as stress, may also be of importance to establish the inflammatory process. It is not unusual that patients report that they have been exposed negative social events some months before to the onset of the disease (Greenberg *et al*., 2008).

During recent years, an association between OLP and hepatitis C virus (HCV) has been described in populations from Japan and some Mediterranean countries. This association has not been observed in northern European countries or the United States. Furthermore, no association has been reported from Egypt and Nigeria, which are countries with a very high HCV prevalence. It has been postulated that the association may be related to a genetic variability between countries. This is in part supported by the observation that specific alleles of the major histocompatibilty complex, such as HLA-DR6, are more prevalent in Italian patients with HCV-related OLP (Greenberg *et al*., 2008).

The lichen planus antigen is unknown, although the antigen may be a self peptide thus making lichen planus a true autoimmune disease. The role of autoimmunity in disease pathogenesis is supported by many autoimmune features of OLP including disease chronicity, adult onset, female predilection, association with other autoimmune diseases, occasional tissue type associations, depressed immune suppressor activity in OLP patients and

the presence of auto-cytotoxic T cell clones in lichen planus lesions (Sugerman *et al*., 1992; Sugerman *et al*., 1993; Sugerman *et al*., 2000).

Sugerman *et al*., (2002) identified up-regulated heat-shock protein (HSP) expression by OLP lesional keratinocytes. Up-regulated HSP expression by oral mucosal keratinocytes may be a common final pathway linking a variety of exogenous agents (systemic drugs, contact allergens, mechanical trauma, bacterial or viral infection) in the pathogenesis of OLP. In this context, HSP expressed by oral keratinocytes may be auto-antigenic in OLP (Sugerman *et al*., 1995). Susceptibility to OLP may result from dysregulated HSP gene expression by stressed oral keratinocytes or from an inability to suppress an immune response following self-HSP recognition (Sugerman *et al*., 1992).

Both antigen-specific and non-specific mechanisms are hypothesized to be involved in the pathogenesis of OLP. Antigen-specific mechanisms in OLP include antigen presentation by basal keratinocytes and antigen-specific keratinocyte killing by $CD8^+$ cytotoxic T cells. Non-specific mechanisms include mast cell degranulation and matrix metalloproteinase (MMP) activation in OLP lesions. These mechanisms may combine to cause T cell accumulation in the superficial lamina propria, basement membrane disruption, intra-epithelial T cell migration and keratinocyte apoptosis in OLP (Sugerman *et al*., 2002; Roopashree *et al*., 2010).

1.1.3. Epidemiology:

Lichen planus is a relatively common disorder, estimated to affect 0.5% to 2% of the general population (McCreary and McCartan, 1999). It is a chronic, inflammatory disease that affects mucosal and cutaneous tissues. Oral lichen planus (OLP) occurs more frequently than the cutaneous form and tends to be more persistent and more resistant to treatment (Mollaoglu, 2000). Lichen planus is one of the most common oral diseases that manifest itself in the oral cavity (Silverman and Bahl, 1997).

A retrospective study by Oliveira Alves *et al*., (2010) showed a relatively high prevalence of OLP in the population studied, with a predominance of the disease among middle-aged women (Brown *et al*., 1993; Ismail *et al*., 2007; Oliveira Alves *et al*., 2010). Children are only rarely affected (Jungell, 1991; Laeijendecker *et al*., 2005; Patel *et al*., 2005; Shekhar *et al*., 2010) characteristically the lesions are symmetrical, involving the buccal mucosa, tongue, gingiva, floor of the mouth, lips, and palate (Jungell and Malmstrom, 1996).

No relationship with tobacco or alcohol consumption was observed (Oliveira Alves *et al*., 2010).

1.1.4. Clinical features:

Lichen planus affects most frequently the oral mucosa, but it may also involve other mucosa and the skin (Farhi and Dupin, 2010).

Several types of OLP have been described, the two main types being reticular and erosive OLP (Mollaoglu, 2000). Oral lichen planus may

contain both red and white elements and provide, together with the different textures, the basis for clinical classification of this disorder. The white and red components of the lesion can be a part of the following textures; reticulum, papules, plaque-like, bullous, erythematous and ulcerative (Greenberg *et al.*, 2008).

The most known clinical characteristics of LP are lesions with fine crossed white-grayish lines, called Wickham's striae (Pindborg *et al.*, 1997). To establish a clinical diagnosis of OLP, reticular or papular textures have to be present. If, in addition, plaque-like, bullous, erythematous, or ulcerative areas are present, the OLP lesion is designated accordingly (Greenberg *et al.*, 2008).

The explanation of the different clinical manifestations of OLP is related to the magnitude of the subepithelial inflammation. A mild degree of inflammation may provoke the epithelium to produce hyperkeratosis, whereas more intense inflammation will lead to partial or complete deterioration of the epithelium, histopathologically perceived as atrophy, erosion, or ulceration (Greenberg *et al*., 2008).

The reticular form of OLP is the most common form (Xue *et al*., 2005; Oliveira Alves *et al*., 2010; Al-Bayati, 2012). It characterized by fine white lines or striae (Pindborg *et al*., 1997). The striae may form a network but can also show annular (circular) patterns (Figure 1-1). The striae often display a peripheral erythematous zone, which reflect the subepithelial inflammation. Although reticular OLP may be encountered in all regions of the oral mucosa, most frequently this form is observed bilaterally in the buccal

mucosa and rarely on the mucosal side of the lips. Reticular OLP can sometimes be observed at the vermilion border (Greenberg *et al*., 2008).

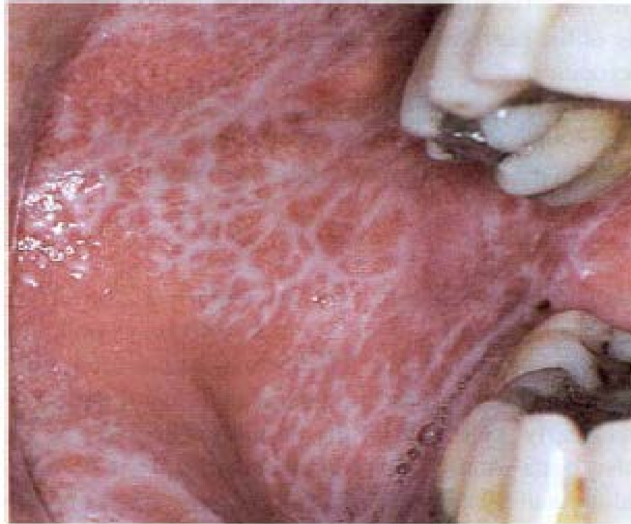


Figure (1-1): Lichen planus, striate pattern. This is the most common site and type of lesion, a lacy network of white striae on the buccal mucosa. The lesions are usually symmetrically distributed (Cawson and Odell, 2002)

The papular type of OLP is usually present in the initial phase of disease. It is clinically characterized by small white dots, which in most occasions intermingle with reticular form (Greenberg *et al*., 2008).

Plaque-type OLP shows homogeneous well demarcated white plaque often, but not always, surrounded by striae. Plaque-type lesions may clinically be very similar to homogeneous oral leukoplakias. The difference between these two mucosal disorders is simultaneous presence of reticular or papular structures in the case of plaque-like OLP. This form is most often encountered in smokers, and following cessation, the plaque may disappear and convert into the reticular type of OLP (Greenberg *et al*., 2008).

Erythematous (atrophic) OLP is characterized by a homogeneous red area. When this type of OLP is present in the buccal mucosa or in the palate, striae are frequently seen in the periphery (Pindborg *et al*., 1997; Greenberg *et al*., 2008).

Ulcerative lesions are the most disabling form of OLP. Clinically the fibrin-coated ulcers are surrounded by an erythematous zone frequently displaying radiating white striae (Figure 1-2, 1-3). This appearance may reflect a gradient of the intensity of subepithelial inflammation that is that is the most prominent at the center of the lesions. As for the erythematous form of OLP, affected patient complains of smarting sensation in conjunction with food intake (Greenberg *et al*., 2008).

The bullous form is very unusual but may appear as bullous structures surrounded by reticular network (Greenberg *et al*., 2008).

Erosive and bullous lesions of lichen planus occur in the severe form of the disease when extensive degeneration of the basal layer of epithelium causes a separation of the epithelium from the underlying connective tissue (Walsh *et al*., 1990). In some cases, the lesions start as vesicles or bullae, this has been classified as "bullous lichen planus"; in a majority of cases, the disease is characterized by ulcers and is called "erosive lichen planus." Both of these disorders are variations of the same process and should be considered together (Greenberg and Glick, 2003).

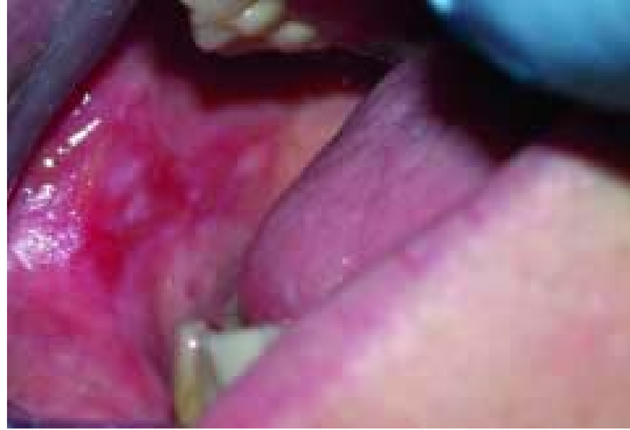


Figure (1-2): Erosive oral lichen planus involving the buccal mucosa (Edwards and Kelsch, 2002)

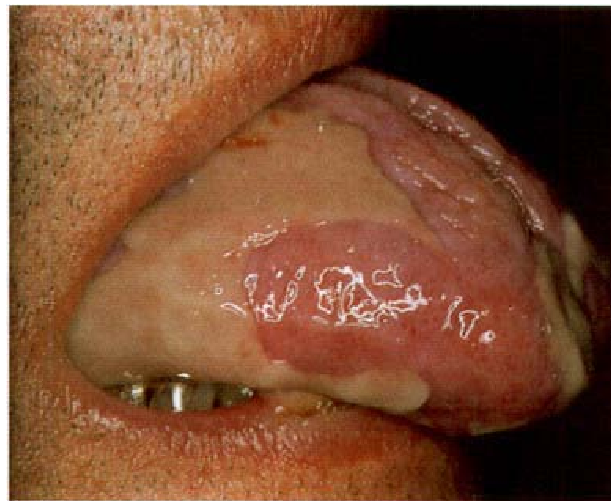


Figure (1-3): Severe erosive lichen planus. Thick plaques of fibrin cover extensive ulcers on the dorsum of the tongue (Cawson and Odell, 2002)

Atrophic or erosive lichen planus involving the gingivae results in desquamative gingivitis, a condition characterized by bright red edematous patches that involve the full width of the attached gingivae (Figure 1-4). Lichen planus must be distinguished histologically from other diseases that cause desquamative gingivitis, such as mucous membrane pemphigoid and pemphigus. A significant number of cases of erosive lichen planus present with a picture of desquamative (Greenberg and Glick, 2003).

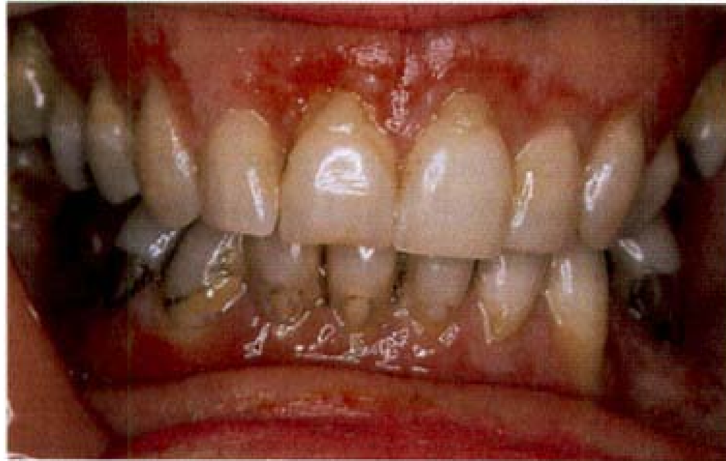


Figure (1-4): Desquamative gingivitis caused by lichen planus. A well defined band of patchy erythema extends across the full width of the attached gingiva around several teeth. This change may be localized or widespread. Within the red areas faint white flecks and striae are sometimes visible (Cawson and Odell, 2002)

Essentially there are two forms of oral lesions; reticular and erosive (Neville *et al*., 2002). The atrophic, ulcerative, and bullous forms of the disease are referred to as erosive lichen planus (Silverman and Bahl, 1997).

Approximately two thirds of OLP patients report oral discomfort (Eisen, 1993). Typically, the reticular, papular, plaque-like forms of OLP are asymptomatic, although the patient may experience a feeling of roughness (Greenberg *et al*., 2008). Most cases of symptomatic OLP are associated with atrophic (erythematous) or erosive (ulcerative) lesions. Symptoms vary from mucosal sensitivity to continuous debilitating pain (Rojo-Moreno *et al*., 1998).

1.1.5. Histopathology:

Oral lichen planus is diagnosed clinically by means of a biopsy for histopathological analysis (Ismail *et al*., 2007).

The histological criteria include the existence of a band of lymphocytic inflammatory infiltrate in the subepithelial connective tissue, hydropic degeneration of the basal layer and the absence of epithelial dysplasia (Figure 1-5; 1-6). If the above three criteria are met, the lesion is considered a typical lichen planus from a histological perspective; and as for those that do not meet one of the histological criteria, they are considered to be lesions that are histologically compatible with lichen planus (González *et al*., 2011).

The differential diagnosis between lichen planus and lichenoid reaction will be based on the combination of the clinical and histological aspects previously mentioned. Thus, all of the clinical and histological criteria must be met in the case of lichen planus. Conversely, lichenoid reaction includes patients with typical lichen planus clinically but not histologically, patients with typical lichen planus histologically but not clinically, and patients who are both clinically and histologically only compatible with lichen planus (Van der Meij and Van der Waal, 2003).

Degenerating basal keratinocytes form colloid (Civatte, hyaline, cytoid) bodies that appear as homogenous eosinophilic globules. The ultrastructure of colloid bodies suggests that they are apoptotic keratinocytes (Dekker *et al.*, 1997; Shimizu *et al.*, 1997; Bloor *et al.*, 1999; Neppelberg *et al.*, 2001).

Degeneration of basal keratinocytes and disruption of the epithelial basement membrane and basal keratinocyte anchoring elements in OLP produce weaknesses at the epithelial-connective tissue interface which may result in histological cleft formation (Max-Joseph space) and, rarely, clinical blistering of the oral mucosa (bullous lichen planus) (Sugerman and Savage, 2002).

Parakeratosis, acanthosis, and "saw-tooth" rete peg formation may be seen (Sugerman and Savage, 2002) (Figure 1-7). B-cells and plasma cells are infrequent in OLP, and immunoglobulin and complement deposits are not a consistent feature. Some cases show fibrinogen and fibrin deposition in a linear pattern in the basement membrane zone. Colloid bodies may be positive for fibrin, IgM, C3, C4, and keratin. Laminin and fibronectin staining may be absent in areas of heavy fibrin deposition and colloid body formation, suggesting basement membrane damage in these areas. Immunofluorescent findings in OLP are not diagnostic (Scully *et al.*, 1998).

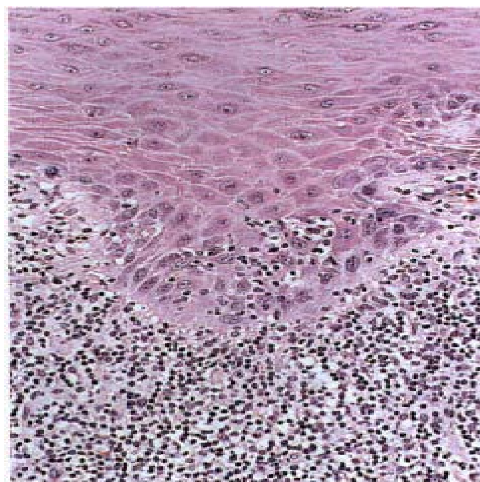


Figure (1-5): The basement membrane is thickened and lymphocytes from the dense infiltrate below emigrate into the basal cells of the epithelium where they are associated with focal basal cell degeneration (Cawson and Odell, 2002)

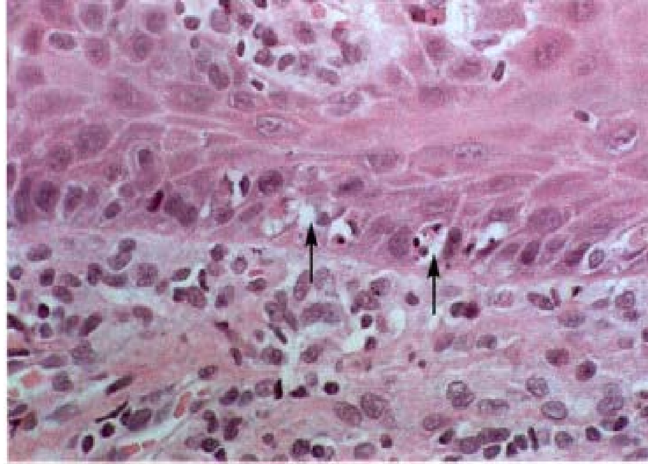


Figure (1-6): Lymphocytes infiltrating the basal cells are associated with basal cell apoptosis, loss of a prominent basal cell layer and prickle cells abutting the basement membrane. A cluster of apoptotic bodies is visible (arrows), each consisting of a shrunken bright pink cell with a condensed and fragmented nucleus (Cawson and Odell, 2002)

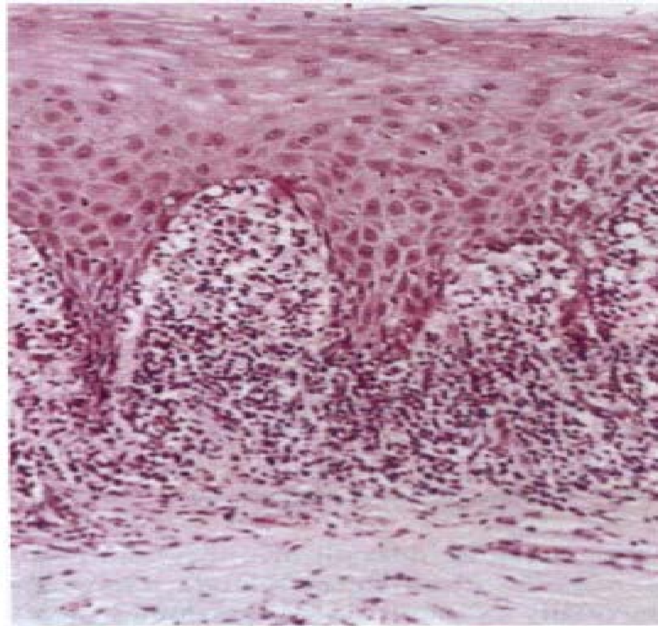


Figure (1-7): The rete ridges have the characteristic pointed (saw-tooth) outline which is frequent in the skin but uncommon in mucosal lichen planus (Cawson and Odell, 2002)

Erosions merely show destruction of the epithelium, leaving only the fibrin-covered, granulating connective tissue floor of the lesion. Diagnosis depends on seeing atrophic lesions or striae nearby (Cawson and Odell, 2002). Biopsy of the erosive lesions shows hydropic degeneration of the

basal layer of epithelium. This can help to distinguish it from mucous membrane pemphigoid, which is also a subepithelial lesion but which shows an intact basal layer, or from pemphigus vulgaris, in which acantholysis is demonstrated (Greenberg and Glick, 2003).

Typical histological features of atrophic lesions are; severe thinning and flattening of the epithelium, destruction of basal cells, and compact band-like, subepithelial inflammatory infiltrate hugging the epitheliomesenchymal junction (Cawson and Odell, 2002).

1.1.6. Diagnosis:

The diagnostic criteria for OLP are based on clinical and histopathologic features of the condition (Van Der Meij and Van Der Waal, 2003).

The diagnosis of reticular lichen planus can often be made based on the clinical findings alone. The interlacing white striae appearing bilaterally on the posterior buccal mucosa are virtually pathognomonic. Erosive lichen planus is sometimes more challenging to diagnose (based on clinical features alone) than the reticular form. If the typical radiating white striae and erythematous, atrophic mucosa are present at the periphery of well-demarcated ulcerations on the posterior buccal mucosa, the diagnosis can sometimes be rendered without the support of histopathologic findings. However, a biopsy is often necessary to rule out other ulcerative or erosive diseases (Neville *et al*., 2002).

Seifi *et al*., (2010) showed that there is a good agreement between the clinical and pathological diagnosis of oral lesions and the study showed 86.3% clinico-pathological agreement in lichen planus.

1.1.7. Lichenoid Reactions:

Lesions of OLP often present a diagnostic dilemma. In addition to the patterns and forms enumerated above, a number of lesions can simulate OLP and are designated as "lichenoid" (Myers *et al*., 2002). Oral lichenoid lesions can follow the administration of a systemic drug (Table 1-1), placement of a dental restoration, or provision for a denture (Sugerman *et al*., 2000).

Flavorings, especially cinnamates in toothpaste, may also trigger lichenoid contact sensitivity reactions (Porter and Scully, 2000; Yiannis *et al*., 2000).

Most of these lesions are unilateral and usually regress after removal of the causative (or precipitating) factor. In general, bilateral lesion distribution is a key clinical diagnostic feature of OLP, while unilateral distribution is a detractor rendering true OLP less likely (Eisenberg, 1994; Yiannis *et al*., 2000). In many cases, a cause for the oral lichenoid lesions cannot be identified and the diagnosis by exclusion is “idiopathic OLP” (Sugerman and Savage, 2002).

Some features which suggest a drug reaction are; onset associated with starting a drug, unilateral lesions or unusual distributions, unusual severity, widespread skin lesions, and localized lesion in contact with restoration (Cawson and Odell, 2002).

A drug history can be one of the most important aspects of the assessment of a patient with an oral or oral-and-skin lichenoid reaction. Drug-induced lichenoid reactions may resolve promptly when the offending drug is eliminated. However, many lesions take months to clear; in the case of a reaction to gold salts, 1 or 2 years may be required before complete resolution (Greenberg and Glick, 2003).

Histopathologically, lichenoid drug eruptions may show a deep as well as superficial perivascular lymphocytic infiltrate rather than the classic bandlike infiltrate of lichen planus, and eosinophils, plasma cells, and neutrophils may also be present in the infiltrate (Greenberg and Glick, 2003).

Table 1-1: Medications associated with mucosal lichenoid reactions (Bernstein, 1999)

Drug class
Antimalarials Hydrochloroquine Quinidine Quinine
Nonsteroidal anti-inflammatory drugs (NSAIDs) Indomethacin Naproxen Phenylbutazone
Diuretics Furosemide Hydrochlorothiazide
Antihypertensives *Angiotensin-converting enzyme inhibitor*s Captopril Enalapril *Beta-blockers* Propranolol
Antibiotics Penicillin Sulfonamides Tetracycline
Antifungals Ketoconazole
Heavy metals Bismuth Chromium Mercury Nickel
Miscellaneous Allopurinol Carbamazepine Lithium Lorazepam Methyldopa Oral contraceptives

1.1.8. Cutaneous form:

Lichen planus is a common skin disease but skin lesions are uncommon in those who complain of oral symptoms (Cawson and Odell, 2002). Oral lichen planus occurs more frequently than the cutaneous form and tends to be more persistent and more resistant to treatment (Nagpal *et al*., 2011). Cutaneous lesions may be encountered in approximately 15% of patients with OLP (Greenberg *et al*., 2008).

Although cutaneous and oral lichen planus share similar histopathological features, oral LP often follows a recalcitrant course while LP skin lesions tend to be self-limiting (Lage *et al*., 2011).

Skin lesions typically form purplish papules; 2-3 mm across with a glistening surface marked by minute fine striae and are usually itchy (Cawson and Odell, 2002). The papules may be discrete or coalesce to form plaques. The patients report relief following intense scratching of the lesions but trauma may aggravate the disease, which is referred to as a Koebner phenomenon (Greenberg *et al*., 2008). Typical sites are the flexor surface of the forearms and especially the wrists. Skin lesions help, but are not essential, to confirm the diagnosis of oral lichen planus (Cawson and Odell, 2002). (Figure 1-8)

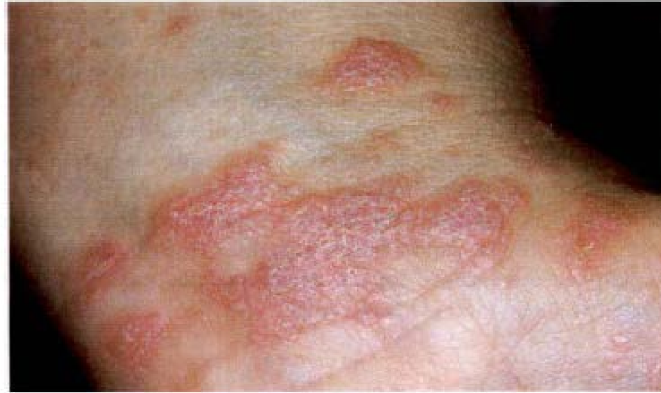


Figure (1-8): Dermal lichen planus. The flexor surface of the wrists is a characteristic site. The lesions consist of confluent papules with a pattern of minute white striae on their surface. (Cawson and Odell, 2002)

1.1.9. Treatment:

There is no known cure for OLP (Rhodus *et al*., 2003; Sahebjamee and Arbabi-Kalati, 2005; Sharma *et al*., 2008; Dalirsani *et al*., 2010) therefore, the management of symptoms guides therapeutic approaches. Corticosteroids have been the most predictable and successful medications for controlling signs and symptoms. Topical and/or systemic corticosteroids are prescribed electively for each patient after orientation to OLP and by patient choice (Greenberg and Glick, 2003). Alternative therapies include topical and systemic retinoids, griseofulvin, cyclosporine, and surgery (Eisen, 1993).

Lesions of OLP undergo periods of exacerbation and quiescence. The quiescent, asymptomatic reticular and plaque forms do not warrant pharmacological intervention, while the erythematous and erosive forms are associated with a high degree of morbidity. Treatment is therefore aimed at reducing the severity and length of these episodic outbreaks (Rhodus *et al*., 2003).

Oral lichen planus patients should be reviewed every month during active treatment and lesions monitored for reduction in mucosal erythema and ulceration and alleviation of symptoms. Active treatment should be continued and alternative therapies tried until erythema, ulceration and symptoms are controlled. The elimination of mucosal erythema and ulceration leaving residual asymptomatic reticular or papular lesions may be considered an end-point of current OLP therapy (McCartan and McCreary, 1999).

Due to the chronicity of OLP and the increased risk of oral cancer, OLP patients should be reviewed at least six-monthly and lesions re-biopsied as indicated by their clinical presentation and previous histological findings. Oral lichen planus patients should be advised to attend whenever there is an exacerbation of symptoms or a change in lesion presentation (Sugerman and Savage, 2002).

Topical corticosteroids are the mainstay in treating mild to moderately symptomatic lesions (Edwards and Kelsch, 2002). A usual course of steroid therapy should not exceed 10-14 days, with an interim period approximately the same before re-instituting the steroid therapy. Steroid therapy may include any of the following;

Low: Hydrocortisone gel or ointment, 1.0% (Rhodus *et al*., 2003)

Intermediate: Betamethasone valerate ointment 0.1% Triamcinolone acetonide ointment 0.1% (Rhodus *et al*., 2003)

Potent: Dexamethasone oral rinse, 0.5 mg/5 ml (Rhodus *et al*., 2003), Fluocinonide gel, 0.05% (Voûte *et al*., 1993; Greenberg and Glick, 2003; Rhodus *et al*., 2003).

Ultra-potent: Clobetasol propionate ointment, 0.05% (Lozada-Nur *et al*., 1991; Rhodus *et al*., 2003), Halobetasol propionate ointment, 0.05% (Rhodus *et al*., 2003).

Systemic steroids are rarely indicated for brief treatment of severe exacerbations or for short periods of treatment of recalcitrant cases that fail to respond to topical steroids (Greenberg and Glick, 2003). Interestingly, topical corticosteroids have been found to be equally or more effective than systemic corticosteroids or the combination of the two (Silverman *et al*., 1991; Carbone *et al*., 2003).

1.1.10. Potential for Malignancy:

It is increasingly believed that inflammatory cells and cytokines in the stroma might contribute more to tumour development and progression than to an effective antitumour response (Balkwill and Mantovani, 2001; O'Byrne and Dalgleish, 2001).

Oral lichen planus is one of the most prevalent oral mucosal lesions with an increased potential for malignant development (Mattsson, 2002).

Its malignant transformation is controversial (Gonzalez-Moles *et al*., 2008) ranging from 0.4 to 5.6% (Lo Muzio *et al*., 1998; Scully *et al*., 1998). The mean length of time for OLP to transform to squamous cell carcinoma (SCC) is 7.2 years (Figure 1-9) (Lozada-Nur and Miranda, 1997; Onofre *et al*., 1997). While this issue is still discussed, patients with LP should be

strictly followed-up to allow the early diagnosis of a possible malignant lesion (Gonzalez-Moles *et al*., 2008).

Oral lichen planus has by the World Health Organization (WHO) been classified as a precancerous lesion (Nylander *et al*., 2011). There was insufficient evidence to conclude about the potentially premalignant nature of OLP (Axelrod *et al*., 2006).

The cause of increased oral cancer risk in OLP patients is unknown, although the oral mucosa affected by OLP may be compromised to the extent of being more sensitive to exogenous mutagens in tobacco, alcohol, betel quid and *Candida albicans*. Alternatively, the chronic inflammatory response and simultaneous epithelial wound healing response in OLP may increase the likelihood of cancer-forming gene mutations. The latter hypothesis is supported by recent important findings that link chemical mediators of T cell inflammation to tumourigenesis. These studies showed that macrophage migration inhibitory factor (MIF), released from T cells and macrophages, suppresses the transcriptional activity of the p53 tumour suppressor protein (Hudson *et al*, 1999).

Normal p53 function is central to the prevention of many cancers including oral SCC (Sugerman and Savage, 1999). Hence, blocking p53 function by MIF (and possibly other inflammatory mediators) may underlie the increased risk of oral cancer in OLP patients (Sugerman and Savage, 2002).

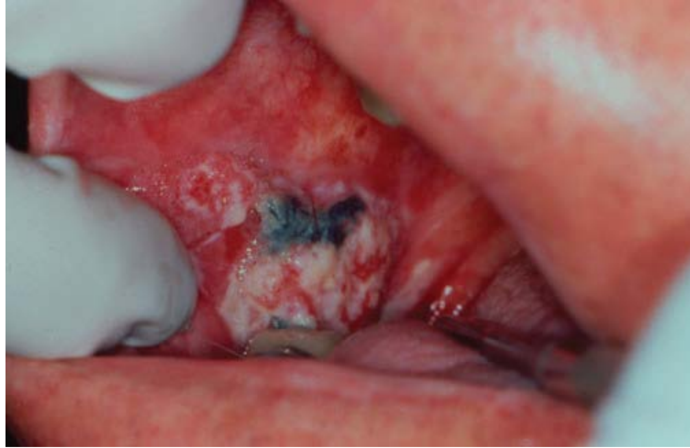


Figure (1-9): Squamous cell carcinoma forming on the buccal mucosa of a patient with erosive lichen planus (Greenberg and Glick, 2003)

1.2. Apoptosis:

Apoptosis is a regulated process of physiological cell death in which cells are triggered to activate intracellular proteins that lead to the death of the cell. Apoptosis results from the action of intracellular cysteine protease called caspases which are activated following cleavage and lead to endonuclease digestion of DNA and disintegration of the cell skeleton. There are two major pathways by which caspases can be activated. The first is by signaling through membrane proteins such as Fas receptor via their intracellular death domain. An example of this mechanism is shown by activated cytotoxic T cells expressing Fas ligand which induce apoptosis in target cells. The second pathway is via the release of cytochrome c from mitochondria which then activates caspases. DNA damage induced by irradiation or chemotherapy may act through this pathway. The protein P53 has an important role in sensing DNA damage. It activates apoptosis by

raising the cell level of Bax which then increases cytochrome c release (Hoffbrand *et al*., 2006).

1.2.1. Death receptor-mediated procaspase-activation pathway:

Cell death signals, such as Fas ligand (FasL) and tumor necrosis factor (TNF)-2, can be specifically recognized by their corresponding death receptors, such as Fas or TNF receptor (TNFR)-1, in the plasma membrane. Their binding will in turn activate the death receptors. Fas can bind to the Fas-associated death domain (FADD) (or TNFR-associated death domain, TRADD) and cause FADD aggregation and the emergence of the death effector domain (DED). These exposed DEDs interact with the DEDs in the prodomain of procaspase-8, which will induce the oligomerization of procaspase-8 localized on the cytosolic side of the plasma membrane. The complex of Fas, FasL, FADD and pro-caspase-8 is called the death-inducing signal complex (DISC). In DISC, two linear subunits of procaspase-8 compact to each other followed by procaspase-8 autoactivation to caspase-8 (Fu and Fan, 2002; Arnoult *et al*., 2003; Lü CX *et al*., 2003; Wang *et al*., 2005).

The activation of the downstream pathways of caspase-8 varies with different cell types. In Type I cells (cells of some lymphoid cell lines), caspase-8 is vigorously activated and can directly activate the downstream procaspases (e.g. procaspase-3) (Fu and Fan, 2002; Arnoult *et al*., 2003; Lü CX *et al*., 2003; Wang *et al*., 2005).

In Type II cells (other than Type I cells), caspase-8 is only mildly activated and unable to activate procaspase-3 directly. However, it can

activate the mitochondrion-mediated pathway by truncating Bid (a pro-apoptotic Bcl-2 family member), a kind of proapoptotic protein in the cytosol, into its active form, (tBid). tBid will trigger the activation of the mitochondrion pathway: cytochrome c, apoptosis-inducing factor (AIF) and other molecules are released from mitochondria, and apoptosis will be induced (Fu and Fan, 2002; Arnoult *et al*., 2003; Lü CX *et al*., 2003; Wang *et al*., 2005).

The activation pathway mediated by procaspase-10 is similar to that mediated by procaspase-8. Caspase-10 functions mainly in the apoptosis of lymphoid cells (Wang *et al*., 2001). Figure (1-10)

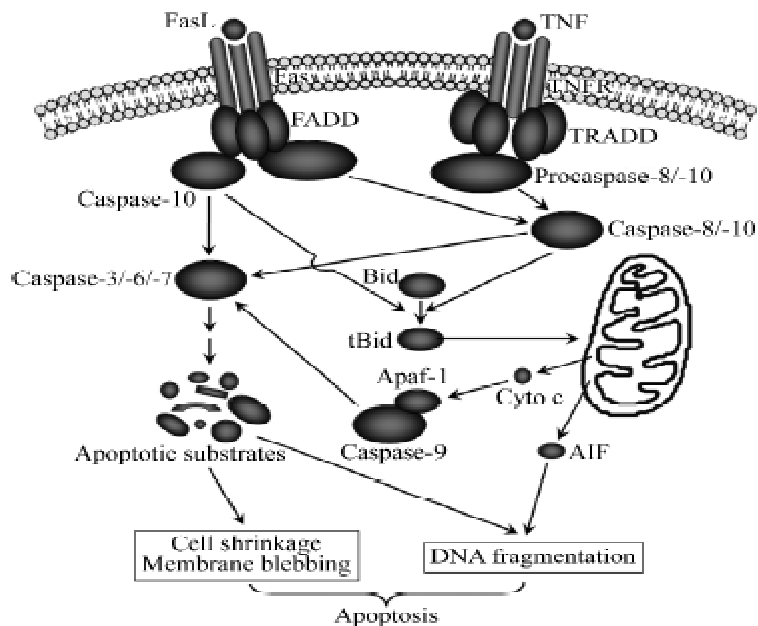


Figure (1-10): Caspase-8/caspase-10-dependent procaspase-activation pathway (Fan *et al*., 2005) *AIF, apoptosis-inducing factor; Apaf-1, apoptotic protease activation factor-1; Cyto c, cytochrome c; FADD, Fas-associated death domain; TNF, tumor necrosis factor; TNFR, TNF receptor; TRADD, TNFR-associated death domain.*

1.2.2. Mitochondrion-mediated procaspase-activation pathway:

When cellular stress occurs, proapoptotic proteins in the cytosol will be activated, which will in turn induce the opening of mitochondrion permeability transition pores (MPTPs). As a result, cytochrome c localized in mitochondria will be released to the cytosol. With the presence of cytosolic dATP (deoxyadenosine triphosphate) or ATP, apoptotic protease activation factor-1 (Apaf-1) oligomerizes. Together with cytosolic procaspase-9, dATP and cytochrome c, oligomerized Apaf-1 can result in the formation of a massive complex known as apoptosome. The apoptosome-bound procaspase-9 is activated (Fan *et al.*, 2001; Arnoult *et al.*, 2003).

Activated caspase-9 can in turn activate procaspase-3 and procaspase-7. The activated caspase-3 will then activate procaspase-9 and form a positive feedback activation pathway (Fan *et al.*, 2005). Figure (1-11)

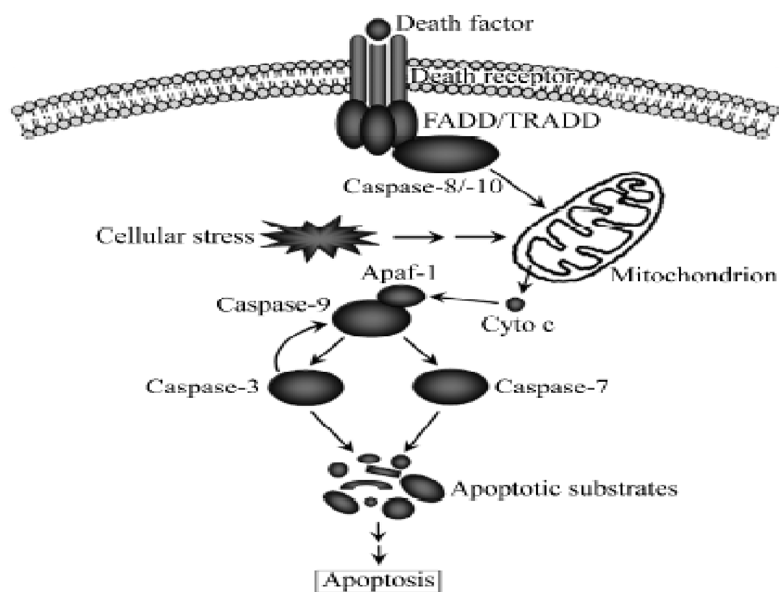


Figure (1-11): Typical mitochondrion-mediated and caspase-dependent pathway (Fan *et al.*, 2005) *Apaf-1, apoptotic protease activation factor-1; Cyto c, cytochrome c; FADD, Fas-associated death domain; TRADD, tumour necrosis factor receptor-associated death domain.*

1.2.3. Bcl-2 family proteins

The members of the Bcl-2 family are a group of crucial regulatory factors in apoptosis. According to functional and structural criteria, the members can be divided into two groups. Group I proteins are all anti-apoptotic proteins, including A1/Bfl1, Bcl-2, Bcl-w, Bcl-xL, Boo/Diva, Mcl-1, NR-13 and Nrf3 in mammals, BHRF-1, E1B19K, Ks-Bcl-2, LMW5-HL and ORF16 in bacteria, and Ced-9 in *C. elegans* (Gross *et al*., 1999; Fu and Fan, 2002; Milosevic *et al*., 2003).

Group II proteins are all proapoptotic proteins, including Bad, Bak, Bax, Bcl-rambo, Bcl-xS, Bid, Bik, Bim, Blk, BNIP3, Bok/Mtd, Hrk and Nip3 in mammals, and Egl-1 in *C. elegans* (Milosevic *et al*., 2003).

Bax and Bak, originally localized in the cytoplasm, can translocate to the mitochondrial outer membrane after an apoptotic program starts. Following the translocation, they will undergo conformation changes, oligomerization and insertion into the mitochondrial outer membrane to elevate the permeability of MPTPs. Group I proteins can bind selectively to the active conformation of Bax to prevent it from inserting into the mitochondrial outer membrane to maintain the normal permeability of MPTPs, and prevent the release of mitochondrial proapoptotic factors, such as cytochrome c, AIF and Smac/DIABLO (Fan *et al*., 2001; Lü *et al*., 2003).

Through cytochrome c, AIF, and others, Bcl-2 family proteins can indirectly regulate the activity of caspases in related apoptotic pathways (Fan *et al*., 2001).

1.2.4. Fas and Fas Ligand:

Nineteen cytokines have been identified as part of the TNF family on the basis of sequence, functional, and structural similarities (Sun and Fink, 2007).

Fas (APO-1, CD95) is a type I integral membrane protein (Smith *et al*., 1994; Nagata and Golstein, 1995), and a member of the tumour necrosis factor (TNF) receptor family. Fas Ligand (FasL/APO-1, CD95L) is a type II integral membrane protein and a member of the TNF family, which includes TNF-α, TNF-β, and the ligands for CD27 (CD70) (Lens *et al*., 1996) and CD40 (CD154) (Clark *et al*., 1996).

Interaction between Fas positive cells and FasL positive cells caused intracellular signal transduction in the former cells, which induces apoptosis of the Fas positive cells (Hanabuchi *et al*., 1994; Arase *et al*., 1995). Fas is expressed on various immunocytes including lymphocytes when they are activated, and FasL is expressed on activated T cells and NK cells (Suda *et al*., 1993; Suda *et al*., 1995).

In the immune system, programmed cell death has been shown to play a critical role in both T and B cell development as well as in the homeostatic regulation of mature peripheral lymphoid populations (Cohen *et al*., 1992). In particular, apoptotic signaling through the TNF family receptor Fas (CD95) and its ligand (CD95L) is central to the regulation of mature lymphocyte growth and differentiation (Nagata and Golstein. 1995). This is evidenced by the abnormal peripheral lymphocyte expansion and multiple autoimmune manifestations of mice or humans bearing mutant forms of either Fas or its ligand (Watanabe-Fukunaga *et al*., 1992; Lynch *et al*., 1994;

Takahashi *et al*., 1994; Fisher *et al*., 1995; Drappa *et al*., 1996; Wu *et al*., 1996; Bettinardi *et al*., 1997).

1.2.5. Soluble Fas and FasL:

Fas and FasL also exist in soluble forms (sFas, sFasL), which can be produced de novo or from conversion of the membrane-bound form by a metalloproteinase (Cascino *et al*., 1995; Kayagaki *et al*., 1995). Both sFasL and sFas may also possess proinflammatory properties, in addition to their apoptotic roles (Joashi *et al*., 2002). Proinflammatory cytokine release is attenuated by pretreatment with steroids, which has potentially beneficial clinical implications (Kawamura *et al*., 1999; Bronicki *et al*., 2000).

Joashi *et al*., (2002) reported that sFas may be a marker of inflammation. This is consistent with other reports, in which sFas has been shown to be elevated in conditions involving an active inflammatory process (Emmenegger *et al*., 2000; Fiorina *et al*., 2000; Kuwano *et al*., 2000; Papathanassoglou *et al*., 2000; Sliwa *et al*., 2000).

Soluble Fas is usually generated by alternative mRNA splicing and lacks a transmembrane domain. By binding to FasL, sFas is thought to inhibit cell-surface Fas-FasL signaling and downregulate Fas-mediated apoptosis (Owen-Schaub *et al*., 1995). This apoptotic Fas-FasL pathway may play a role in tumorogenesis and disease progression (Owen-Schaub *et al*., 1995; Thompson, 1995). The first experimental evidence that the loss of Fas can enhance tumor development has recently been reported (Zornig *et*

al., 1995). Because sFas can functionally antagonize FasL to effectively inactivate cell-surface Fas function, it is conceivable that elevated sFas production may promote tumorigenesis and disease progression (Konno *et al.*, 2000).

Activated lymphocytes have been shown to release soluble forms of FasL (sFasL), which is capable of inducing apoptosis of Fas positive cells (Kayagaki *et al*., 1995; Tanaka *et al*., 1995). Several lines of evidence suggest that sFasL may be involved in the pathogenesis of tissue injury (Matute-Bello *et al*., 1999). Circulating sFasL is elevated in the serum of patients with leukemia (Tanaka *et al*., 1996), lymphoma (Sato *et al*., 1996; Tanaka, *et al*., 1996), and inflammatory diseases (Nozawa *et al*., 1997). Blockade of Fas (CD95) in humans with toxic epidermal necrolysis stops the progression of disease (Viard *et al*., 1998).

From the other hand, the shedding of sFasL has been shown to down-regulate the apoptotic and inflammatory activity of its membrane bound counterpart, suggesting that membrane-bound FasL is the functional form and that shedding of sFasL acts to regulate membrane-bound FasL cytotoxic activity (Suda *et al*., 1997; Schneider *et al*., 1998; Tanaka *et al*., 1998; Hohlbaum and Marshak-Rothstein, 2000). An uncharacterized metalloproteinase cleaves the 40-kD membrane-bound FasL to generate the 26–29 kD-soluble fragment (Mariani *et al*., 1995).

One of the possible explanations to the absence of apoptotic activity of sFasL is trimerization of Fas is not sufficient to transmit FasL-mediated death signal (Schneider *et al*., 1998).

1.3. Apoptosis and oral lichen planus:

1.3.1. Fas and Fas ligand and oral lichen planus:

Oral lichen planus is a T cell-mediated autoimmune disease in which autocytotoxic CD8+ T cells trigger apoptosis of oral epithelial cells (Eisen *et al*., 2005).

The mechanisms used by the CD8+ cytotoxic T cells to trigger keratinocyte apoptosis in OLP are unknown. Possible mechanisms include: (i) T cell secreted TNF-α binding the TNF- α R1 receptor on the keratinocyte surface; (ii) T cell surface CD95L (Fas ligand) binding CD95 (Fas) on the keratinocyte surface; or (iii) T cell secreted granzyme B entering the keratinocyte via perforin-induced membrane pores. All of these mechanisms may activate the keratinocyte caspase cascade resulting in keratinocyte apoptosis (Sugerman *et al*., 2000).

Activated autocytotoxic CD8+ T cells can produce Fas ligand and by binding to Fas lead to apoptosis (Yanatatsaneeji *et al*., 2010). The positivity rates of FasL and granzyme B expression in the lamina propria of the OLP cases were remarkably higher than those in the normal control group; significantly higher rates were noted in atrophic-erosive than in nonerosive OLP cases. The over-expressions of FasL and granzyme B are closely related to the progression of OLP (Shen *et al*., 2004).

The abnormal cell apoptosis plays an important role in the pathogenesis of OLP (Deng *et al*., 2009). Accelerated apoptosis of the keratinocytes that confined within the basal or suprabasal cell layer instead of the superficial cell layer in the control group (Shen *et al*., 2004) and inhibition of

lymphocyte apoptosis may coexist and contribute to the formation and progression of OLP (Shi *et al*., 2010).

In this regard, it has been proposed that a mechanism may be produced that allows T lymphocytes to escape apoptosis, explaining the persistence of the infiltrate (Bascones-Ilundain *et al*., 2006). Thus, Neppelberg *et al*., (2001) demonstrated, a high expression of the apoptosis-inducing Fas/FasL complex, in addition to a lack of lymphocyte apoptosis and proposed that the signal for apoptotic death may not be transmitted to the interior of the cell, despite the permission for apoptosis mediated by the Fas/FasL system, and that this escape from apoptosis may underlie the formation of massive inflammatory infiltrates in OLP. Lei *et al*., (2010) found that T cells, especially CD8+ T cells in OLP and CD4+T cells in atrophic-erosive OLP appeared to escape from activation-induced cell death (AICD), which may account for the persistence of inflammation.

1.3.2. Bcl-2 and oral lichen planus:

A balance between cell proliferation and cell death plays an important part in the homeostasis of normal tissues (Kalemkerian and Ramrath, 1996) and a disarray of this balance is probably present in diseases like OLP (Piattelli *et al*., 2007).

In normal squamous and columnar epithelium, bcl-2 immunoreactivity is seen in the basal cell layers (Ramsay *et al*., 1995; Flohil *et al*., 1996; Ter Hamsel *et al*., 1996; Tjalma *et al*., 1997). Bcl-2 has an important role in

preserving stem cells of keratinocytes (Harada *et al*., 1998) and protects epithelial cells from death (Lu *et al*., 1996).

The strong expression of p53 together with the low expression of bcl-2 in lesions of LP supports the notion that apoptosis could be a potential mechanism of keratinocyte loss and that liquefaction degeneration might be a marker of apoptosis and may suggest a contributory role for these apoptosis-associated proteins in the pathogenesis of LP (Nofal *et al*., 2008).

In OLP, bcl-2 expression by the lymphocytes and macrophages of the infiltrate may contribute to the longevity of these inflammatory cells and, therefore, to chronicity of the lesion (Dekker *et al*., 1997).

Nofal *et al*., (2008) assessed the possible role of apoptosis and its regulatory proteins in pathogenesis and healing of LP by measured bcl-2 and p53 expression in LP lesions before and after treatment with narrow band ultraviolet B phototherapy (NB-UVB) and mentioned that the decreased bcl-2 expression in lymphocytes after treatment may provide evidence that apoptosis of lymphocytes is an important mechanism of the therapeutic action of NB-UVB in LP.

The strong expression of bcl-2 in dermal lymphocytes in lesions of LP before treatment inhibits the apoptosis in lymphocytes that strengthens cell-mediated immune process causing chronicity of the disease (Bal *et al*., 2008). After treatment with NB-UVB, the marked decrease in bcl-2 expression leads lymphocytes to undergo apoptosis which might contribute to healing of the lesions (El-Domyati *et al*., 2007).

1.4. Oxidative Status:

Oxidative stress is an expression used to describe various deleterious processes resulting from an imbalance between the excessive formation of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) and limited antioxidant defences (Turrens, 2003).

In contrast, ROS play a crucial role in normal physiological processes like response to growth factors, immune response and apoptotic elimination of damaged cells (Chapple, 1996). Most cell types have been shown to elicit a small oxidative burst generating low concentrations of ROS when they are stimulated by cytokines, growth factors and hormones (Thannickal and Fanburg, 2000). It is generally accepted that ROS generated by these ligand/receptor-initiated pathways can function as true second messengers and mediate important cellular functions such as proliferation and programmed cell death (Valko *et al.*, 2007).

At the cellular level, oxidant injury elicits a wide spectrum of responses ranging from proliferation to growth arrest, to senescence, to cell death (Martindale and Holbrook, 2002). Figure (1-12)

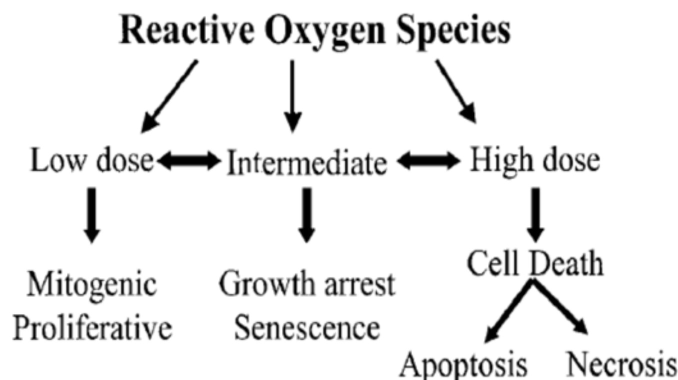


Figure (1-12); Reactive oxygen species (ROS) elicit a wide spectrum of responses. (Martindale and Holbrook, 2002)

The mitochondrion plays a dual role in the cell as a regulator of apoptosis and as a cellular powerhouse, and multiple mitochondrial components are involved both in the regulatory processes of energy metabolism and in stress-induced apoptosis signaling (Saraste, 1999; Ravagnan *et al*., 2002). The mitochondria are sensitive to changes in the redox state of the cell. Several studies have shown that the global shutdown of mitochondrial function under condition of the oxidative stress could contribute to apoptosis (Zamzami *et al*., 1996).

Apoptosis is triggered by two major initiating pathways, designated as the inner/intrinsic/mitochondria mediated and outer/extrinsic/receptor-mediated route, respectively (Green and Kroemer, 2004). It has become evident that there is an intensive cross-talk between the extrinsic (death receptors) and intrinsic (mitochondrial) pathways (Jin and El Deiry, 2005). The tumor suppressor protein p53 is a typical example of such intrinsic-to-extrinsic crosstalk (Haupt *et al*., 2003).

Cellular stress response often implicates activation of the proapoptotic p53-dependent machinery, which determines the threshold level of cellular stress able to trigger apoptosis (Pluquet and Hainaut, 2001; Slee *et al*., 2004). Apart from binding directly to the mitochondrial membrane, p53 enhances transcription of genes coding for death receptors, which increases susceptibility of the cells to receptor-mediated programmed death (Karawajew *et al*., 2005).

1.5. Free radicals and antioxidant:

1.5.1. Free radicals:

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbital (Halliwell and Gutteridge, 1999). This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical (Valko *et al*., 2007).

1.5.1.1. Reactive oxygen species (ROS):

Radicals derived from oxygen represent the most important class of radical species generated in living systems. The addition of one electron to dioxygen forms the superoxide anion radical ($O2^{\bullet}-$) (Miller *et al*., 1990). Superoxide anion, arising either through metabolic processes or following oxygen "activation" by physical irradiation, is considered the "primary" ROS, and can further interact with other molecules to generate "secondary" ROS, either directly or prevalently through enzyme- or metal-catalysed processes (Valko *et al*., 2005).

The production of superoxide occurs mostly within the mitochondria of a cell (Cadenas and Sies, 1998). The mitochondrial electron transport chain is the main source of ATP in the mammalian cell and thus is essential for life. During energy transduction, a small number of electrons "leak" to oxygen prematurely, forming the oxygen free radical superoxide, which has been implicated in the pathophysiology of a variety of diseases (Valko *et al*., 2004; Kovacic *et al*., 2005).

Superoxide rapidly disappears in aqueous solution because of its dismutation reaction in which hydrogen peroxide and oxygen are formed.

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Any system producing superoxide will, as a result of the dismutation reaction, also produce H_2O_2. H_2O_2 is a weak oxidant and a weak reducing agent that is relatively stable in the absence of transition metal ions (Gutteridge *et al*., 1995).

The hydroxyl radical, $^{\bullet}OH$, is the neutral form of the hydroxide ion. The hydroxyl radical has a high reactivity, making it a very dangerous radical with a very short *in vivo* half-life of approximatly 10^{-9} s (Pastor *et al*., 2000). The hydroxyl radical is the most powerful oxidant formed in biological systems and can readily attack any biological molecule. Hydroxyl radicals can attack polyunsaturated fatty acids to initiate lipid peroxidation (Gutteridge *et al*., 1995).

The redox state of the cell is largely linked to an iron (and copper) redox couple and is maintained within strict physiological limits. It has been suggested that iron regulation ensures that there is no free intracellular iron; however, *in vivo*, under stress conditions, an excess of superoxide releases "free iron" from iron-containing molecules. The release of iron by superoxide has been demonstrated for [4Fe–4S] cluster-containing enzymes of the dehydratase-lyase family (Liochev and Fridovich, 1994). (Figure 1-13)

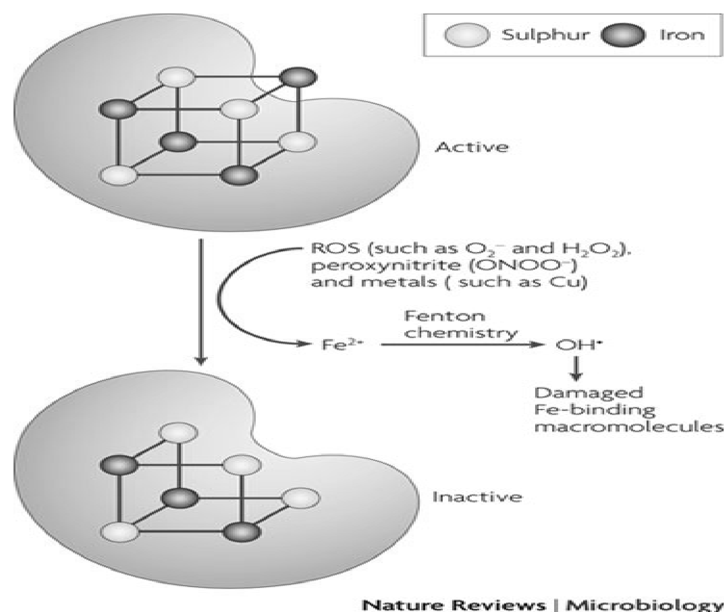
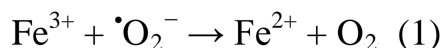


Figure (1-13): Fe–S proteins and oxidative stress (Py and Barras, 2010)

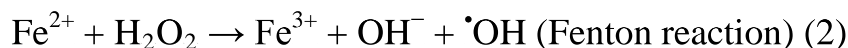
The released Fe^{2+} can participate in the Fenton reaction, generating highly reactive hydroxyl radical ($Fe^{2+}+H_2O_2 \rightarrow Fe^{3+}+{}^{\bullet}OH+OH^-$). Thus under stress conditions, $O_2^{\bullet-}$ acts as an oxidant of [4Fe–4S] cluster-containing enzymes and facilitates $^{\bullet}OH$ production from H_2O_2 by making Fe^{2+} available for the Fenton reaction (Leonard *et al*., 2004; Valko *et al*., 2005). The superoxide radical participates in the Haber–Weiss reaction ($O_2^{\bullet-}+H_2O_2 \rightarrow O_2+{}^{\bullet}OH+OH^-$) which combines a Fenton reaction and the reduction of Fe^{3+} by superoxide, yielding Fe^{2+} and oxygen ($Fe^{3+}+O_2^{\bullet-} \rightarrow Fe^{2+}+O_2$) (Liochev and Fridovich, 2002).

Substantial evidence suggests that conversion of these poorly reactive intermediates of oxygen to highly reactive forms requires the participation of transition metal ions, particularly iron. Iron ions react with hydrogen peroxide (Fenton chemistry) to generate hydroxyl radicals that can damage all organic molecules (Gutteridge, 1994).

The Haber–Weiss reaction (Eq. 3) might provide a means to generate more toxic radicals from the less reactive superoxide and hydrogen peroxide that could be generated enzymatically. The original paper by Haber and Weiss discussed the need for a metal ion catalyst and illustrated that the net reaction creating the hydroxyl radical (Eq. 3) can be broken down into two chemical reactions (Eqs. 1 and 2). Although other transition metal ions are capable of catalyzing this reaction, the iron-catalyzed Haber–Weiss reaction, which makes use of Fenton chemistry, is now considered to be the major mechanism by which the highly reactive hydroxyl radical is generated in biological systems (Liochev, 1999).



$$Fe^{3+} + {}^{\bullet}O_2^{-} \rightarrow Fe^{2+} + O_2 \quad (1)$$



$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + {}^{\bullet}OH \text{ (Fenton reaction) } (2)$$

The net reaction:

$${}^{\bullet}O_2^{-} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^{-} + O_2 \quad (3)$$ (Kehrer, 2000)

1.5.1.2. Reactive nitrogen species (RNS):

Nitric oxide ($NO^{\bullet}$) $\dot{N}=O$ is an abundant reactive radical that acts as an important oxidative biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defence mechanisms, smooth muscle relaxation and immune regulation (Bergendi *et al*., 1999).

Overproduction of reactive nitrogen species is called nitrosative stress (Klatt and Lamas, 2000; Ridnour *et al*., 2004). This may occur when the generation of reactive nitrogen species in a system exceeds the system's ability to neutralise and eliminate them. Nitrosative stress may lead to nitrosylation reaction [R-N=O] that can alter the structure of proteins and so inhibit their normal function (Valko *et al*., 2007).

Cells of the immune system produce both the superoxide anion and nitric oxide during the oxidative burst triggered during inflammatory processes. Under these conditions, nitric oxide and the superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion (ONOO−), which is a potent oxidizing agent that can cause DNA fragmentation and lipid oxidation [$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^{-}$] (Carr *et al*., 2000).

Volk *et al*., (1995) noted the cytotoxic potential of NO against various cells. Investigations by Das *et al*., (1997) have indicated that free radicals including NO may play an important role in ulceration induced by several kinds of stress. Therefore NO is considered to cause erosion and ulceration as a consequence of cell damage. Sunitha and Shanmugam, (2006) proposed that free radicals including NO represent one route of pathogenesis and that excess of salivary NO may have pathophysiological implications for erosive and ulcerative lesions in OLP.

1.5.2. Oxidative stress marker-Malondialdehyde (MDA):

The free-radical oxidation of polyunsaturated fatty acids in biological systems is known as lipid peroxidation (Gutteridge, 1995).

A common approach to estimate oxidative stress *in vivo* is to measure the end products of lipid peroxidation. The most widely used index is plasma malondialdehyde, which is measured by the thiobarbituric acid–reacting substances (TBARS) assay (Belch *et al*., 1991).

First-chain initiation of a peroxidation sequence in a membrane or polyunsaturated fatty acid results from the attack by any species with sufficient reactivity to abstract a hydrogen atom from a methylene (-CH2-) group. Because a hydrogen atom contains only one electron, abstraction leaves behind an unpaired electron on the carbon, -CH-. The presence of a double bond in the fatty acid weakens the C-H bonds on the carbon atom adjacent to the double bond facilitates $H^{\cdot}$ removal. The carbon-centered radical undergoes a molecular rearrangement to form a conjugated diene (Figure 1-14), which then combines with oxygen to form a peroxyl radical, itself able to abstract a hydrogen atom from another fatty acid and thus start a chain reaction. Peroxidation continues to use up the substrate unless a chain-breaking antioxidant. The end products of the chain reaction are a variety of hydroperoxides and cyclicperoxides (Gutteridge, 1995).

Lipid peroxides are fairly stable molecules at physiological temperatures, but their decomposition is catalyzed by transition metals and metal complexes. For example, all redox-active iron complexes present *in vivo* that participate in the Fenton reaction can also promote lipid peroxide

decomposition. Reduced metal complexes [e.g., iron (II) or copper (I)] react with lipid peroxides (LOOH) to give alkoxyl radicals

$$LOOH + M^{n+} \rightarrow LO^{\bullet} + M^{(n+1)+} + OH^{-}$$

Whereas oxidized metal complexes [i.e., iron (III) or copper (II)] react more slowly to produce peroxyl and alkoxyl radicals. Both alkoxyl and peroxyl radicals stimulate the chain reaction of lipid peroxidation by abstracting further hydrogen atoms (Gutteridge, 1995).

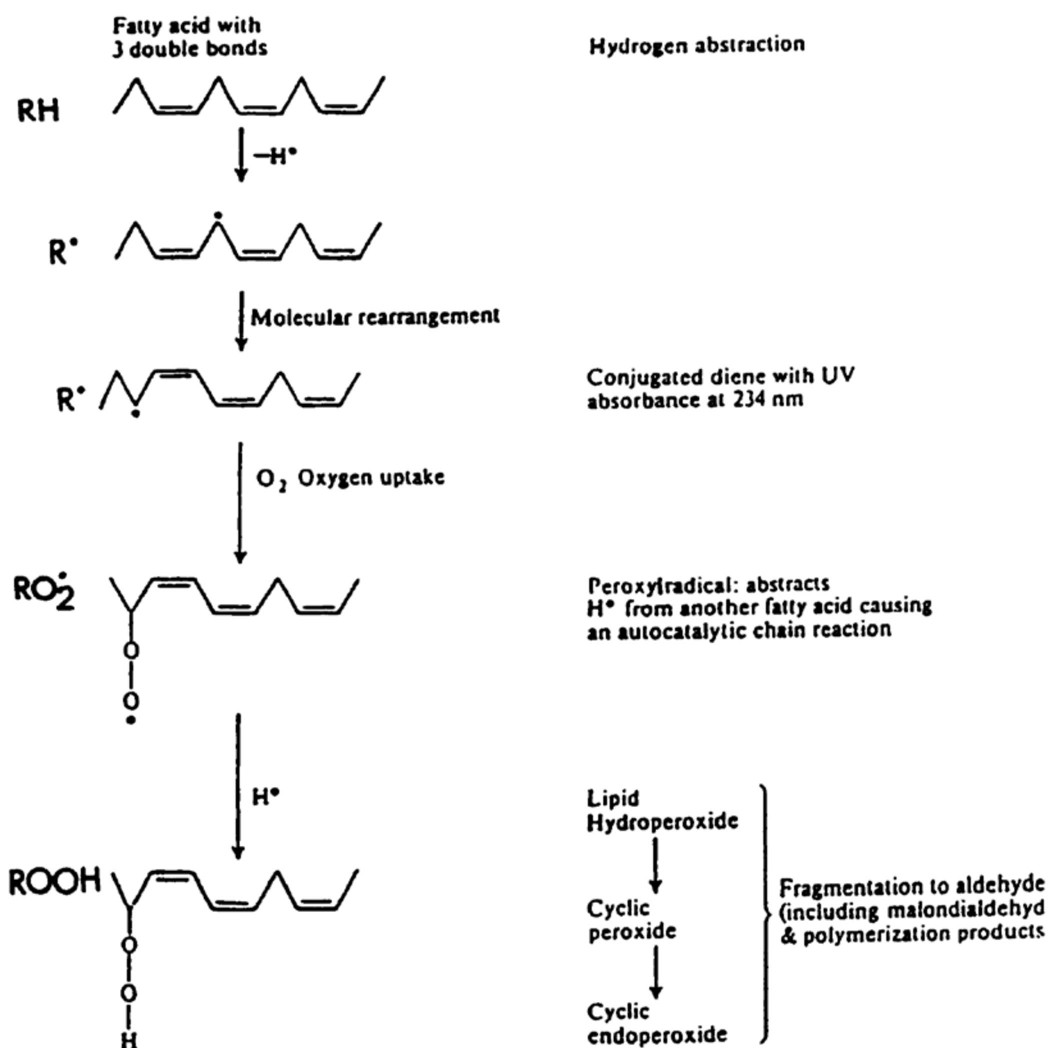


Figure (1-14): lipid peroxidation (Gutteridge, 1995)

Extensive lipid peroxidation in biological membranes causes loss of fluidity, falls in membrane potential, increased permeability to H and other ions, and eventual rupture leading to release of cell and organelle contents. Some end products of peroxide fragmentation are also cytotoxic (Esterbauer *et al*., 1991).

Malondialdehyde can arise from oxidative mechanisms other than lipid peroxidation. Therefore, the test is considered to be a general indicator of oxidative stress rather than a specific marker of lipid peroxidation (Armstrong and Browne, 1994).

1.5.3. Antioxidants:

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents (Sies, 1997). In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell (Davies, 1995; Sies, 1997).

Exposure to free radicals from a variety of sources has led organisms to develop a series of defence mechanisms (Cadenas, 1997). Enzymatic antioxidant defences include superoxide dismutase (SOD), glutathione

peroxidase (GPx), and catalase (CAT). Non-enzymatic antioxidants are represented by ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. This balance is essential for the survival of organisms and their health (Valko *et al*., 2007).

1.5.3.1. Ceruloplasmin (CP):

Ceruloplasmin (CP) is a major multicopper-containing plasma protein that is not only involved in iron metabolism through its ferroxidase activity but also functions as an antioxidant (Inoue *et al*., 1999).

Ceruloplasmin (CP) is abundant in the plasma of vertebrates and is synthesized in the liver (Sato and Gitlin, 1991), but cells other than hepatocytes, such as macrophages, are also known to produce CP (Ehrenwald and Fox, 1996).

Ceruloplasmin may play an important role not only in copper transport but also as a multicopper oxidase to oxidize NO, thus generating RS-NO in the presence of thiol compounds (RSH). In the latter case, CP might function to protect cells and tissues by generating RS-NO from highly toxic free radicals and reactive nitrogen oxides such as hydroxyl radical and peroxynitrite (Inoue *et al*., 1999).

Various studies have confirmed that metals activate signaling pathways and the carcinogenic effect of metals has been related to activation of mainly redox-sensitive transcription factors, involving nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappa B), activator protein 1 (AP-1) and p53. Antioxidants (both enzymatic and non-enzymatic) provide protection against deleterious metal-mediated free radical attacks (Valko *et al*., 2005).

Ceruloplasmin has been implicated in Fe metabolism mostly because of its catalytic oxidation of Fe (II) to Fe (III) (ferroxidase activity) (Osaki and Johnson, 1969) with its subsequent incorporation into apotransferrin (Osaki *et al*., 1966) or into the Fe storage protein ferritin (Van Eden and Aust, 2000). Nevertheless, the physiological role of CP is not well defined but may include extracellular antioxidant activity by promoting Fe mobilization and thus preventing metal-catalyzed free radical tissue damage (Goldstein *et al*., 1979, Graf and Noetzel, 1999).

1.5.3.2. Uric acid (UA):

Uric acid is considered a major antioxidant in human blood (Sautin *et al*., 2007) which contributes as much as 60% to free radical scavenging in human serum (Maxwell *et al*., 1997).

Urate (the soluble form of uric acid in the blood) can scavenge superoxide, hydroxyl radical, and singlet oxygen and can chelate transition metals (Vertuani *et al*., 2004).

Uric acid may assist in the removal of superoxide by preventing against the degradation of superoxide dismutase, the enzyme that is responsible for clearing superoxide from the cell (Pacher *et al*., 2007). Removal of superoxide helps to prevent its reaction with NO, blocking the formation of peroxynitrite (van der Veen *et al*., 1997). Uric acid is a strong peroxynitrite scavenger (Hooper *et al*., 1998).

When ferrous iron reduces H_2O_2 to generate $^{\bullet}OH$, it becomes ferric iron. Vitamin C (ascorbic acid) converts ferric iron back to ferrous iron, itself becoming oxidized ascorbic acid, thus allowing another cycle of $^{\bullet}OH$ generation from renewed ferrous iron (Aisen *et al*., 1990). Uric acid has the ability to bind iron and inhibit iron-dependent ascorbate oxidation, preventing an increased production of free radicals that can further contribute to oxidative damage (Davies *et al*., 1986).

Furthermore, Ascorbate is catalytically oxidized by a coupled iron-ceruloplasmin system, the iron ions functioning as a *red/ox* cycling intermediate between ceruloplasmin and ascorbate. Urate also has the ability to inhibit the iron-ceruloplasmin-dependent ascorbate oxidation by chelating ferric ions. (Løvstad, 1995).

Although some alterations in UA levels may be a consequence of disease, it is likely that UA also plays an important role in the development and prevention of many diseases. Thus, it appears that UA is not an inert organic compound, as has historically been believed, but can instead play a role in many biological functions. Uric acid can be both beneficial, as an antioxidant and free radical scavenger, and deleterious if present at an

elevated level. The manipulation of serum UA levels holds promise in the treatment of a number of diseases. It is noteworthy that because both decreased and elevated UA levels may contribute to the development and progression of a number of disease states, significant alterations in UA levels should be minimized (Kutzing and Firestein, 2008).

Serum UA levels that are below normal concentrations have also been linked to a variety of disease states, including multiple sclerosis, optic neuritis, Parkinson's disease, and Alzheimer's disease (Church and Ward, 1994; Toncev *et al*., 2002; Knapp *et al*., 2004; de Lau *et al*., 2005; Kim *et al*., 2006). In these inflammatory diseases, a decreased UA concentration may not be able to prevent the toxicity by reactive oxygen and nitrogen species that form as a result of the inflammation. Peroxynitrite, in particular, is believed to have a significant negative impact on cellular function and survival. Under normal physiological conditions, there is a low production of peroxynitrite, resulting in a minimal amount of oxidative damage. However, a small increase in NO and superoxide formation produces a much larger increase in peroxynitrite formation. Even a slight increase in peroxynitrite production can result in substantial oxidation that can lead to tissue destruction and can damage a number of processes that are critical for normal cellular function (Pacher *et al*., 2007).

1.5.3.3. Oxidized Glutathione (GSSG):

Glutathione (GSH) is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella *et al*., 2003).

Glutathione acts as electron carriers. When electrons are lost from GSH (i.e. on oxidation by ROS or Glutathione peroxidase), the molecule (GSH) becomes oxidized (GS-) and two such molecules become linked (dimerised) by a disulphide bridge to form Glutathione disulphide or oxidized Glutathione (GSSG). This linkage is reversible upon re-reduction (Doolen, 2011).

In healthy cells and tissues, more than 90% of total Glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulphide or oxidized form (GSSG) (Anna *et al*.,2003).

Intracellular GSH status appears to be a sensitive indicator of the cell's overall health, and of its ability to resist toxic challenge. Experimental GSH depletion can trigger suicide of the cell by a process known as apoptosis (Slater *et al*., 1995; Duke *et al*., 1996). Formation and release of GSSG from the cell can be used as an indicator of the flux mediated by glutathione peroxidase, and an increase in the production of GSSG is considered to be a reliable index of oxidative stress (Adams *et al*., 1983; Ishikawa and Sies, 1984).

Increased oxygen free radical production, which may contribute to several human diseases (Cross *et al*., 1987), is associated with low plasma GSH/GSSG ratios (Paolisso *et al*., 1992). The GSSG/GSH ratio may be a sensitive indicator of oxidative stress (Kidd, 1997).

It has been established that a decrease in GSH concentrations may be associated with aging and the pathogenesis of many diseases (Cecchi *et al*., 1999; Bonnefont-Rousselot *et al*., 2000; Rahman *et al*., 2000). Furthermore, recent data suggest that those elderly with low GSH concentrations are in poorer health than those with higher GSH (Julius *et al*., 1994).

1.6. Oxidative status and oral lichen planus:

It has been demonstrated that the imbalances in free radical levels and reactive oxygen species with antioxidants may play a key role in the onset and development of several inflammatory oral pathologies (Battino *et al*., 2002).

The exact etiology and pathogenesis of Lichen planus remains unclear (Aly and Shahin, 2010). Anshumalee *et al*., (2007), Anshumalee and Shashikanth, (2007) and Sezer *et al*., (2007) reported that ROS may be involved in the pathogenesis of LP.

Aly and Shahin (2010) suggested that increased ROS and lipid peroxidation in LP may enhance the inflammatory response by immunological mechanisms.

One major intracellular target of oxidative stress is the transcription factor NF-$_{\kappa}$B (Barnes and Karin, 1997; Mercurio and Manning, 1999; Baldwin, 2001; Tak and Firestein, 2001). NF-$_{\kappa}$B plays a critical role in

mediating immune and inflammatory responses and apoptosis (Evans *et al*., 2002).

Reactive oxygen species also seem to play an important role in mediating Fas-dependent apoptosis (Aronis *et al*., 2003) sustained by the observation that Fas-induced apoptosis was completely abolished by antioxidants such as glutathione (Gulbins *et al*., 1996). It was found that oxidative stress and increased inflammatory mediators produce the release of effector molecules including granzyme that can promote local tissue damage (Hendel *et al*., 2010). Furthermore, ROS stimulate MMP-9 secretion in human fetal membranes (Buhimschi *et al*., 2000). These factors implicated in the induction of histological alteration present in oral lichen planus are influenced by oxidative stress (Scrobota *et al*., 2011).

1.7. Saliva as a diagnostic fluid for oral lichen planus:

Saliva offers an alternative to serum as a biologic fluid that can be analyzed for diagnostic purposes. Saliva can be considered as gland-specific saliva and whole saliva. Gland-specific saliva is secretions of individual salivary glands: parotid, submandibular, sublingual, and minor salivary glands whereas whole saliva is a complex mix of fluids from salivary glands and gingival crevicular fluid, containing oral bacteria and food debris (Gupta *et al*., 2011).

The use of saliva as a diagnostic tool presents many advantages: it is easy to collect, by a non-invasive technique which can be performed at home; no special equipment is needed for collection. From children to seniors, saliva can be used as a diagnostic fluid because collection of this fluid is associated with fewer compliance problems compared with blood collection (Kaufman and Lamster, 2002).

Antioxidants are present in all body fluids including saliva. Saliva may constitute a first line of defense against oxidative stress and has protective effects against microorganisms, toxins and oxidants (Battino *et al*., 2002; Nagler *et al*., 2002).

Human saliva has a total antioxidant capacity higher than blood plasma (Ziobro and Bartosz, 2003). Saliva is an important defense system of body. Recently studies have shown that saliva has its own antioxidant system including UA, superoxide dismutase, glutathione peroxidase and catalase. Uric acid constitutes around 70% of total antioxidant capacity of saliva (Moore *et al*., 1994; Nagler *et al*., 2002; Karincaoglu *et al*., 2005).

Saliva that is deficient in antioxidants, such as urate and ascorbic acid, may not be able to prevent against the potentially harmful RNS that can promote tissue damage and mutagenesis (Pietraforte *et al*., 2006).

Chapter Two

Subjects, Materials and Methods

Subjects, Materials and Methods:

2.1. Sample groups (subjects):

Eighty (80) subjects were participated in this study, they were divided into 2 groups:-

1- Oral lichen planus (OLP) patients group: forty eight (48) patients with OLP. A diagnosis of oral lichen planus was made based on clinicopathologic correlation by oral medicine specialists.

All of the OLP patients were diagnosed clinically, and the diagnoses were confirmed through histopathologic examination according to the modified WHO diagnostic criteria for OLP (van der Meij and van der Waal, 2003). ***(Appendix I)***

2- Control group: this includes thirty two (32) healthy looking volunteers who were age-matched with the patients.

Samples collection and histopathological study was done in the College of Dentistry/University of Baghdad during the period from October 2011 to October 2012, while the laboratory work was done in the College of Medicine/Al-Mustansiriya University.

Exclusion criteria were applied to exclude the subjects that had received any systemic treatment suppressing the immune system such as systemic steroids or other immunosuppressive drugs, as well as NSAIDs, antimalarials, diuretics, antihypertensives, antibiotics, antifungals for the last 4 weeks, and topical medications for the last 2 weeks prior to sample collection. Also, patients with a history of trauma or any surgery 4 weeks prior to sampling, those suffering from any systemic or dermatological

disease affecting the immune system or any malignancy, and subjects with specific habits such as smoking were excluded.

2.2. Clinical Examination:

Intraoral examination was done for each individual using sterile dental mirror with artificial light.

The procedure of the examination of oral soft tissues was done in sequence according to the directions suggested by the W.H.O (1997).

The examination was performed systematically in the following sequence:

- Labial mucosa and labial sulci (upper and lower).
- Labial part of the commissures and buccal mucosa (right and left).
- Tongue (dorsal and ventral surfaces, margins).
- Floor of the mouth.
- Hard and soft palate.
- Alveolar ridges/gingiva (upper and lower).

2.2.1. Data collection:

A data extraction form was designed to collect the following information from patient records: age, sex and disease severity.

Scores for signs and symptoms were calculated to quantitate the severity of OLP depending on disease activity scoring sheet by Kings College London Dental Institute with modification. **(*Appendix II*)**

2.3. Sample collection:

Blood and saliva were collected from each subject at a fixed daily time i.e 8-11 am.

2.3.1. Blood sample:

About 6 ml of venous blood sample was aspirated from anticubital vein of each individual, using disposable syringes with 21 gauge stainless steel needle. The whole blood was collected in sterile polyethylene tubes. Samples were allowed to clot for 30 minutes before centrifugation at 3000 rpm for 10 minutes then the supernatant serum was aspirated and transferred immediately into Eppendorf tubes and frozen at -20 °C for subsequent analysis (Figure 2-1). Haemolyzed samples were discarded.

2.3.2. Saliva sample:

About 3-4 ml of unstimulated (resting) whole saliva was collected. An individual was asked to rinse his/her mouth thoroughly with water to allow removal of debris. The first mouth- full of saliva was discarded to allow clearance of water, and then the patients were asked to spit all the saliva into plastic polyethylene tubes. The collected saliva was centrifuged at 3000 rpm for 10 minutes; the clear supernatants were separated and stored frozen at (-20 °C) until assayed. (Figure 2-1)

Figure (2-1): Serum and saliva collection and transferred into Eppendorf tubes

2.4. Instruments, Equipments, and Materials:

2.4.1. Instruments and Equipments:

1. Diagnostic instruments: dental mirror, probe, tweezer.
2. Surgical instruments for biopsy: cartridge-type local anaesthetic syringe, fine single use two-sided needles, scalpel blades (no. 15), tweezer, scissor, 3/0 suture material, gauze.
3. Plate reader, Bioelisa Reader, EL_X800-Biokit, USA
4. Automated plate washer, Bioelisa washer, EL_X50-Biokit, USA
5. Incubator, LAB-LINE, BARNSTEAD USA
6. Spectrophotometer, UV-1800, SHIMADZU, JAPAN
7. Centrifuge, KARL KOLB, BUCHSCHLAG-Frankfurt, W.Germany
8. Refrigerator
9. Pipettes and pipette tips
10. Graduated cylinders
11. Absorbent paper
12. Plane tube
13. Eppendorf tube

14. Disposable syringes with 21 gauge stainless steel needle
15. Electronic balance. (Figure 2-2)
16. Vortex (Mixer), LABINCO BV, NETHERLANDS. (Figure 2-3)
17. Water bath, GFL, F.G. BODE&CO, Laboratory-Equipment, Hamburg. (Figure 2-4)
18. Deep freezer

Figure (2-2): Electronic balance

Figure (2-3): Vortex

Figure (2-4): Water bath

2.4.2. Materials:

1. Human B-cell leukemia/lymphoma 2, Bcl-2 ELISA Kit Catalog No. CSB-E08853h
2. Human Oxidized Glutathione (GSSG) ELISA Kit Catalog No. CSB-E13735h
3. Nitric Oxide Colorimetric BioAssay™ Kit Catalog No.N2577-01
4. CD95 Human ELISA Kit (ab100513)
5. Fas Ligand Human ELISA Kit (ab100515)
6. Uric acid colorimetric assay kit (SPINREACT/Spain)
7. Trichloroacetic acid (TCA).
8. 2 -thiobarbituric acid (TBA).
9. Crystalline p-phenylenediamine.
10. Glacial acetic acid.
11. Sodium acetate trihydrate.
12. Sodium azide.
13. Distilled water.
14. Cartridges of local anaesthetic solution
15. Fixing solution.

2.5. Estimation of apoptotic activities markers:

2.5.1. Estimation of Human soluble Fas:

2.5.1.1. Principles of the assay:

The Fas kit [ab100513 CD95 Human Enzyme-Linked Immunosorbent Assay (ELISA)] is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human CD95 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human CD95 coated on a 96-well plate. Standards and samples are pipetted into the wells and CD95 present in a

sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human CD95 antibody is added. After washing away unbound biotinylated antibody, Horseradish Peroxidase (HRP)-conjugated streptavidin is pipetted to the wells. The wells are again washed, a tetramethyl-benzidine (TMB) substrate solution is added to the wells and color develops in proportion to the amount of CD95 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2.5.1.2. Assay Method

1. All reagents and samples were brought to room temperature (18 - 25°C) before use.

2. A hundred 100µl of each standard and sample were added into appropriate wells and covered well and incubated over night at 4°C with gentle shaking.

3. The plate was washed 4 times with 1x Wash Solution by filling each well with Wash Buffer (300 µl) using autowasher. After the last wash, the plate was blotted against clean paper towels.

4. A hundred 100µl of 1x prepared biotinylated antibody was added to each well and incubated for 1 hour at room temperature with gentle shaking.

5. The wash was repeated as in step 3.

6. A hundred 100µl of prepared Streptavidin solution was added to each well and incubated for 45 minutes at room temperature with gentle shaking.

7. The wash was repeated as in step 3.

8. A hundred 100µl of TMB was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking.

9. A fifty 50µl of Stop Solution was added to each well and read at 450 nm immediately.

2.5.1.3. Data Analysis

The mean absorbance for each duplicate standard was calculated. The standard curve was plotted with standard concentration on the X-axis and absorbance on the Y-axis. The best-fit straight line was drawn through the standard points. ***(Appendix III)***

2.5.2. Estimation of Human Soluble Fas Ligand:

2.5.2.1. Principles of the assay:

The Fas Ligand kit (ab100515 Human Fas Ligand ELISA) is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human Fas Ligand in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human Fas Ligand coated on a 96-well plate. Standards and samples are pipetted into the wells and Fas Ligand present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Fas Ligand antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted into the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Fas Ligand bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2.5.2.2. Assay Method

1. All reagents and samples were brought to room temperature (18 - 25°C) before use.

2. A hundred 100μl of each standard and sample were added into appropriate wells. Well was covered and incubated over night at 4°C with gentle shaking.

3. The plate was washed 4 times with 1xWash Solution by filling each well with Wash Buffer (300 μl) using autowasher. After the last wash, any remaining Wash Buffer was removed by aspirating. The plate was inverted and bloted against clean paper towels.

4. A hundred 100μl of 1x prepared biotinylated antibody was added to each well and incubated for 1 hour at room temperature with gentle shaking.

5. The solution was discarded and the wash was repeated as in step3.

6. A hundred 100μl of prepared Streptavidin solution was added to each well and incubated for 45 minutes at room temperature with gentle shaking.

7. The wash was repeated as in step 3.

8. A hundred 100μl of TMB was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking.

9. Stop solution (sulfuric acid) (50 μl) was added to each well and read at 450 nm immediately. (Figure 2-5, 2-6)

2.5.2.3. Data Analysis

The mean absorbance for each set of duplicate standards was calculated. The standard curve was plotted with standard concentration on the X-axis and absorbance on the Y-axis. The best-fit straight line was drawn through the standard points. (***Appendix IV)***

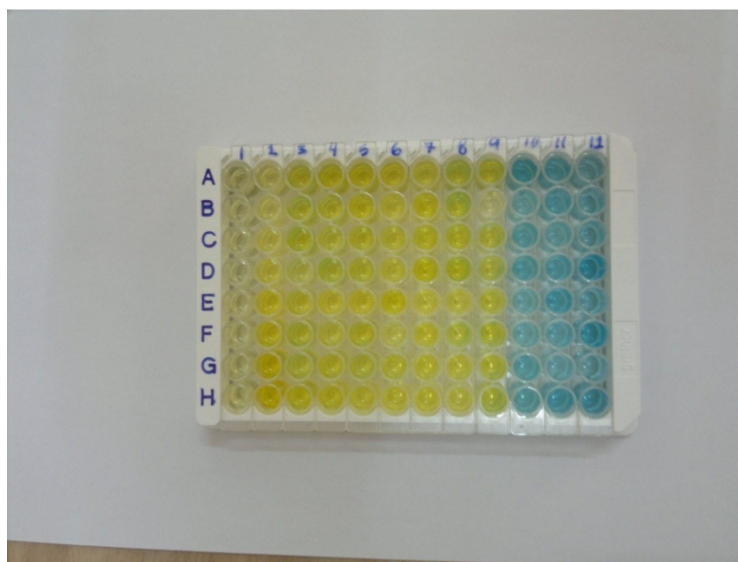


Figure (2-5): Stop solution (sulfuric acid) was added to each well

Figure (2-6): The Plate reader and printer

2.5.3. Estimation of Human Bcl-2

2.5.3.1. Principles of the assay:

The microtiter plate provided in this kit has been pre-coated with an antibody specific to Bcl-2. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody

preparation specific for Bcl-2 and Avidin conjugated to HRP is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain Bcl-2, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Bcl-2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.5.3.2. Assay Method

1. A hundred 100µl of Standard or Sample was added per well and covered with the adhesive strip and incubated for 2 hours at 37°C. (Figure 2-7)

2. The liquid of each well was removed without washing.

3. Biotin-antibody working solution (100µl) was added to each well and incubated for 1 hour at 37°C.

4. The plate was washed 3 times by filling each well with Wash Buffer (200µl) and let it stand for 150 seconds (Figure 2-8). The remaining drops were removed by patting the plate on a paper towel.

5. HRP-avidin working solution (100µl) was added to each well and the microtiter plate was covered with a new adhesive strip and incubated for 1 hour at 37°C.

6. The aspiration and wash were repeated five times as step 4.

7. TMB Substrate (90µl) was added to each well and incubated for 15-30 minutes at 37°C in the dark.

8. A fifty 50µl of Stop Solution to each well was added when the first four wells containing the highest concentration of standards develop obvious blue color.

9. The optical density of each well was determined within 5 minutes, using a microplate reader set to 450 nm.

2.5.3.3. Data Analysis

The mean absorbance for each duplicate standard was calculated. A standard curve was constructed by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and a best fit line was drawn through the points on the graph. (***Appendix V)***

Figure (2-7): Incubator

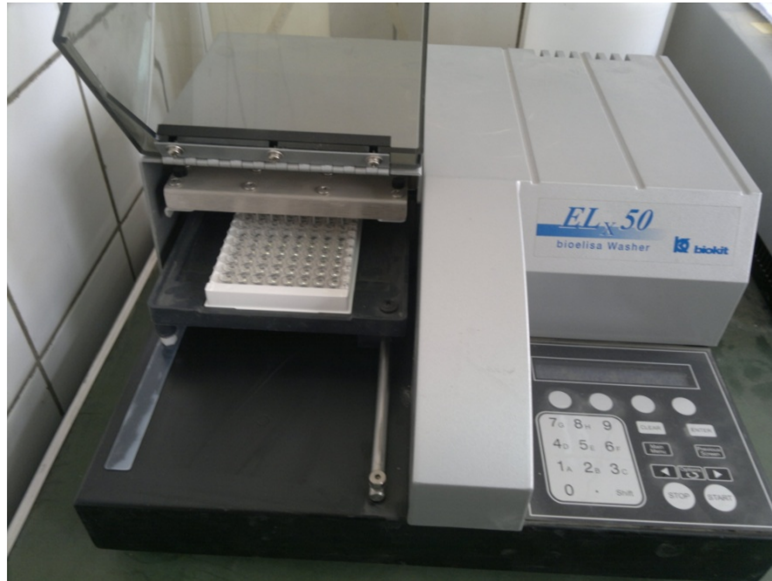


Figure (2-8): automated washer

2.6. Estimation of oxidative stress markers:

2.6.1. MDA assessment:

2.6.1.1. Principle of the assay:

Lipid peroxidation end products, particularly MDA react with thiobarbituric acid under acidic conditions and heating to a pink color that measured spectrophotometricaly at 532 nm (Shah and Walker, 1988).

2.6.1.2. Reagents:

17.5% w/v trichloroacetic acid (TCA).

0.6% w/v 2-thiobarbituric acid (TBA).

70% w/v trichloroacetic acid (TCA)

2.6.1.3. Assay Method:

1. The following was pipetted into a glass test tube and mixed well.

0.5 ml sample.

0.5 ml 17.5% TCA.

0.5 ml 0.6% TBA.

2. The mixture was incubated in boiling water bath for 30 minutes.

3. The mixture was left to cool at room temperature.

4. After cooling, 1ml of 70% TCA was added with mixing to stop reaction, and then allowed to stand at room temperature for 30 minutes.

5. The mixture was centrifuged at 3000 r.p.m. for 15 minute, and then the clear supernatant was aspirated (Figure 2-9) and read at 532 nm against blank (Figure 2-10). The blank was prepared by adding 0.5 ml distilled water instead of test sample and processed similarly.

2.6.1.4. Calculation:

The MDA concentration was calculated using the molar extinction coefficient of 1.56 X 10^5 cm^{-1} mol^{-1}

$$MDA\ (\mu mol\,/\,L) = \frac{Absorbance\ of\ sample}{1.56 \times 10^{5}\ cm^{-1}\ mol^{-1}} \times 10^{6} \times \text{Dilution factor}$$

Dilution factor = 4

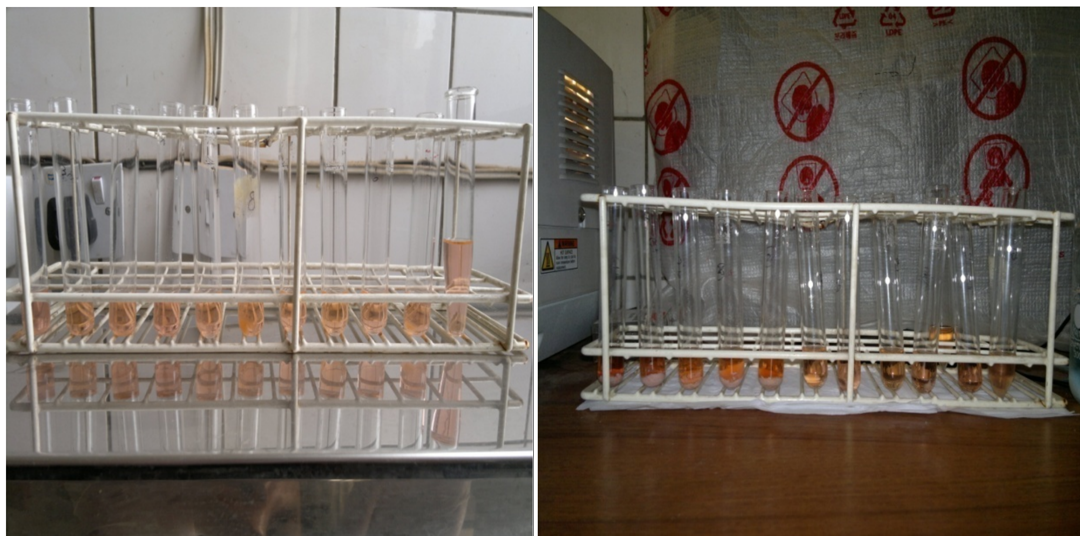


Figure (2-9): The clear supernatant after centrifugation

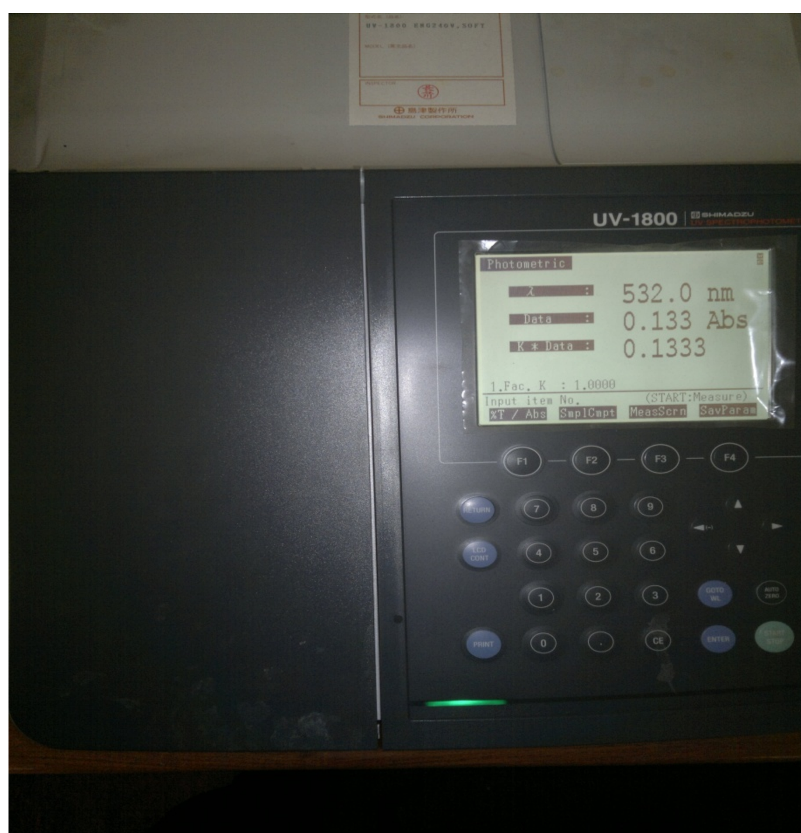


Figure (2-10): Spectrophotometer

2.6.2. Nitric Oxide assessment:

2.6.2.1. Principles of the assay:

Since most of the NO is oxidized to nitrite ($NO2^-$) and nitrate ($NO3^-$), the concentrations of these anions have been used as a quantitative measure of NO production. N2577-01 provides an accurate and convenient measurement of total nitrate/nitrite concentration in a simple two-step process (Figure 2-11). The first step is to convert nitrate to nitrite utilizing nitrate reductase. The second step involves addition of the Griess Reagents which convent nitrite into a deep purple azo compound. The amount of the azo chromophore accurately reflects the total nitric oxide amount in samples. The detection limit of the assay is ~0.1nmol nitrite/well or 1uM.

2.6.2.2. Assay Method

1. A standard (0, 2, 4, 6, 8, 10 µl) was added to each well and adjust to 85ul with Assay Buffer.

2. Eighty five 85µl of sample was added to each unknown well.

3. Five 5µl of the Nitrate Reductase mixture was added to each well (standards and unknowns).

4. The enzyme cofactor *(N2577-01B)* (5µl) was added to each well (standards and unknowns).

5. The plate was covered and incubated at room temperature for 1 hr to convert nitrate to nitrite.

6. The enhancer *(N2577-01J)* (5µl) was added to each well and incubated 10 min (standards and unknowns).

7. Griess Reagent R1 *(N2577-01E)* (50µl) was added to each well (standards and unknowns).

8. Griess Reagent R2 *(N2577-01F)* (50µl) was added to each well (standards and unknowns).

9. The color was developed for 10 min. at room temperature. The color is stable for about an hour.

10. The absorbance was read at 540nm using a plate reader.

2.6.2.3. Data Analysis

The mean absorbance for each duplicate standard was calculated. A standard curve was constructed by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and a best fit line was drawn through the points on the graph. (***Appendix VI)***

Figure (2-11): Nitric Oxide Kit

2.7. Estimation of antioxidants:

2.7.1. Estimation of Ceruloplasmin activity:

2.7.1.1. Principle:

Ceruloplasmin catalyzed oxidation of P-Phenylenediamine to give blue-violet color that measured spectrophotometricaly at 525 nm (Menden *et al*, 1977).

2.7.1.2. Reagents:

50mg Crystalline p-phenylenediamine.

1 ml Glacial acetic acid.

8.15 g Sodium acetate trihydrate.

100 mg Sodium azide.

1. Substrate solution: This was prepared by dissolving 50mg of p-phenylenediamine in 5 ml deionized water containing 1 ml of glacial acetic acid. In another container, 8.15 g sodium acetate trihydrate was dissolved in 30 ml of deionized water then added to the first solution and mixed well and the volume was completed to 50 ml with deionized water. This solution is stable for three hours if kept refrigerated in dark bottle.

2. The inactivating solution: This was prepared by dissolving 100 mg of sodium azide in 500 ml of deionized water then kept cold in refrigerator prior to use. This solution is stable for one week.

2.7.1.3. Procedure:

1. One ml of substrate was pipetted into glass test tube and incubated at 37 C° for one minute.

2. Sample (0.1 ml) was added, mixed immediately, and then incubated at 37 C° for exactly 15 minutes. At the end of the incubation period, the tubes were removed and placed in ice water bath for 30 seconds.

3. Five ml of cold inactivating solution was added, mixed, and then the temperature was brought to 25 C° in a 27 C° water bath for 5 minutes.

4. The absorbance was read in spectrophotometer at 525 nm against the blank, which was prepared by combining 1 ml of substrate, 5 ml of cold inactivating solution and 0.1ml of deionized water, and then the mixture was incubated as above. (Figure 2-12)

2.7.1.4. Calculation:

The activity of caeruloplasmin is calculated using the extinction coefficient 0.68 cm^{-1} mol^{-1}

$$\text{Caeruloplasmin activity (g /l)} = \frac{\text{Absorbance of sample}}{0.68}$$

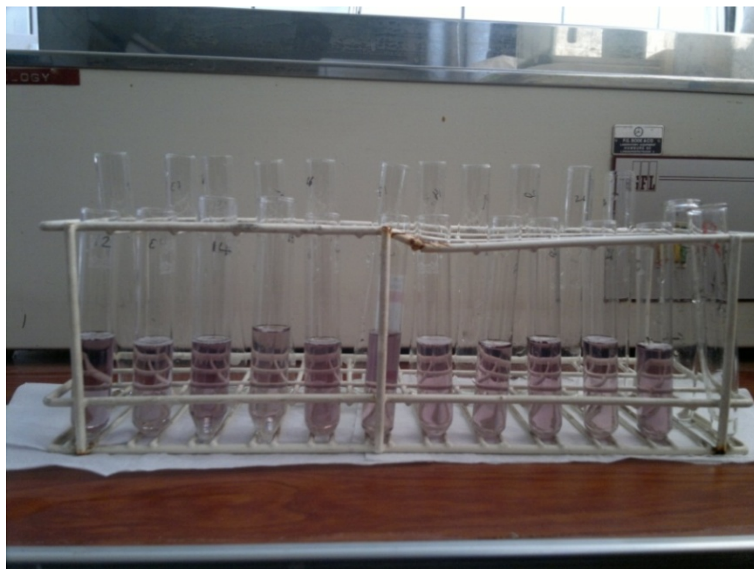
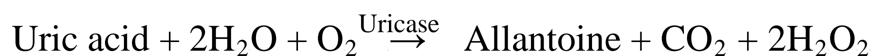


Figure (2-12): Estimation of ceruloplasmin activity

2.7.2. Estimation of Uric acid:

2.7.2.1. Principles of the assay:

Uric acid is oxidized by uricase to allantoine and hydrogen peroxide ($2H_2O_2$), which under the influence of POD, 4–aminophenazone (4- AP) and 2-4 Dichlorophenol sulfonate (DCPS) forms a red quinoneimine compound:



$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + 2H_2O_2$$

$$2H_2O_2 + \text{4-AP} + \text{DCPS} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

The intensity of the red color formed is proportional to the uric acid concentration in the sample. (Figure 2-13, 2-14)

2.7.2.2. Assay Method

1. Assay conditions:

Wavelength: . . . . .520 nm (490-550)

Cuvette: . . . . 1 cm light path

Temperature . . . . 37ºC / 15-25ºC

2. The instrument was adjusted to zero with distilled water.

3. The following was pipetted into a glass test tube:

	Blank	Standard	Sample
Working reagent (ml)	1.0	1.0	1.0
Standard (µl)	-------	25	-------
Sample (µl)	-------	-------	25

4. All of the above were mixed and incubated for 10 min at 15-25ºC.

5. The absorbance (A) of the samples and standard was read against the blank. The colour is stable for at least 30 minutes.

2.7.2.3. Data Analysis:

$$\frac{(A)\text{Standard}}{(A)\text{Sample}} \text{ X } 6 \text{ (Standard conc.)} = \text{mg/dl uric acid in the sample}$$

Figure (2-13): Uric acid kit

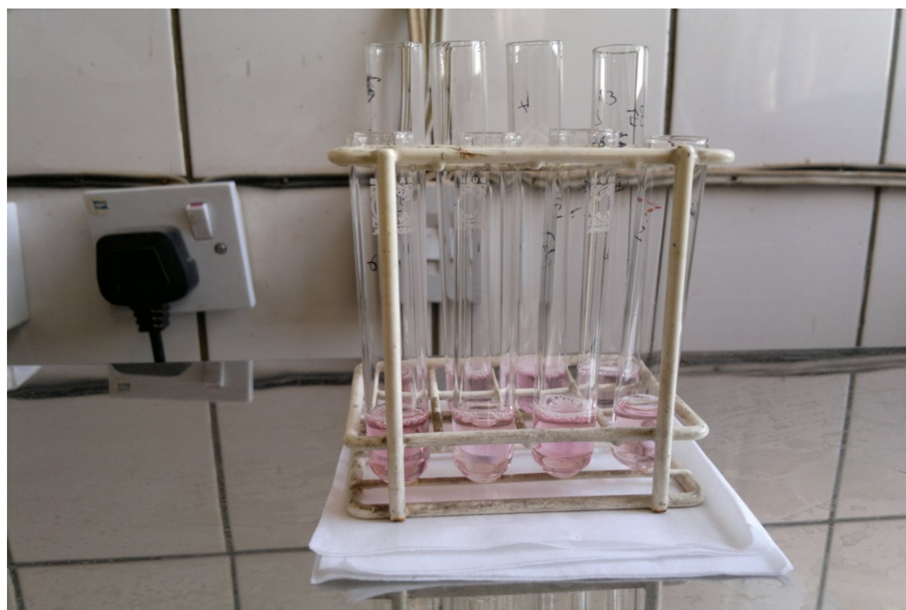


Figure (2-14): Uric acid estimation

2.7.3. Estimation of Human Oxidizided Glutathione

2.7.3.1. Principles of the assay:

This assay employs the competitive inhibition enzyme immunoassay technique. An antibody specific to GSSG has been pre-coated onto a microplate. Standards or samples are added to the appropriate microtiter plate wells with HRP-conjugated GSSG and incubated. A competitive inhibition reaction is launched between GSSG (Standards or samples) and HRP-conjugated GSSG with the pre-coated antibody specific for GSSG. The more amount of GSSG in samples, the less antibody bound by HRP-conjugated GSSG. Then the substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of GSSG in the sample. The color development is stopped and the intensity of the color is measured. (Figure 2-15)

2.7.3.2. Assay Method

1. Standard or Sample (50µl) was added per well.

2. HRP-conjugate working solution (50µl) was added to each well and mixed well and then incubated for 40 minutes at 37°C.

3. The plate was washed 5 times by filling each well with Wash Buffer (200µl) and let it stand for 10 seconds. After the last wash, the remaining drops were removed by patting the plate on a paper towel.

4. TMB Substrate (90µl) was added to each well and mixed well, then incubated for 20 minutes at 37°C in the dark.

5. Stop Solution (50µl) was added to each well.

6. The optical density of each well was determined within 10 minutes, using a microplate reader set to 450 nm.

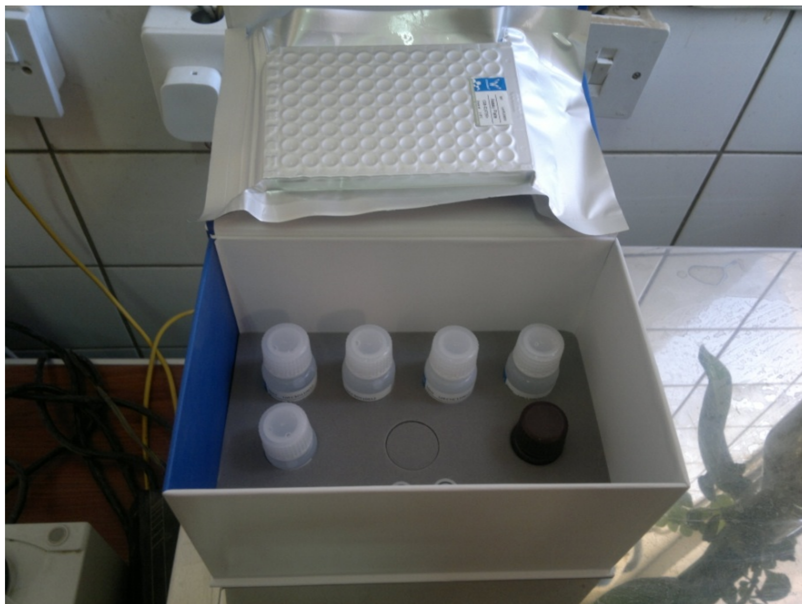


Figure (2-15): GSSG kit

2.7.3.3. Data Analysis

The mean absorbance for each duplicate standard was calculated. A standard curve was constructed by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and a best fit line was drawn through the points on the graph. (***Appendix VII***)

2.8. Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-20 (Statistical Packages for Social Sciences- version 20).

Data were presented in simple measures of frequency, percentage, mean, standard deviation, range (minimum-maximum values), and 5th – 99th percentiles.

The significance of difference of different means (quantitative data) was tested using analysis of variance (ANOVA) for more than two groups and using independent student-t-test for difference between two means, while different percentages (qualitative data) were tested using chi-square test (χ^2-test) with application of Yates correction or Fisher Exact test whenever applicable. Pearson correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation. The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) with value <0.3 represent no correlation, 0.3 - <0.5 represent weak correlation, 0.5 - <0.7 moderate strength, >0.7 strong correlation. Statistical significance was considered whenever the P value was equal or less than 0.05.

Chapter Three

Results

Results:

3.1. Clinical findings:

Eighty subjects were participated in this study.

3.1.1. Age and sex:

1- Oral lichen planus (OLP) patients group: forty eight (48) patients with OLP, 25 of the patients were females (52.1%) and 23 were males (47.9%) with an age range of (30-69) years and mean age of (50.9±10.4 years).

The study group was divided into two subgroups according to the clinical presentation of the lesions, 21 patients were with reticular form of OLP (43.75%) and 27 patients were with erosive form (56.25%).

2- Control group: - thirty two (32) healthy subjects, 20 were females (62.5%) and 12 were males (37.5%) with an age range of (30-65) years and mean age of (49.1±9.8 years). (Table 3-1)

Table (3-1): Distribution of the study groups according to the age and sex

		Patients		Controls		P value
		No	%	No	%	
Age (years)	30--39	6	12.5	5	15.6	0.901
	40--49	10	20.8	8	25.0	
	50--59	22	45.8	14	43.8	
	60--69 years	10	20.8	5	15.6	
	Mean±SD (Range)	50.9±10.4 (30-69)		49.1±9.8 (30-65)		
Sex	Males	23	47.9	12	37.5	0.358
	Females	25	52.1	20	62.5	

When the group of patients with reticular form was compared with patients with erosive form, no statistically significant differences were found in terms of the mean age and sex. (Table 3-2)

Table (3-2): Distribution of reticular and erosive OLP patients group according to the age and sex

Clinical Type	Number	Sex		*chi square test*	Mean age
		Male	Female		
Reticular	21	12	9	$X^2=0.429$ $p=0.513$	49.95 ±10.8
Erosive	27	11	16	$X^2=0.926$ $p=0.336$	51.74 ±10.5
					t-test=0.578 *P=0.566*

3.2. Oral findings:

3.2.1. Site affected:

The present study revealed that the buccal mucosa was the most affected site (91.7%) followed by gingiva (22.2%), tongue (14.8%) then lip (4.8%).

In erosive form, the buccal mucosa represented (88.9%) of affected sites followed by gingiva (22.2%), then tongue (14.8%). (Table 3-3) (Figure 3-1, 3-2, 3-3)

In reticular form, the buccal mucosa represented (95.2%) of affected sites followed by lip (4.8%). (Table 3-3) (Figure 3-1, 3-4)

Table (3-3): Distribution of OLP patients according to the site of lesion

Site affected	Reticular	%	Erosive	%	TOTAL	%
Buccal Mucosa	20	95.2%	24	88.9%	44	91.7%
Gingiva	0	0%	6	22.2%	6	22.2%
Tongue	0	0%	4	14.8%	4	14.8%
lip	1	4.8%	0	0%	1	4.8%

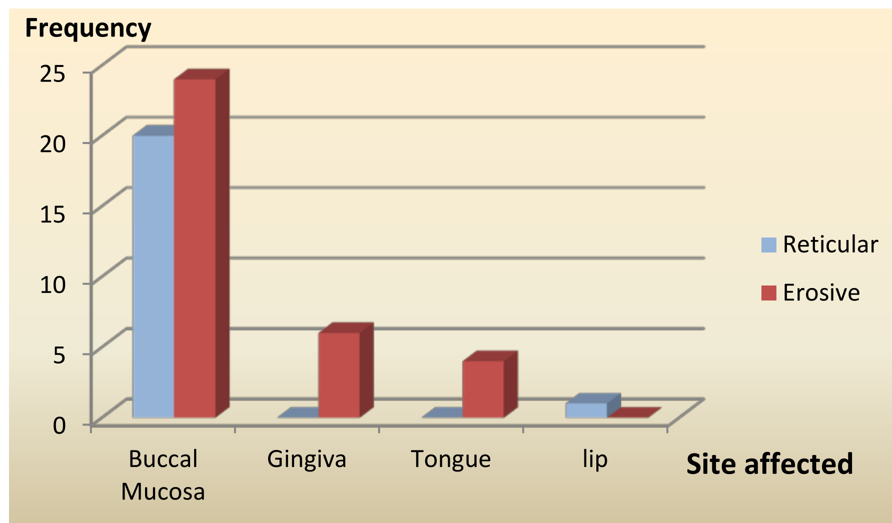


Figure (3-1): Distribution of OLP patients according to the site of lesion

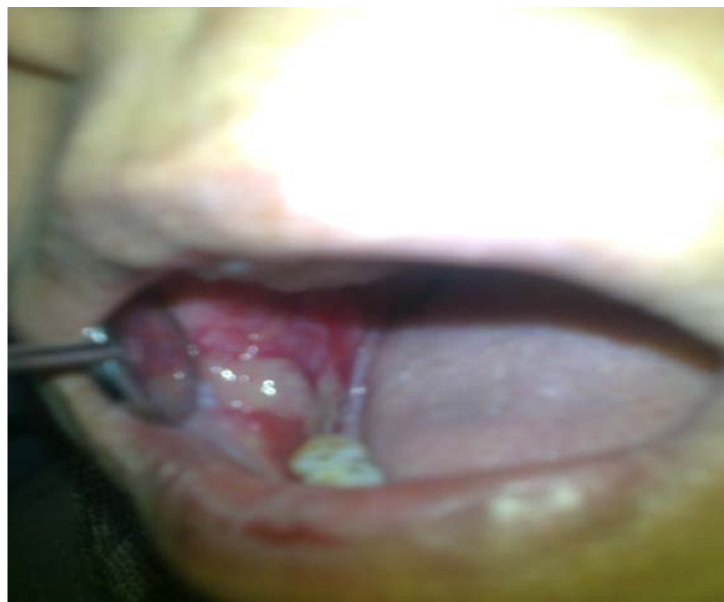


Figure (3-2): Erosive oral lichen planus involving the buccal mucosa

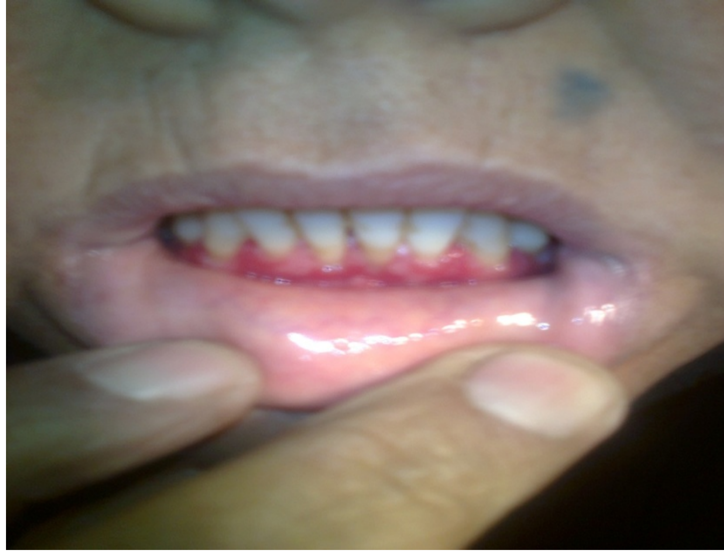


Figure (3-3): Desquamative gingivitis

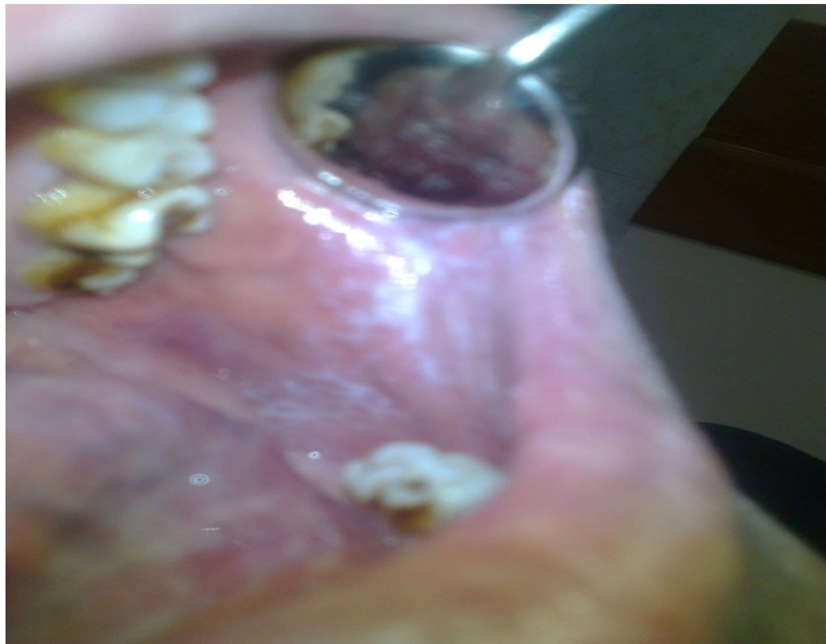


Figure (3-4): Reticular oral lichen planus involving the buccal mucosa

3.2.2. Number of sites affected:

The present study showed that 74.1% of patients with erosive form of OLP presented with one anatomical site affected and 25.9% of the patients were with multiple-site oral involvement, whereas all patients with reticular form presented with one anatomical site affected. (Table 3-4) (Figure 3-5)

Table (3-4): Distribution of OLP patients according to the number of affected site

No. of sites affected	Reticular	%	Erosive	%	TOTAL	%
One anatomical site	21	100%	20	74.1%	41	85.4%
More than one site	0	0%	7	25.9%	7	14.6%

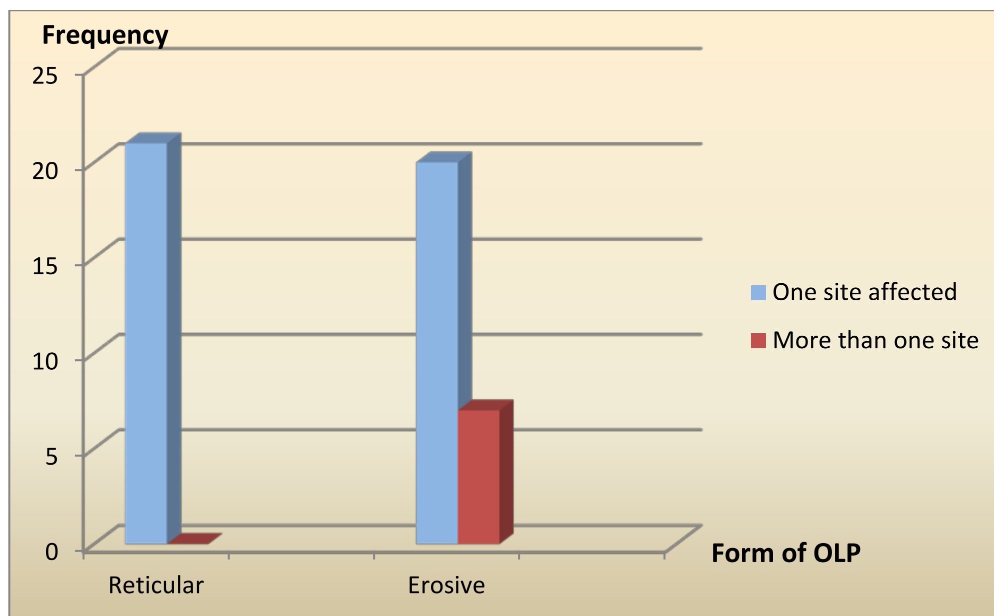


Figure (3-5): Distribution of OLP patients according to the number of affected site

3.2.3. Extension of the lesion:

With respect to the extension of the lesion, the results showed that 70.4% of patients were of severe extension in erosive OLP patients compared to 42.9% in reticular form and 29.6% of patients were of light extension in erosive form compared to 57.1% in reticular form. (Table 3-5) (Figure 3-6)

Table (3-5): Distribution of OLP patients according to the extension of the lesion

Size of lesion	Reticular	%	Erosive	%	TOTAL	%
Light extension	12	57.1%	8	29.6%	20	41.7%
Severe extension	9	42.9%	19	70.4%	28	58.3%
chi square test	$X^2=0.429$ $P=0.513$		$X^2=4.481$ $P=0.034$*		$X^2=1.333$ $P=0.248$	

*Significant using Pearson Chi-square test at 0.05 level

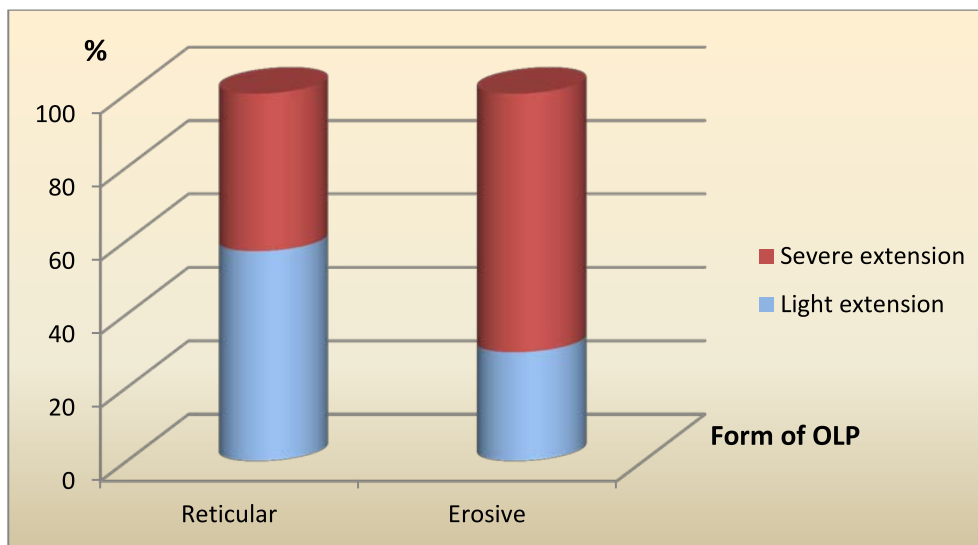


Figure (3-6): Distribution of OLP patients according to the extension of the lesion

3.2.4. Intensity of pain:

Symptomatic OLP were associated with erosive form. Symptoms varied from mucosal sensitivity which represented 88.9% to continuous debilitating pain 11.1%. (Table 3-6) (Figure 3-7)

Table (3-6): Distribution of OLP patients according to the intensity of pain

Intensity of pain	Reticular	%	Erosive	%
No pain	21	100%	0	0%
Mucosal sensitivity	0	0%	24	88.9%
Continuous debilitating pain	0	0%	3	11.1%

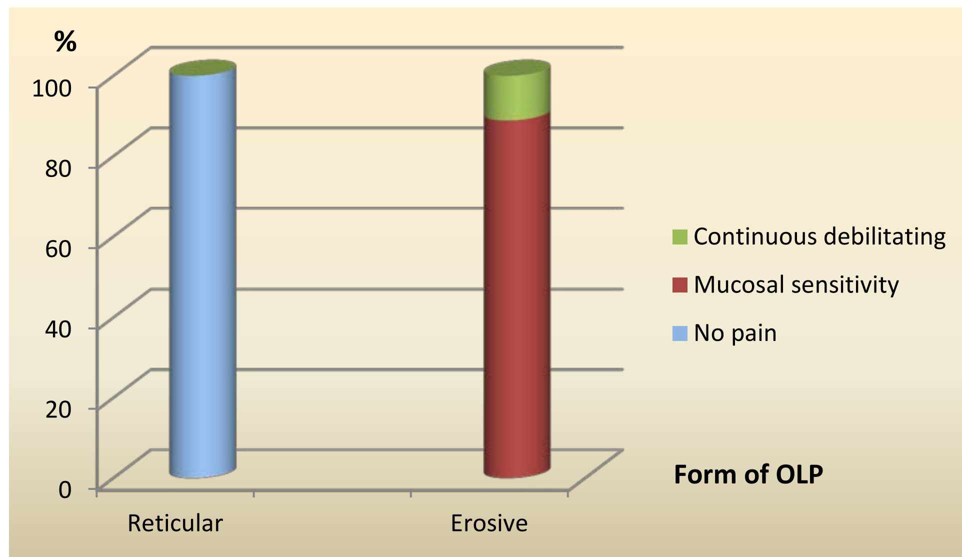


Figure (3-7): Distribution of OLP patients according to the intensity of pain

3.2.5. Distribution of reticular and erosive OLP patients according to the severity:

According to the total disease severity scores, the reticular OLP patients group has been divided into two subgroups, (52.4%) of patients were of score 2 and (47.6%) of patients were of score 4. (Table 3-7)

With respect to erosive OLP patients group, four subgroups have been formed. (29.6%) of patients were of score 3, (33.3%) of patients were of score 5, (18.5%) of patients were of score 6 and (18.5%) of patients were of score 7. (Table 3-7)

Table (3-7): Distribution of reticular and erosive OLP patients according to severity scores

		No	%
Types	Reticular	21	43.8
	Erosive	27	56.3
Reticular severity	2	11	52.4
	4	10	47.6
Erosive severity	3	8	29.6
	5	9	33.3
	6	5	18.5
	7	5	18.5

3.3. Laboratory findings:

3.3.1. Apoptotic markers:

3.3.1.1. Soluble Fas:

The mean of serum sFas in patients with OLP (3181.17±702.01pg/ml) was higher than that of control group (2958.04±607.94 pg/ml), but it did not reach a significant level by t-test ($p>0.05$). (Table 3-8) (Figure 3-8)

The mean of saliva sFas in patients with OLP (483.33±218.80 pg/ml) was higher than that of control group (396.08±189.56 pg/ml), but it did not reach a significant level by t-test ($p>0.05$). (Table 3-9) (Figure 3-8)

Table (3-8): Mean and SD of serum sFas with t-test between OLP patients and controls

Serum Soluble Fas (pg/ml)	Patients	Controls
No	48	32
Mean±SD	3181.17±702.01	2958.04±607.94
Standard Error of Mean	101.327	107.470
Mode	3178.7	2938.7
Range	1738.7-4885.3	1985.3-4265.3
Percentile 05th	2018.7	2045.3
25th	2755.3	2568.7
50th (Median)	3188.7	2888.7
75th	3678.7	3348.7
95th	4318.7	4232.0
99th	4885.3	4265.3
P value	0.146	

Table (3-9): Mean and SD of saliva sFas with t-test between OLP patients and controls

Saliva Soluble Fas (pg/ml)	Patients	Controls
Mean±SD	483.33±218.80	396.08±189.56
Standard Error of Mean	31.580	33.510
Mode	460.2	336.0
Range	105.2-1053.5	16.0-763.5
Percentile 05th	155.2	18.5
25th	311.8	301.8
50th (Median)	459.3	420.6
75th	631.4	502.7
95th	891.8	741.0
99th	1053.5	763.5
P value	0.069	

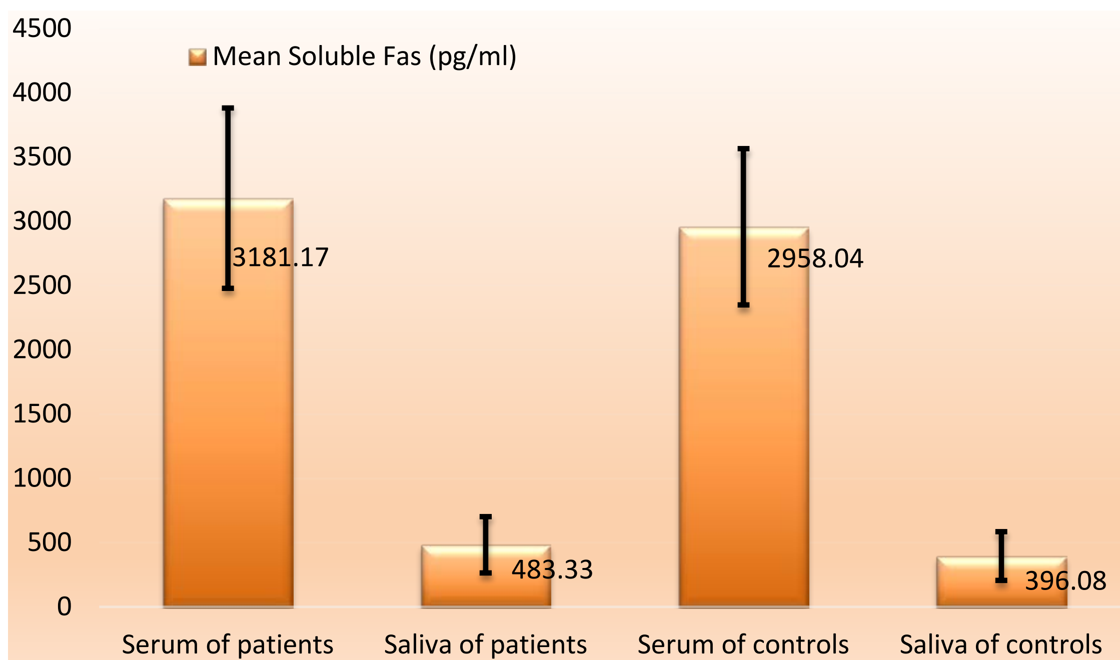


Figure (3-8): The mean of Fas conc. in serum and saliva of OLP patients and control groups

3.3.1.2. Fas Ligand:

The mean of serum Fas ligand in patients with OLP (504.537±168.432 pg/ml) was higher than that of control group (465.833±140.926 pg/ml), but it did not reach a significant level by using t-test ($p>0.05$). (Table 3-10) (Figure 3-9)

The present study showed that sFasL was not detected in saliva samples of both OLP patients and control subjects.

Table (3-10): Mean and SD of serum sFasL with t-test between OLP patients and controls

Serum Soluble Fas Ligand (pg/ml)	Patients	Controls
No	48	32
Mean±SD	504.537±168.432	465.833±140.926
Standard Error of Mean	24.311	24.912
Mode	556.7	258.9
Range	254.4-932.2	107.8-778.9
Percentile 05th	258.9	223.3
25th	353.3	414.4
50th (Median)	498.9	476.7
75th	577.8	548.9
95th	852.2	681.1
99th	932.2	778.9
P value	0.287	

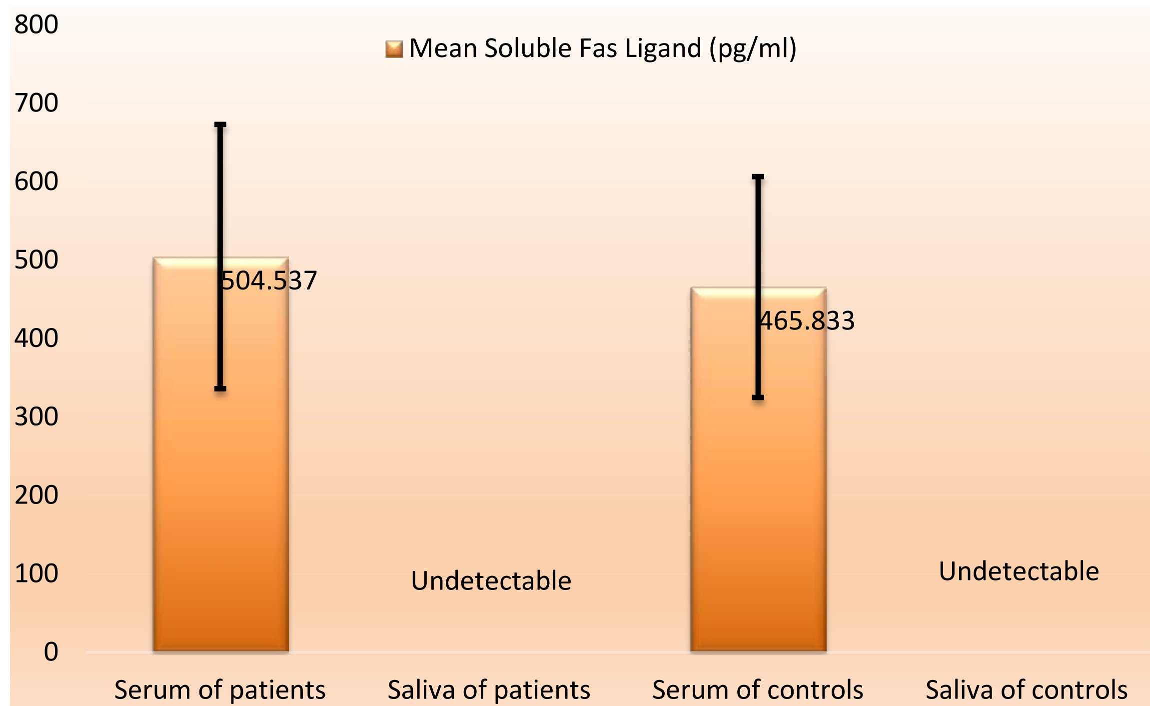


Figure (3-9): The mean of sFas ligand conc. in serum of OLP patients and control groups

3.3.1.3. Bcl-2:

The present study showed that the mean of serum Bcl-2 in patients with OLP (3.750±2.244 ng/ml) was lower than that of control group (4.117±1.713 ng/ml), but also it did not reach a significant level by using t-test ($p>0.05$). (Table 3-11) (Figure 3-10)

Also Bcl-2 was not detected in saliva samples of the study groups.

Table (3-11): Mean and SD of serum Bcl-2 with t-test between OLP patients and controls

Serum Bcl-2 (ng/ml)	Patients	Controls
No	48	32
Mean±SD	3.750±2.244	4.117±1.713
Standard Error of Mean	0.324	0.303
Mode	1.57	3.26
Range	0.56-13.90	1.29-8.32
Percentile 05th	1.05	1.48
25th	2.20	3.26
50th (Median)	3.43	3.66
75th	4.71	4.98
95th	6.39	7.83
99th	13.90	8.32
P value	0.435	

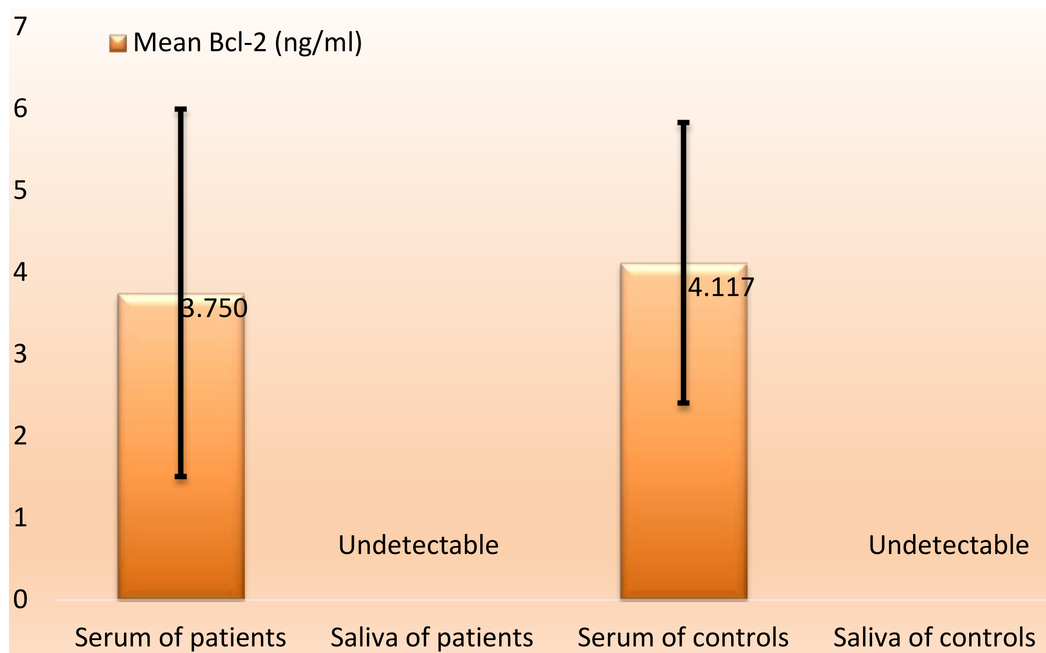


Figure (3-10): Mean levels of Bcl-2 conc. in serum of OLP and control group

3.3.2 Oxidative stress markers:

3.3.2.1. MDA:

The present study showed that the mean level of serum MDA in patients with OLP (4.725±1.634 µmol/l) was significantly higher ($p<0.001$) by using t-test than that of control group (1.626±0.712 µmol/l). (Table 3-12) (Figure 3-11)

The mean level of saliva MDA in patients with OLP (0.972±0.433 µmol/l) was significantly higher ($p<0.05$) by using t-test than that of control group (0.732±0.358 µmol/l). (Table 3-13) (Figure 3-11)

Table (3-12): Mean and SD of serum MDA with t-test between OLP patients and controls

Serum MDA (µmol/l)	Patients	Controls
No	48	32
Mean±SD	4.725±1.634	1.626±0.712
Standard Error of Mean	0.236	0.126
Mode	2.88	1.88
Range	2.68-9.48	0.52-3.48
Percentile 05th	2.88	0.60
25th	3.44	1.04
50th (Median)	4.32	1.62
75th	6.10	1.98
95th	7.20	3.44
99th	9.48	3.48
P value	0.0001**	

** Highly Significant using Students-t-test for difference between two independent means at 0.01 level.

Table (3-13): Mean and SD of saliva MDA with t-test between OLP patients and controls

Saliva MDA (µmol/l)	Patients	Controls
Mean±SD	0.972±0.433	0.732±0.358
Standard Error of Mean	0.074	0.070
Mode	0.88	0.56
Range	0.48-2.12	0.12-1.40
Percentile 05th	0.52	0.32
25th	0.64	0.48
50th (Median)	0.84	0.58
75th	1.24	1.04
95th	2.00	1.40
99th	2.12	1.40
P value	0.026*	

*Significant using Students-t-test for difference between two independent means at 0.05 level.

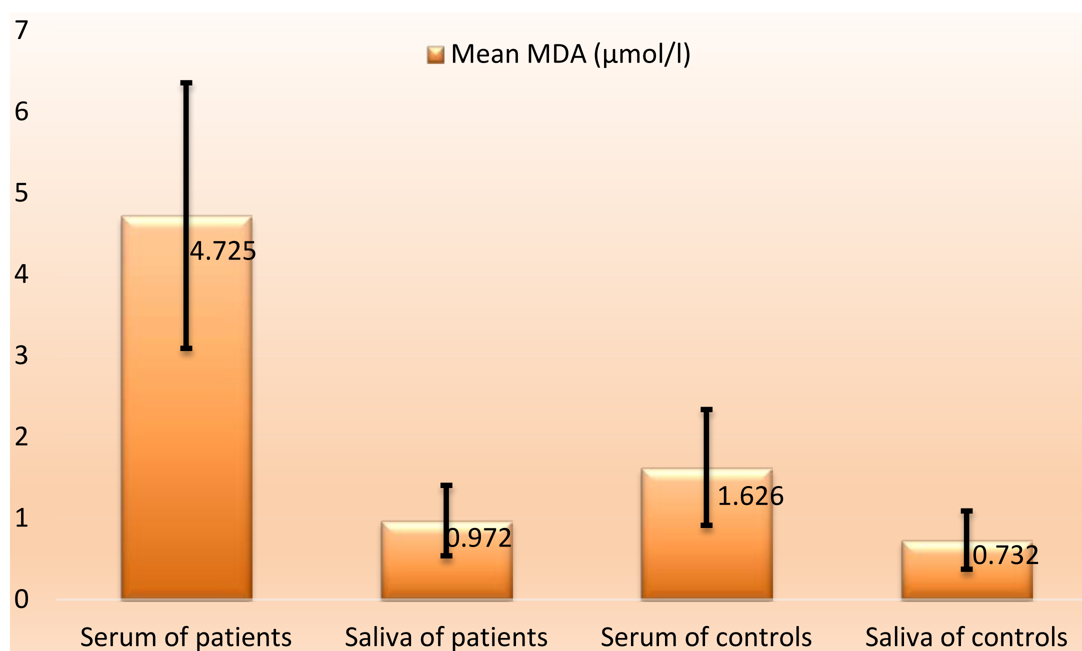


Figure (3-11): Mean of MDA conc. in serum and saliva of OLP patients and control groups

3.3.2.2. Nitric oxide (NO):

The present study showed that the mean of serum NO in patients with OLP (0.042±0.023 nmol/µl) was non-significantly difference (P>0.05) compared with control group (0.045±0.011 nmol/µl). (Table 3-14) (Figure 3-12)

It has been shown that the mean of saliva NO in patients with OLP (0.061±0.072 nmol/µl) was also non-significantly difference (P>0.05) compared with controls (0.042±0.039 nmol/µl). (Table 3-15) (Figure 3-12)

Table (3-14): Mean and SD of serum NO with t-test between OLP patients and controls

Serum Nitric oxide (nmol/µl) [mM]	Patients	Controls
No	48	32
Mean±SD	0.042±0.023	0.045±0.011
Standard Error of Mean	0.003	0.003
Mode	0.038	0.045
Range	0.011-0.125	0.019-0.056
Percentile 05th	0.017	0.019
25th	0.022	0.035
50th (Median)	0.038	0.048
75th	0.053	0.053
95th	0.085	0.056
99th	0.125	0.056
P value	0.623	

Table (3-15): Mean and SD of saliva NO with t-test between OLP patients and controls

Saliva Nitric oxide (nmol/µl) [mM]	Patients	Controls
Mean±SD	0.061±0.072	0.042±0.039
Standard Error of Mean	0.010	0.008
Mode	0.002	0.034
Range	0.002-0.274	0.007-0.157
Percentile 05th	0.003	0.007
25th	0.014	0.014
50th (Median)	0.033	0.032
75th	0.080	0.050
95th	0.244	0.124
99th	0.274	0.157
P value	0.240	

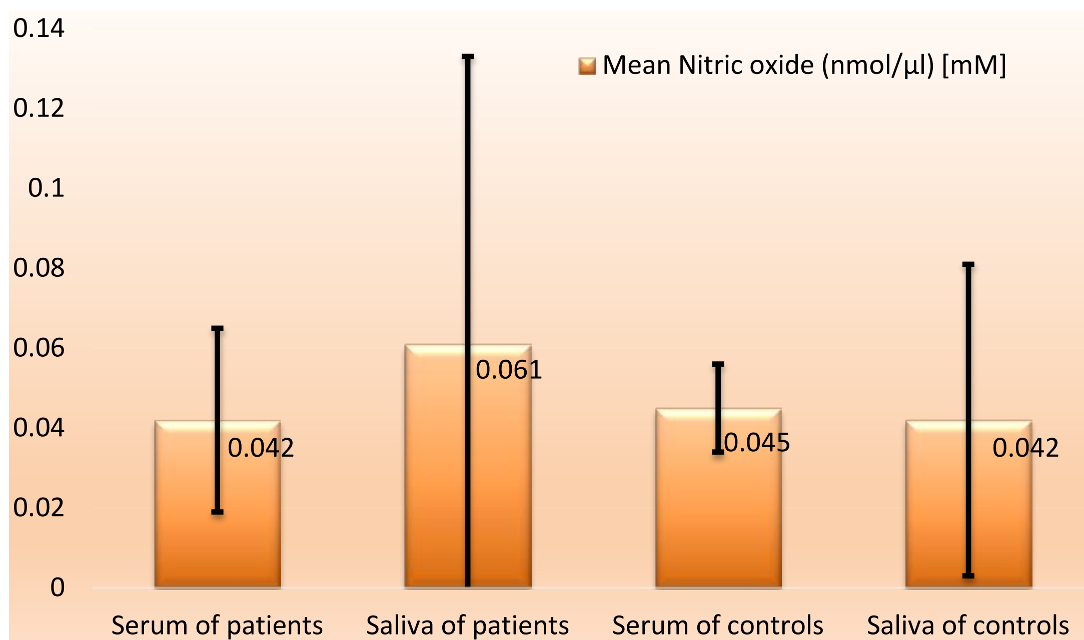


Figure (3-12): Mean of nitric oxide conc. in serum and saliva of OLP patients and control groups

3.3.3. Antioxidant:

3.3.3.1. Ceruloplasmin:

The present study revealed that the mean of serum ceruloplasmin in patients with OLP (0.408±0.101 g/l) was significantly higher ($p<0.001$) by using t-test than that of control group (0.311±0.105 g/l). (Table 3-16) (Figure 3-13)

The mean of saliva ceruloplasmin in patients with OLP (0.014±0.009 g/l) was significantly higher ($p<0.01$) using t-test than that of control group (0.009±0.009 g/l). (Table 3-17) (Figure 3-13)

Table (3-16): Mean and SD of serum ceruloplasmin with t-test between OLP patients and controls

Serum Ceruloplasmin (g/l)	Patients	Controls
No	48	32
Mean±SD	0.408±0.101	0.311±0.105
Standard Error of Mean	0.015	0.018
Mode	0.299	0.224
Range	0.274-0.710	0.188-0.619
Percentile 05th	0.299	0.202
25th	0.338	0.240
50th (Median)	0.393	0.292
75th	0.448	0.346
95th	0.613	0.618
99th	0.710	.619
P value	0.0001**	

** Highly significant using Students-t-test for difference between two independent means at 0.01 level.

Table (3-17): Mean and SD of saliva ceruloplasmin with t-test between OLP patients and controls

Saliva Ceruloplasmin (g/l)	Patients	Controls
Mean±SD	0.014±0.009	0.009±0.009
Standard Error of Mean	0.001	0.002
Mode	0.013	0.003
Range	0.003-0.056	0.002-0.035
Percentile 05th	0.004	0.003
25th	0.008	0.003
50th (Median)	0.013	0.006
75th	0.018	0.010
95th	0.034	0.034
99th	0.056	0.035
P value	0.01**	

** Highly significant using Students-t-test for difference between two independent means at 0.01 level.

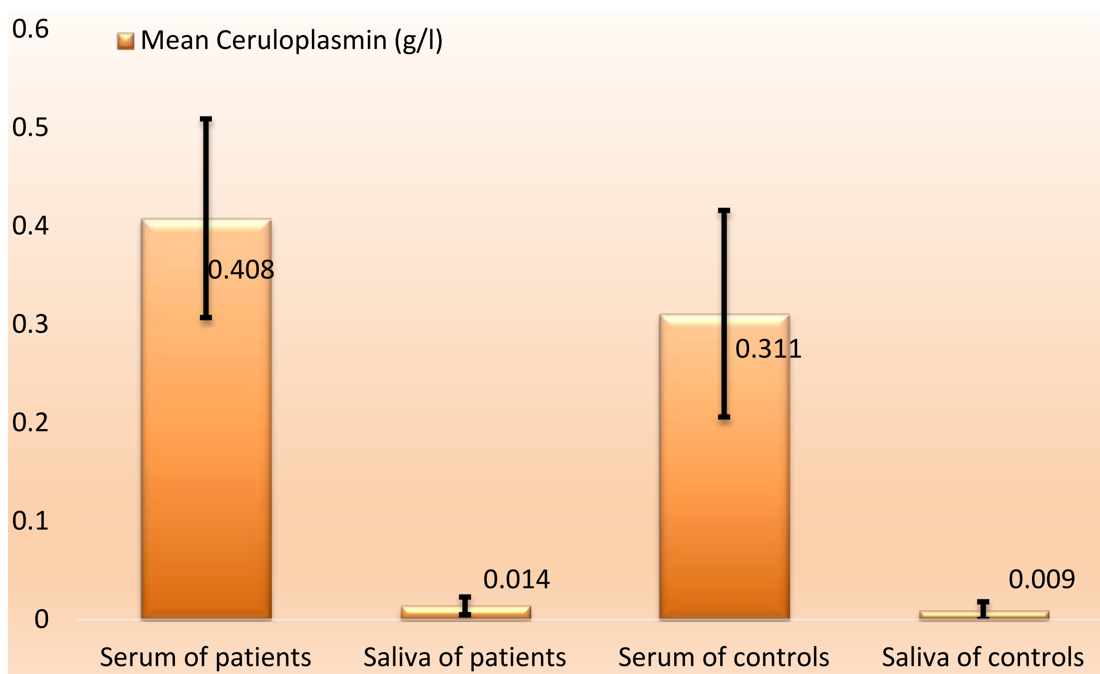


Figure (3-13): Mean of ceruloplasmin conc. in serum and saliva of OLP patients and control groups

3.3.3.2. Uric acid:

The results showed that the mean of serum UA in patients with OLP (5.474±1.855 mg/dl) was lower than that of control group (5.910±1.731 mg/dl), but it did not reach a significant level by using t-test ($p>0.05$). (Table 3-18) (Figure 3-14)

The mean of saliva UA in patients with OLP (3.185±1.951 mg/dl) was lower than that of control group (3.201±1.830mg/dl), but it did not reach a significant level by using t-test ($p>0.05$). (Table 3-19) (Figure 3-14)

Table (3-18): Mean and SD of serum uric acid with t-test between OLP patients and controls

Serum Uric Acid (mg/dl)	Patients	Controls
No	48	32
Mean±SD	5.474±1.855	5.910±1.731
Standard Error of Mean	0.277	0.316
Mode	3.17	4.05
Range	1.30-9.71	3.29-9.44
Percentile 05th	3.07	3.45
25th	4.11	4.43
50th (Median)	5.44	6.12
75th	6.28	6.80
95th	8.75	9.40
99th	9.71	9.44
P value	0.310	

Table (3-19): Mean and SD of saliva uric acid with t-test between OLP patients and controls

Saliva Uric Acid (mg/dl)	Patients	Controls
Mean±SD	3.185±1.951	3.201±1.830
Standard Error of Mean	0.282	0.324
Mode	1.79	3.08
Range	0.02-6.97	0.33-6.84
Percentile 05th	0.29	0.40
25th	1.79	1.86
50th (Median)	2.61	3.08
75th	4.68	3.91
95th	6.75	6.29
99th	6.97	6.84
P value	0.972	

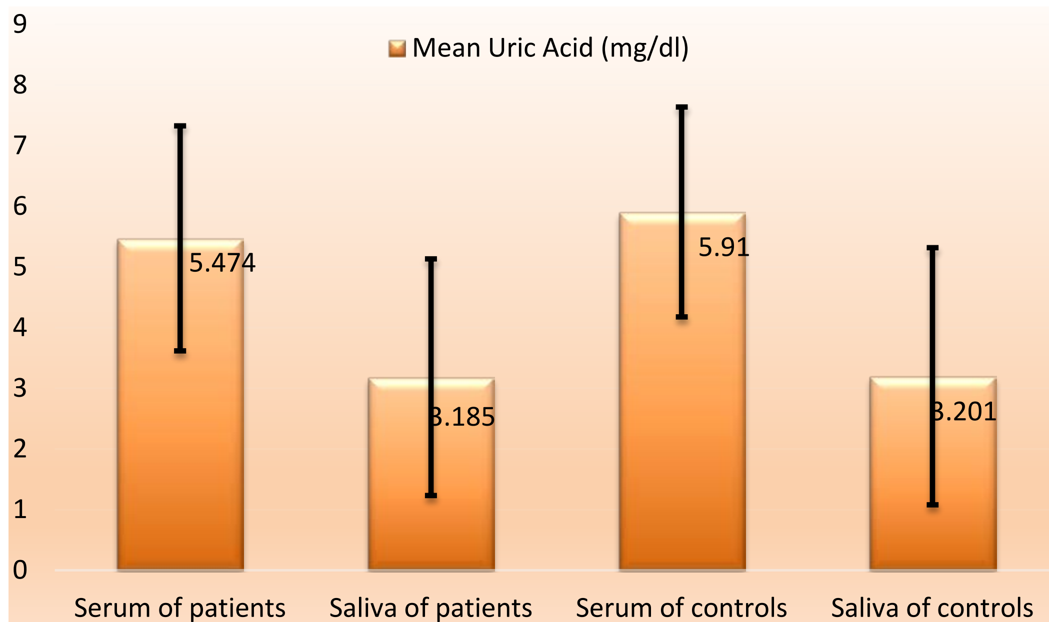


Figure (3-14): Mean of Uric acid conc. in serum and saliva of OLP patients and control groups

3.3.3.3. Oxidized glutathione:

The results showed that there was no significant difference (P>0.05) in mean of serum GSSG (19857.3±1414.9 pg/ml) in patients with OLP compared with controls (20150.0±1383.9 pg/ml). (Table 3-20) (Figure 3-15)

Statistical analysis using t-test showed no significant difference (P>0.05) was found between the mean of saliva GSSG in patients with OLP (162.55±61.26 pg/ml) and that in control group (164.25±49.92 pg/ml). (Table 3-21) (Figure 3-15)

Table (3-20): Mean and SD of serum GSSG with t-test between OLP patients and controls

Serum GSSG (pg/ml)	Patients	Controls
No	48	32
Mean±SD	19857.3±1414.9	20150.0±1383.9
Standard Error of Mean	204.224	252.672
Mode	19175.0	20925.0
Range	15425.0-21925.0	14425.0-21675.0
Percentile 05th	17675.0	18175.0
25th	19050.0	19675.0
50th (Median)	20050.0	20425.0
75th	20925.0	20925.0
95th	21925.0	21675.0
99th	21925.0	21675.0
P value	0.373	

Table (3-21): Mean and SD of saliva GSSG with t-test between OLP patients and controls

Saliva GSSG (pg/ml)	Patients	Controls
Mean±SD	162.55±61.26	164.25±49.92
Standard Error of Mean	8.842	8.824
Mode	207.2	153.8
Range	2.2-223.8	40.5-215.5
Percentile 05th	8.8	48.8
25th	164.7	149.7
50th (Median)	181.3	180.5
75th	203.8	203.0
95th	213.8	212.2
99th	223.8	215.5
P value	0.896	

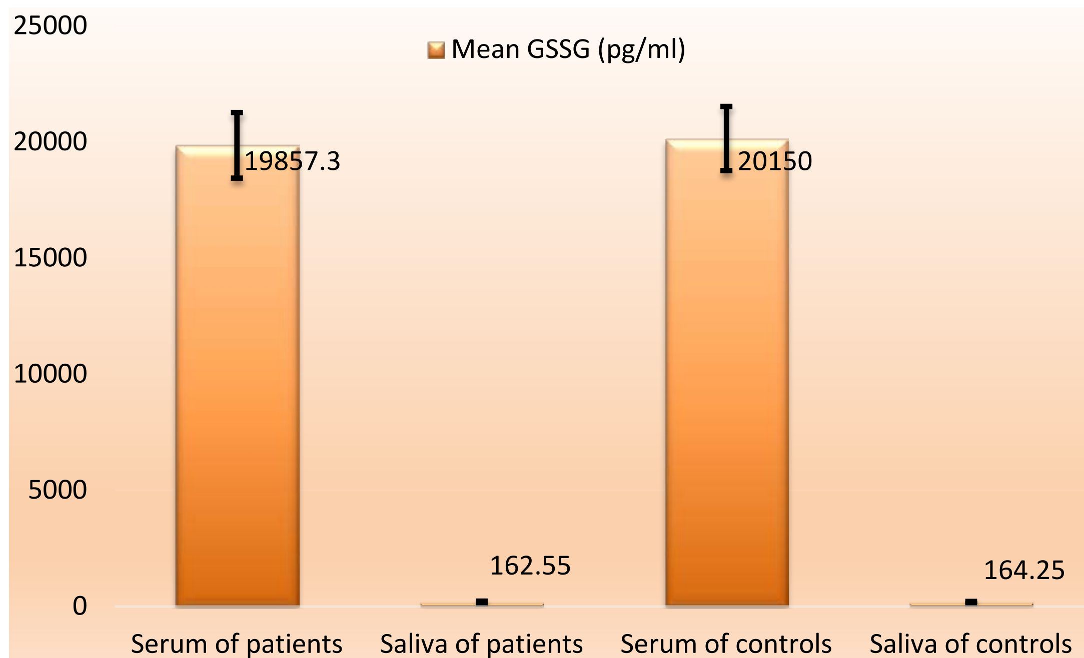


Figure (3-15): Mean of GSSG conc. in serum and saliva of OLP patients and control groups

3.3.4. Laboratory findings in reticular and erosive OLP:

The present study showed that there was no statistically significant difference in serum and saliva of all parameters between patients with reticular and patients with erosive form of OLP, with the exception of sFas and NO which showed that the means of sFas and NO in the saliva of patients with erosive OLP form were significantly higher than that in patients with reticular form. (Table 3-22)

Table (3-22): Means and significant level of serum and saliva parameters between reticular and erosive forms of OLP patients group

	Types		P value
	Reticular	Erosive	
Bcl-2 (ng/ml) Serum	3.908±2.806	3.627±1.735	0.672
Saliva	-	-	
Soluble Fas (pg/ml) Serum	3339.302±728.548	3058.173±668.334	0.171
Saliva	387.548±180.750	557.821±219.621	0.006**
Soluble Fas Ligand (pg/ml) Serum	494.762±145.576	512.140±186.666	0.727
Saliva	-	-	
MDA (µmol/l) Serum	4.703±1.818	4.742±1.512	0.935
Saliva	0.994±0.426	0.956±0.448	0.804
Ceruloplasmin (g/l) Serum	0.418±0.130	0.400±0.072	0.549
Saliva	0.013±0.007	0.015±0.011	0.394
Uric Acid (mg/dl) Serum	5.906±2.175	5.129±1.512	0.165
Saliva	3.363±1.726	3.047±2.132	0.584
GSSG (pg/ml) Serum	19425.000±1468.418	20193.519±1300.750	0.061
Saliva	170.262±58.158	156.549±64.000	0.448
Nitric oxide (nmol/µl) [mM] Serum	0.041±0.019	0.043±0.026	0.808
Saliva	0.036±0.045	0.080±0.083	0.033*

*Significant using Students-t-test for difference between two independent means at 0.05 level.

** Highly significant using Students-t-test for difference between two independent means at 0.01 level.

3.3.5. Laboratory findings according to severity scores:

3.3.5.1. Reticular OLP:

There was no significant difference (p>0.05) in serum and saliva parameters in relation to severity of reticular OLP, with the exception of serum ceruloplasmin which was significantly higher in patients with reticular OLP of severity score 4 (p<0.05). Table (3-23)

Table (3-23): Means and significant level of serum and saliva parameters in relation to severity scores in patients with reticular OLP

	Reticular severity		P value
	2	4	
Bcl-2 (ng/ml) Serum	4.552±3.417	3.199±1.859	0.281
Saliva	-	-	
Soluble Fas (pg/ml) Serum	3412.000±615.135	3259.333±863.432	0.644
Saliva	406.909±211.057	366.250±148.811	0.619
Soluble Fas Ligand (pg/ml) Serum	502.121±183.866	486.667±96.911	0.815
Saliva	-	-	
MDA (µmol/l) Serum	4.436±1.437	4.996±2.206	0.495
Saliva	1.080±0.475	0.930±0.406	0.536
Ceruloplasmin (g/l) Serum	0.362±0.075	0.479±0.152	0.036*
Saliva	0.011±0.009	0.014±0.004	0.308
Uric Acid (mg/dl) Serum	5.763±2.261	6.081±2.188	0.754
Saliva	3.912±1.917	2.759±1.327	0.129
GSSG (pg/ml) Serum	19970.455±1312.527	18825.000±1453.922	0.073
Saliva	150.348±74.440	192.167±18.476	0.101
Nitric oxide (nmol/µl) [mM] Serum	0.041±0.018	0.041±0.021	0.922
Saliva	0.048±0.055	0.023±0.027	0.221

*Significant using Students-t-test for difference between two independent means at 0.05 level.

3.3.5.2. Erosive OLP:

There was no significant difference (p>0.05) in serum and saliva of the study parameters in relation to severity scores of patients with erosive form of OLP. (Table 3-24)

Table (3-24): Means and significant level of serum and saliva parameters in relation to severity scores of patients with erosive form of OLP

	Erosive severity				**P value**
	3	5	6	7	
Bcl-2 (ng/ml) Serum	2.813±1.551	4.113±1.910	3.374±1.952	4.307±1.319	0.357
Saliva	-	-	-	-	
Soluble Fas (pg/ml) Serum	2889.5±630.585	3100.2±601.367	2916.0±897.770	3394.7±671.228	0.590
Saliva	630.271±300.9	587.296±198.0	434.833±205.1	511.833±23.6	0.441
Soluble Fas Ligand (pg/ml) Serum	521.67±134.711	547.28±239.497	491.33±205.280	454.44±173.810	0.847
Saliva	-	-	-	-	
MDA (µmol/l) Serum	4.890±1.792	5.324±1.518	3.608±1.106	4.592±0.982	0.239
Saliva	1.147±0.550	0.809±0.217	1.027±0.845	0.940±0.255	0.580
Ceruloplasmin (g/l) Serum	0.389±0.070	0.413±0.075	0.399±0.098	0.396±0.064	0.928
Saliva	0.016±0.010	0.013±0.008	0.012±0.006	0.022±0.019	0.438
Uric Acid (mg/dl) Serum	4.860±1.379	5.803±1.842	4.903±0.727	4.482±1.267	0.408
Saliva	3.840±2.326	2.904±2.063	3.534±2.134	1.550±1.616	0.285
GSSG (pg/ml) Serum	20081.3±1802.5	20063.9±1199.7	20675.0±984.3	20125.0±1051.8	0.855
Saliva	145.917±66.163	162.722±68.729	153.167±76.017	165.833±57.475	0.943
Nitric oxide (nmol/µl) [mM] Serum	0.042±0.026	0.036±0.021	0.036±0.018	0.062±0.036	0.288
Saliva	0.058±0.067	0.060±0.051	0.089±0.103	0.142±0.119	0.279

3.4. The correlation between serum and saliva parameters in OLP patients:

The study showed that there was no statistically significant correlation between serum and saliva measurements of all parameters (sFas, CP, GSSG, MDA and UA) (p>0.05) in patients with OLP (Figure 3-16, 17, 18, 19 and 20) except with NO which showed a positive highly significant linear correlation (r=0.449, p<0.001).

Table (3-25): Correlation coefficient and significant level between serum and saliva parameters in OLP group

Patients		Salivary							
		Bcl-2 (ng/ml)	Soluble Fas (pg/ml)	Soluble Fas Lig (pg/ml)	MDA (µmol/l)	Ceruloplasmin (g/l)	Uric Acid (mg/dl)	GSSG (pg/ml)	N oxide (nmol/µl) [mM]
Serum Bcl-2 (ng/ml)	r	-							
	P	-							
Soluble Fas (pg/ml)	r		-0.019						
	P		0.899						
Soluble Fas Ligand (pg/ml)	r			-					
	P			-					
MDA (µmol/l)	r				0.053				
	P				0.768				
Ceruloplasmin (g/l)	r					-0.029			
	P					0.843			
Uric Acid (mg/dl)	r						0.230		
	P						0.129		
GSSG (pg/ml)	r							-0.017	
	P							0.907	
Nitric oxide (nmol/µl) [mM]	r								0.449**
	P								0.001

**Correlation is significant at the 0.01 level.

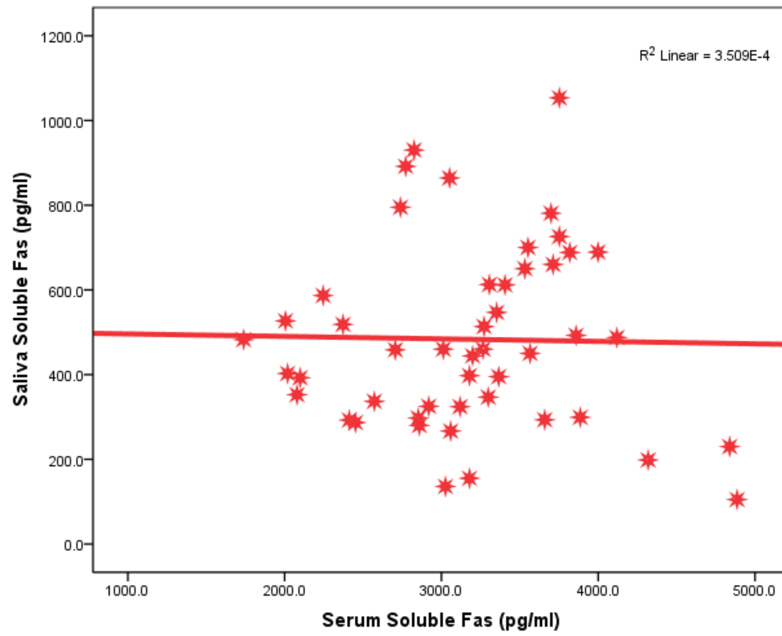


Figure (3-16): Correlation between serum and saliva sFas in OLP patients group

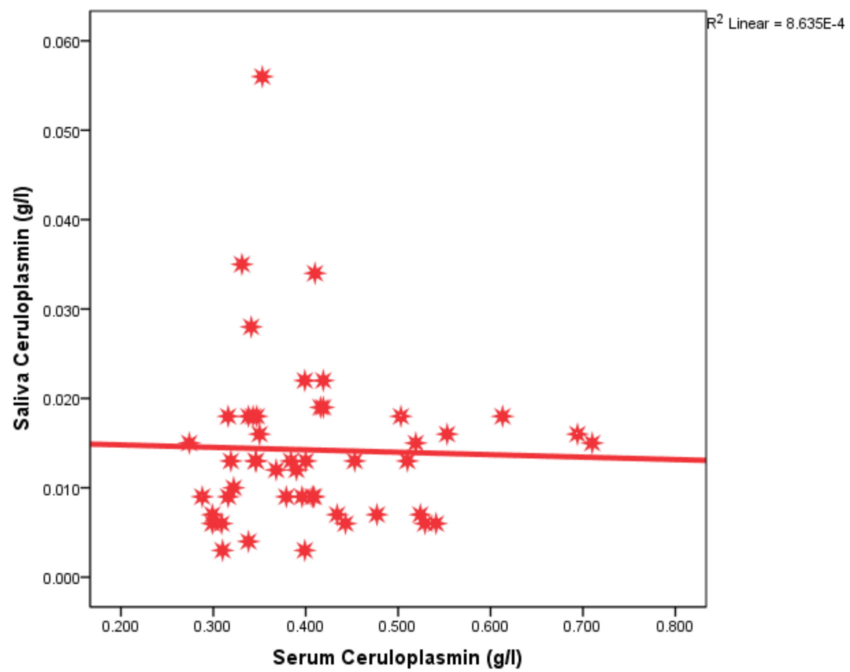


Figure (3-17): Correlation between serum and saliva ceruloplasmin in OLP patients

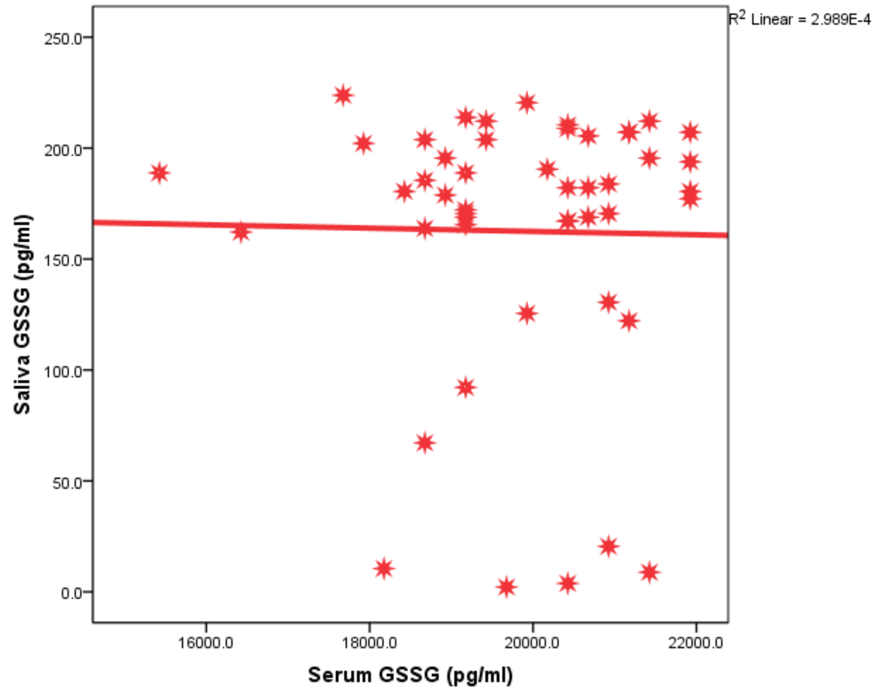


Figure (3-18): Correlation between serum and saliva GSSG in OLP patients.

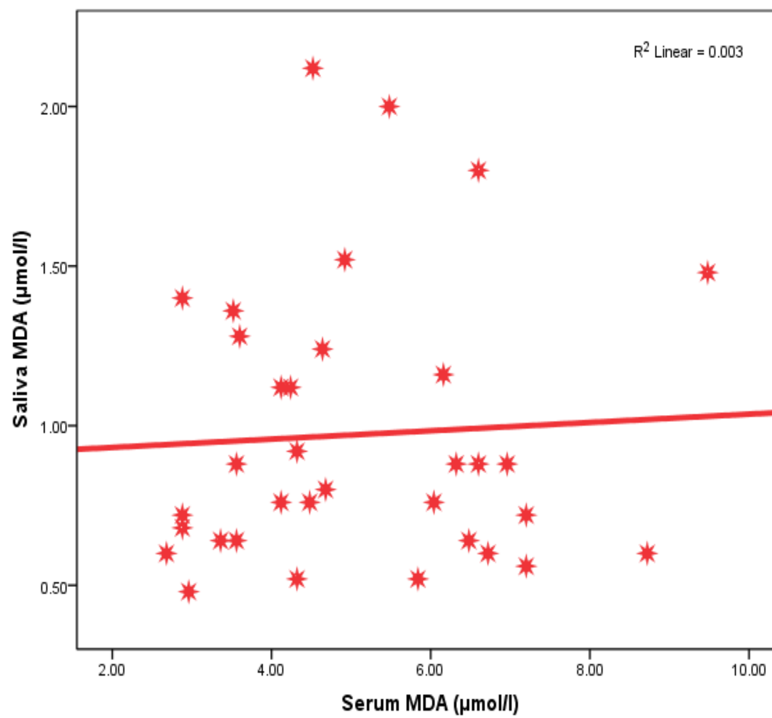


Figure (3-19): Correlation between serum and saliva MDA in OLP patients.

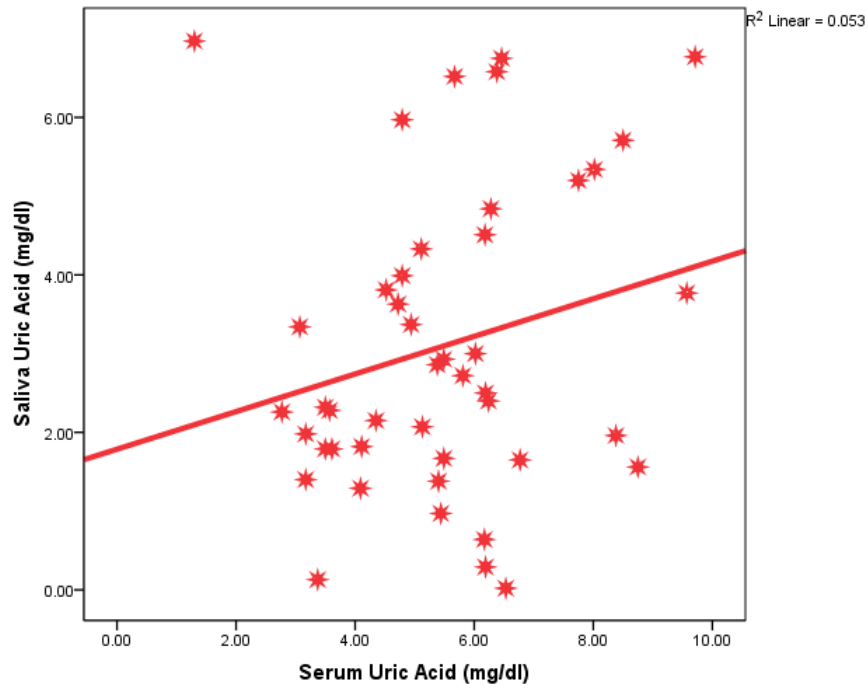


Figure (3-20): Correlation between serum and saliva uric acid in OLP patients

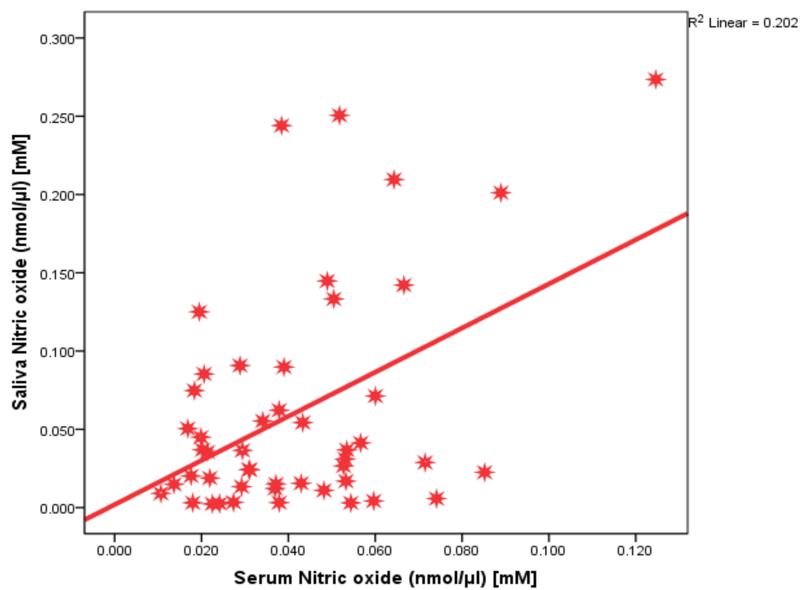


Figure (3-21): Correlation between serum and saliva nitric oxide in OLP patients.

Chapter Four
Discussion

Discussion:

Oral lichen planus (OLP) is a relatively common chronic inflammatory disease (Lombardi *et al*., 2001) characterized by relapses and remissions. There is currently no cure for OLP. Treatment is aimed primarily at reducing the length and severity of symptomatic outbreaks (Sahebjamee and Arbabi-Kalati, 2005).

The initial event in OLP lesion formation and the factors that determine OLP susceptibility are unknown. Clearly, more work is required for a full understanding of the etiology and pathogenesis of OLP (Sugerman *et al*., 2002).

According to reports, 1-2% of OLP patients develop oral squamous cell carcinoma (OSCC) in the long run. While World Health Organization (WHO) classifies OLP as “a potentially malignant disorder,” it is still a matter of debate which mechanisms drive OLP to such a condition (Georgakopoulou *et al*., 2012).

Due to the possibility of this chronic disease to cause significant discomfort and due to its malignant potentially, it is important to make a lot of studies that shed light on the issues.

4.1. Clinical findings:

The present study showed that no cutaneous lesions associated with OLP patients which was consistent with the previous studies mentioned that skin lesions were uncommon in those who complain of oral lichen planus (Cawson and Odell, 2002; Greenberg *et al*., 2008).

The present study showed that the disease had most often been reported in middle-aged adults which was consistent with previous studies by Brown *et al*., 1993; Ismail *et al*., 2007 and Oliveira Alves *et al*., 2010.

4.2. Oral findings:

The results of this study showed that, in both reticular and erosive forms of OLP patients, the buccal mucosa was commonly affected which was consistent with previous studies by Bagán-Sebastián *et al*., 1992; Gorsky *et al*., 1996; Greenberg *et al*., 2008; Oliveira Alves *et al*., 2010 and Munde *et al*., 2013.

The present study showed that most OLP patients had single-site involvement; the same result was reported by the previous studies carried out by Castells *et al*., 2010 and Al-bayati, 2012. In contrast, Xue *et al*., 2005 and Oliveira Alves *et al*, 2010 mentioned that most of the patients studied presented multiple oral lesions.

When the group of patients with reticular form was compared with patients with erosive form, no statistically significant differences were found in terms of the mean age and sex. Erosive lesions showed significantly extensive lesions which was matched with previous study done by Seoane *et al*., 2004.

As had been reported in previous study by Oliveira Alves *et al*., 2010, the present study showed that the reticular lesions were asymptomatic. In contrast, patients with erosive form showed painful symptoms varied from mucosal sensitivity to continuous debilitating pain.

4.3. Laboratory findings:

4.3.1. Apoptotic markers:

Hypothesizing that loss of basal cells in oral lichen planus is due to apoptosis (Dekker *et al*., 1997), serum and saliva of OLP patients were assessed for apoptosis-regulating proteins (positive regulator) Fas/Fas-ligand and anti-apoptotic (negative regulator) Bcl-2.

4.3.1.1. Soluble Fas (sFas):

Accelerated apoptosis of the keratinocytes and inhibition of lymphocyte apoptosis may coexist and contribute to the formation and progression of OLP (Shi *et al*., 2010). In this regard, it has been proposed that a mechanism may be produced that allows T lymphocytes to escape apoptosis, explaining the persistence of the infiltrate (Bascones-Ilundain *et al*., 2006). The sFas release may be one possible mechanism involved in T cells escape from apoptosis leading to their accumulation locally in OLP (Abdel-Rahman *et al*., 2008).

Fas-mediated apoptosis plays a vital role in the immune system. Dysfunction in this apoptosis system is most likely to be involved in the initiation and/or exacerbation of various immunological disorders

(Watanabe-Fukunaga *et al*., 1992; Lynch *et al*., 1994; Takahashi *et al*., 1994). Among its putative mechanisms sFas, which is a soluble splice variant of Fas, would function as an inhibitor of Fas/FasL interactions (Cheng *et al*., 1994). Elevated serum levels of sFas may block apoptosis of autoreactive lymphocytes, which could be important in the pathogenesis of autoimmune diseases (Tomokuni *et al*., 1997), that's where T-cell activation results in expression of FasL on the activated T cell. In an autocrine loop, they can activate their own Fas and commit suicide (activation-induced cell death, AICD) (Lawen, 2003).

Furthermore, the normal oral mucosa may be an immune privileged site, similar to the eye, testis, and placenta. Oral keratinocyte CD95L or TNF-a triggering T-cell apoptosis via CD95 or TNF R1, respectively which may prevent excessive T-cell infiltration in the normal oral mucosa, while failure of such a mechanism may result in OLP (Sugerman *et al*., 2002).

Sklavounou *et al*., (2000 and 2004) reported significantly higher serum levels of the soluble form of the Fas protein (sFas) in patients with OLP compared with healthy controls and suggested that the elimination of autoreactive lymphocytes by Fas/FasL-mediated apoptosis may be blocked by an over-regulation of sFas that may antagonize the Fas membrane-bound form in OLP lesions.

The present study found that sFas was non-significantly higher in serum and saliva of OLP patient group when compared with the control group.

Different apoptotic levels are involved in the erosive/reticular switch in OLP, determining different clinical presentations. Decreased apoptosis in inflammatory infiltrates may contribute to the persistence of T lymphocytes, worsening the attack to the epithelium in erosive OLP (Brant *et al*., 2012).

Brant *et al*., (2008) showed that significant negative correlation between the epithelial thickness and intensity of apoptosis and suggested that apoptosis is responsible for the decrease in epithelial thickness. The thinner epithelia observed in the erosive form of OLP compared to the reticular form seems to indicate that the former corresponds to a more active stage of the disease, while the latter corresponds to a more quiescent phase (Karatsaides *et al*., 2003).

Emmenegger *et al*., (2000); Fiorina *et al*., (2000); Kuwano *et al*., (2000); Papathanassoglou *et al*., (2000); Sliwa *et al*., (2000); Joashi *et al*., (2002) reported that sFas may be a marker of inflammation.

In this study, serum sFas level showed no significant difference between the two clinical forms of OLP patients which was consistent with Sklavounou *et al*., 2004.

With respect to saliva, no previous studies dealing with sFas in saliva of patients with OLP has been found. The present study showed that salivary sFas was significantly higher in erosive OLP when compared with reticular form which may be due to salivary cytokine-rich environment in erosive OLP patients facilitates the release of sFas in saliva from affected area.

Based on the results of the present and previous studies, sFas considered as a marker of inflammation needs further advanced studies.

4.3.1.2. Soluble Fas Ligand (sFasL):

Activated lymphocytes have been shown to release soluble forms of FasL (sFasL), which is capable of inducing apoptosis of Fas positive cells (Kayagaki *et al*., 1995; Tanaka *et al*., 1995). Several lines of evidence suggest that sFasL may be involved in the pathogenesis of tissue injury (Matute-Bello *et al*., 1999).

On the other hand, termination of a successful immune response could therefore be effectively achieved by silencing the driving T helper cells by T suppressor cells. As T helper cells recycle their T cell receptors (TCRs) and process TCR idiotype peptides (id) on to MHC-1, CD8 T cells with appropriate anti-id TCR might bind to and inactivate the T helper cell by a cytotoxic mechanism (Samaranayake, 2002). The shedding of sFasL has been shown to down-regulate the apoptotic and inflammatory activity of its membrane bound counterpart, suggesting that membrane-bound FasL is the functional form and that shedding of sFasL acts to regulate membrane-bound FasL cytotoxic activity (Suda *et al*., 1997; Schneider *et al*., 1998; Tanaka *et al*., 1998; Hohlbaum *et al*., 2000).

In the same context, inadequate CD95 expression by infiltrating T-cells may cause OLP (Sugerman *et al*., 2002). This leads us to suggest that over-regulation of sFasL may be involved in the pathogenesis of OLP by blocking the removal of autoreactive lymphocytes by Fas/FasL-mediated apoptosis.

From the foregoing, it is clear to us that there are several points of view about the impact of sFasL on apoptosis and inflammatory activity.

The present study revealed that serum sFasL level was non-significantly higher in OLP patients group when compared with controls and in erosive OLP when compared with reticular form.

On the other hand, salivary sFasL cannot be detected in the study groups.

On the extent of our knowledge, there were no previous studies dealing with sFasL in both serum and saliva of OLP patients.

4.3.1.3. Bcl-2:

The present study revealed that there was no significant difference in serum Bcl-2 level between OLP patients group and healthy control one which was consistent with previous study by Sklavounou *et al*., 2004.

When compared between the two forms of OLP, there was non-significantly decreased in serum Bcl-2 level in erosive OLP compared with reticular form which was inconsistent with the results of Sklavounou *et al*., (2004) who mentioned that erosive OLP exhibited significantly decreased Bcl-2 serum levels, compared with reticular OLP.

Normal suppressor T lymphocyte function is an essential element in the maintenance of self-tolerance, and deficient cell-mediated suppressor activity is implicated in the pathogenesis of auto-immune diseases (Sugerman *et al*., 1992). Suppressor T cells control the course and size of

specific cell-mediated immune reactions by modulation of T cell responses (Dorf and Benacerraf, 1984). Since cell-mediated immune reactions are implicated almost exclusively in the immunopathogenesis of OLP (Walsh *et al*., 1990), defective cell-mediated suppression may play an important role in the pathogenesis of OLP, especially in lesion progression (Sugerman *et al*., 1992).

Sugerman *et al*., (1994) suggested that the balance between immunological help and suppression may determine the clinical behavior of the disease.

Sklavounou *et al*., (2004) suggested that a possible downregulation of the Bcl-2 protein in the atrophic-erosive form of OLP may be associated with T cells apoptosis susceptibility in the subepithelial infiltrate. Inadequate immunosuppression may promote hyperactive immune responses in OLP and a possible selective apoptosis of putative immunosuppressive T cells in OLP may be associated with promotion of the disease activity.

With respect to saliva, Bcl-2 cannot be detected in saliva samples of the study groups.

4.3.2. Oxidative stress markers and antioxidants:

Aerobic life is connected with continuous production of free radicals, particularly reactive oxygen species (ROS) (Van Wijk *et al*., 2008) which are ubiquitous, highly reactive, diffusible molecules, including superoxide anions, hydrogen peroxide, hydroxyl radical, and nitric oxide (Fridovich,

1997). Free radicals cause tissue damage by a variety of different mechanisms which include; DNA damage, lipid peroxidation, protein damage, oxidation of important enzymes and stimulation of pro inflammatory cytokines release (Pendyala *et al*., 2008). Cells possess an enzymatic and non-enzymatic antioxidant system to maintain redox homeostasis. Oxidant–antioxidant imbalance resulting in excessive accumulation of ROS is defined as oxidative stress (Scrobota *et al*., 2011).

It has been demonstrated that the imbalances in free radical levels and reactive oxygen species with antioxidants may play a key role in the onset and development of several inflammatory oral pathologies (Battino *et al*., 2002).

Oral lichen planus (OLP) is a chronic inflammatory oral mucosal disease of unknown etiology (Sugerman and Savage, 2002). Anshumalee and Shashikanth, (2007); Anshumalee *et al*., (2007) and Sezer *et al*., (2007) reported that ROS may be involved in the pathogenesis of LP.

Aly and Shahin, (2010) suggested that increased ROS and lipid peroxidation in LP may enhance the inflammatory response by immunological mechanisms.

According to the mentioned facts, oxidative stress may play a role in pathogenesis of OLP but it is unclear if oxidants trigger the disease, or if they are produced as a secondary consequence of the disease and tissue damage.

4.3.2.1. Oxidative stress markers:

4.3.2.1.1. MDA:

One of the major presentations of oxidative stress is lipid peroxidation (Haliwell and Gutteridge, 2002). Peroxidation of lipid rich membranes alters their fluidity and signaling efficiency, leading to inflammatory changes and to aberrant cell proliferation responses (Briganti and Picardo, 2003; Sander *et al*., 2004). Malondialdehyde (MDA), the end product of lipid peroxidation, is considered a good marker of free radical–mediated damage and oxidative stress (Kasperska-Zajac *et al*., 2008).

The present study showed that serum MDA, a lipid peroxidation product and a marker of oxidative stress, was significantly higher in OLP patients than in controls. These findings were in accordance with studies by Sezer *et al*. 2007; Rai *et al*. 2008; Aly and Shahin 2010; Upadhyay *et al*., 2010 and Scrobota *et al*., 2011.

Regarding saliva, salivary MDA levels may be directly affected by systemic oxidative stress, since MDA levels were significantly increased in saliva of OLP, which reflects a high oxidative stress status among OLP patients, which were consistent with previous studies by Agha-Hosseini *et al*., 2009 and Ergun *et al*., 2011, whilst Agha-Hosseini *et al*., (2012) showed that there was no significant difference in saliva MDA levels between OLP patients group and control subjects .

The present study showed that there was no relation between the clinical types of OLP and serum and saliva MDA levels. In other words, the

clinical types did not exert a significant influence on serum and saliva MDA levels.

The increase in MDA levels observed in this study supports the concept that free radical mediated lipid peroxidation may be involved in the patho-physiologic mechanisms of OLP.

4.3.2.1.2. Nitric oxide (NO):

Peroxynitrite is generated by the diffusion controlled reaction of superoxide and NO (Radi *et al*., 2001).

Superoxide is quickly removed by the scavenging enzyme superoxide dismutase, and NO is removed by rapid diffusion into the red blood cells where it is converted via a reaction with oxyhemoglobin to nitrite. Because of the high concentration of NO that is produced *in vivo*, along with the rapid reaction rate of NO with superoxide, NO is able to outcompete superoxide dismutase for reaction with superoxide whenever both species are present (Beckman, 1996).

Furthermore, ceruloplasmin, which is one of the most important antioxidant proteins in serum (Pacht and Davis, 1988), may play an important role as a multicopper oxidase to oxidize NO, thus generating RS-NO in the presence of thiol compounds (RSH) (Inoue *et al*., 1999).

The short half-life of NO in biological systems and its loss due to reaction with superoxide makes accurate quantitative measurements of NO difficult (Tschudi *et al*., 1996).

Up to our knowledge, no previous studies were reported on serum NO in OLP patients.

The present study may be a reflective of the above-mentioned facts which showed that there was no significant differences in serum NO level between OLP patients group and controls and between the two forms of OLP patients.

With respect to saliva, nerves endings, salivary gland endothelial cells or macrophages in response to oral bacterial products are the possible cellular source of salivary NO (Sunitha and Shanmugam, 2006).

Both T lymphocytes and macrophages, implicated in the etiology of LP, serve to point a possible role of NO in the mediation and cell injury in this disease. Nitric oxide is released after immunological stimulation by an enzyme, the inducible nitric oxide synthase, which originally described in macrophages. The expression of inducible nitric oxide synthase and hence NO could be increased in cellular infiltrate seen in erosive OLP when compared with normal mucosa and thus may contribute to elevated levels of NO in the saliva of these patients. It is proposed that excess of salivary NO may have pathophysiological implications for erosive and ulcerative lesions in OLP (Sunitha and Shanmugam, 2006).

Volk *et al*., (1995) noted the cytotoxic potential of NO against various cells. Investigations by Das *et al*., (1997) have indicated that free radicals including NO may play an important role in ulceration induced by several kinds of stress.

While salivary NO level showed no significant difference between OLP patients and control groups, its level was significantly higher in erosive OLP patients than in reticular form which may reflect a cytotoxic potential of salivary NO and its association with promotion of the disease activity.

4.3.2.2. Antioxidants:

4.3.2.2.1. Ceruloplasmin (CP):

The source of circulating CP has been almost exclusively assigned to CP secreted by hepatocytes (Banha *et al*., 2008). Human monocytic cells have also been shown to produce and secrete their own CP on activation (Mazumder *et al*., 1997).

Human peripheral blood lymphocytes express the transcripts for both CP molecular isoforms. During infection and inflammation characterized by active proliferation of circulating lymphocytes, CP concentration in serum increases, suggesting that the expression of the CP gene represents an essential part of host response to immunological stress (Hellman and Gitlin, 2002).

Metals such as iron, copper, chromium, vanadium and cobalt are capable of redox cycling in which a single electron may be accepted or donated by the metal. This action catalyzes reactions that produce reactive radicals and can produce reactive oxygen species (Genevieve, 2012).

Iron is essential for a variety of cellular functions, but its levels and bioavailability must be tightly regulated because of its toxic redox activity. A number of transporters, binding proteins, reductases, and ferroxidases help maintain iron homeostasis to prevent cell damage. The multi-copper ferroxidase CP converts toxic ferrous iron (Fe^{2+}) to its nontoxic ferric form (Fe^{3+}) and is required for iron efflux from cells (Jeong and David, 2006).

Multiple mechanisms have been proposed to explain CP antioxidant activity, including scavenging of superoxide and other reactive oxygen species (Goldstein *et al*., 1979), and inhibiting the Fenton reaction by conversion of Fe^{2+} to Fe^{3+} (ceruloplasmin is also called "ferroxidase") (Gutteridge, 1985; Samokyszyn *et al*., 1989). The ability of CP to block Cu^{2+}-mediated lipid oxidation suggests that alternate antioxidant mechanisms must also pertain (Gutteridge, 1983). There is evidence that CP as an antioxidant blocks protein (Krsek-Staples and Webster, 1993) and DNA damage (Gutteridge *et al*., 1980), and that it affords protection against free radical-initiated cell injury and lysis (Lovstad, 1982).

Extensive medline search failed to reveal any study concerning serum and saliva CP in OLP patients.

According to these facts, the significant increase in serum and saliva CP levels in this study may represent a compensatory antioxidant defense system to counteract oxidative stress.

By comparison between the two forms of OLP, The present study showed that there was no effect of clinical type of OLP on serum and saliva CP levels.

4.3.2.2.2. Uric acid:

The present study showed that there was non-significantly decreased in serum UA level in OLP patients than controls and in erosive form compared to reticular one.

Uric acid is produced from purines by the enzyme xanthine oxidase via the purine metabolism pathway (Waring *et al*., 2000). Uric acid is a weak acid distributed throughout the extracellular fluid as sodium urate. The amount of urate in the blood depends on the dietary intake of purines, urate biosynthesis, and the rate of urate excretion (Kutzing and Firestein, 2008).

Uric acid is considered a major antioxidant in human blood (Sautin *et al*., 2007) which contributes as much as 60% to free radical scavenging in human serum (Maxwell *et al*., 1997).

Uric acid can react with a variety of oxidants, including superoxide anion, hydrogen peroxide, hydroxyl radical, and peroxynitrite. From among these oxidants, uric acid is known to react preferentially with peroxynitrite

(Robinson *et al*., 2004; Kuzkaya *et al*., 2005). These reactions are thought to be initiated by the donation of an electron by uric acid to generate the urate radical, followed by its irreversible degradation to a variety of endproducts (Volk *et al*., 1990; Robinson *et al*., 2004). Paradoxically, uric acid can become a pro-oxidant by forming radicals in reactions with other oxidants, and these radicals seem to target predominantly lipids (LDL and membranes) rather than other cellular components (Sautin and Johnson, 2008) and may thus be a marker of oxidative stress (Becker, 1991; Strazzullo and Puig, 2007) but it may also have a therapeutic role as an antioxidant (Becker *et al*., 1991; Hayden and Tyagi, 2004). Thus, it is unclear whether the increased or decreased concentrations of UA in diseases associated with oxidative stress are a protective response or a primary cause (Al-Rawi, 2011).

This confusion reflects the complex internal environment of the human body, so it is difficult to predict or explain the changes in the level of UA.

In regard to saliva, human saliva has a total antioxidant capacity higher than blood plasma (Ziobro and Bartosz, 2003). Uric acid constitutes around 70% of total antioxidant capacity of saliva (Nagler *et al*., 2002; Karincaoglu *et al*., 2005).

While there was a meaningful drop in saliva levels of UA in the aforementioned studies by Battino *et al*., (2008) and Miricescu *et al*., (2011), the present study showed that there was a non-significantly decreased in saliva UA level in OLP patients than controls and in erosive form compared

to reticular, which may be due to the consumption effect of oxidative stress or as a result of a reflection of its serum level.

4.3.2.2.3. Oxidized glutathione:

Up to our knowledge, no previous studies were reported on serum and saliva GSSG in OLP patients.

The present study showed that there was no significant difference in serum and saliva GSSG level between OLP patient and control groups and between the two clinical forms of OLP.

Glutathione status is homeostatically controlled, being continually self-adjusting with respect to the balance between GSH synthesis (by GSH synthetase enzymes), its recycling from GSSG (by GSH reductase), and its utilization (by peroxidases, transferases, transhydrogenases, and transpeptidases) (Kidd, 1997).

Reduced glutathione (GSH), as a cosubstrate of glutathione peroxidase, plays an essential protective role against oxygen-reactive species that may be generated under various conditions. This protective mechanism results in increased formation of GSSG, which is actively transported across the cell membrane, so that its intracellular concentrations are kept low (Chance *et al*., 1979).

The liver is the main storage locale for GSH (actually exporting GSH to the other organs). The hepatocytes are highly specialized to synthesize GSH from its precursors or to recycle it from GSSG, as well as to utilize GSH against potential toxicants (Deleve and Kaplowitz, 1990). Glutathione is homeostatically controlled, both inside the cell and outside. While providing GSH for their specific needs, the liver parenchymal cells export GSH to the outside, where it serves as systemic source of —SH/reducing power (Kidd, 1997).

Repletion of glutathione appears to be central to intrinsic adaptive strategies for meeting the challenge of sustained (or acute) oxidative stress (Kidd, 1997). The GSSG/GSH ratio is homeostatically controlled and the liver works as a safety valve to maintain the level of GSSG within the narrow limits.

4.4. Saliva as a diagnostic specimen:

The use of saliva as a diagnostic fluid is a relatively recent trend. This is not surprising when one considers its many advantages and the fact that saliva contains a wide array of constituents (Soo-Quee Koh and Choon-Huat Koh, 2007)

Whole saliva contains locally produced as well as serum-derived markers that have been found to be useful in the diagnosis of a variety of systemic disorders. However, levels of certain markers in saliva are not always a true reflection of the levels of these markers in serum. The transfer

of serum constituents which are not part of the normal salivary constituents into saliva is related to the physicochemical characteristics of these molecules. Salivary composition can be determined by the method of collection and the degree of stimulation of salivary flow. Furthermore, salivary proteolytic enzymes can affect the stability of certain diagnostic markers. Some particles are also degraded during intracellular diffusion into saliva (Kaufman and Lamster, 2002).

When the concentration of specific component in saliva strongly correlates the serum one, a serum source is accepted. However, the lack of a high correlation between concentrations of a component in saliva and in serum does not necessarily reject the serum origin. It may simply reflect variability in the diffusion process or local synthesis for the component (Nagler, 2008).

These limitations opposed the diagnostic potential of saliva. Blood still remains the best body fluid for evaluation of many biomarkers reflecting systemic processes and substitution should be used with caution (Williamson *et al*., 2012).

The facts mentioned may reflect what the present study revealed that there was no significant correlation between the most biomarkers in serum and saliva of OLP patients.

4.5. Laboratory findings and severity of OLP:

This study is the first of its kind dealing with the number of affected site, the extension of the lesion and the intensity of pain which translated, depending on the scoring system; to the coded number refer to the severity of erosive and reticular forms of OLP to compare between serum and saliva levels of parameters.

The present study found that there was no significant difference in serum and saliva parameters in relation to severity scores of erosive form of OLP patient group.

With respect to reticular form, there was no significant difference in serum and saliva parameters in relation to severity scores, with the exception of serum CP levels which was significantly increased in relation to severity scores.

In other words, the number of affected sites, the extension of the lesion and the intensity of pain did not exert a meaningful influence on the serum and saliva levels of apoptotic and most of the oxidative status parameters.

Chapter Five
Conclusions and Suggestions

Conclusions and Suggestions:

5.1. Conclusions

1- Oral lichen planus mostly reported in middle-aged adults, most patients had single-site involvement and the buccal mucosa was the most affected site. Symptomatic OLP patients were associated with erosive form which showed extensive lesions.

2- Oxidative status play a role in the pathogenesis of oral lichen planus represented by increased serum and saliva MDA and CP levels.

3- Serum and saliva levels of NO, UA and GSSG in OLP patients group showed no statistically significant differences compared with controls. Upon the comparison between the two forms of OLP (reticular and erosive), no statistically significant differences were found in all oxidative status biomarkers (MDA, NO, CP, UA and GSSG) in serum and saliva, with the exception of NO in saliva which was significantly higher in erosive form.

4- No statistically significant differences in serum levels of apoptotic biomarkers (sFas, sFasL and Bcl-2) between OLP patients group and controls and between the two forms of OLP patients.

5- Soluble FasL and Bcl-2 cannot be detected in saliva of OLP patients. On the other hand, Saliva sFas showed no statistically significant difference compared with control subjects and saliva sFas level was significantly higher in erosive OLP than in reticular form

6- No statistically significant correlation was found between serum and saliva measurements of all parameters in OLP patients group, with the exception of NO. So Saliva is not always a reliable indicator of the internal environment of the body.

7- Saliva can be considered as a diagnostic body fluid for oxidative status assay for OLP. Salivary NO and sFas can be used as markers of disease activity.

8- Number of affected site, extension of the lesion and intensity of pain did not exert a meaningful influence on the serum and saliva levels of apoptotic and most of the oxidative status parameters.

5.2. Suggestions:

1- Studying the expression of apoptotic biomarkers in tissues of OLP patients and compare the results with that of serum and saliva.

2- Studying other oxidative stress markers like 8-hydroxy-deoxyguanosine and antioxidants like superoxide dismutase, catalase and vitamins A, C, and E in saliva of OLP patients and compare the results with that of serum.

3- Studying the effects of treatment of OLP patients on oral findings and on serum and saliva oxidative stress biomarkers and antioxidants.

4- Studying the effects of treatment of OLP patients by activation of anti-inflammatory genes and suppression of inflammatory genes in immune cells.

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Appendices

Appendix I:

Set of modified WHO diagnostic criteria of oral lichen planus (OLP) and oral lichenoid lesions (OLL) (Van der Meij, Van der Waal, 2003)

Clinical criteria:

– Presence of bilateral, more or less symmetrical lesions

– Presence of a lacelike network of slightly raised gray-white lines (reticular pattern)

– Erosive, atrophic, bullous and plaque-type lesions are only accepted as a subtype in the presence of reticular lesions elsewhere in the oral mucosa

In all other lesions that resemble OLP but not complete the aforementioned criteria the term (clinically compatible with) should be used.

Histopathological criteria:

– Presence of a well-defined band-like zone of cellular infiltration that is confined to the superficial part of the connective tissue, consisting mainly of lymphocytes

– Signs of 'liquefaction degeneration' in the basal cell layer

– Absence of epithelial dysplasia

When the histopathological features are less obvious, the term (histopathologically compatible with) should be used.

Final diagnosis OLP or OLL:

To achieve a final diagnosis clinical as well as histopathological criteria should be included. A diagnosis of OLP requires fulfillment of both clinical and histopathological criteria.

The term OLL will be used under the following conditions:

(1) Clinically typical of OLP but histopathologically only ‘compatible with’ OLP,

(2) Histopathologically typical of OLP but clinically only ‘compatible with’ OLP,

(3) Clinically ‘compatible with’ OLP and histopathologically ‘compatible with’ OLP

Appendix II ***Case sheet***

No: *Date:* *Gender:* *Age:*

Patient's Name: Address:

Disease Severity Score:

Site Score:

Site (score values)	**Site score**
Outer lips (0-1)	
Inner lips (0-1)	
R Buccal Mucosa (0 or 1 [<50%] or 2 [>50%]) L Buccal Mucosa (0 or 1 [<50%] or 2 [>50%])	
Gingivae (0-1 each segment) Lower R (distal) Lower central Lower L (distal) Upper R (distal) Upper central Upper L (distal)	
Dorsum tongue (0 or 1 or 2)	
R ventral tongue (0-1) L ventral tongue (0-1)	
Floor of mouth (0 or 1 or 2)	
Hard palate (0 or 1 or 2)	
Soft palate (0 or 1 or 2)	

Surface Area Score (extension of the lesion):

0 = no lesion at site
1 = less than 50% of area affected (light extension)
2 = greater than 50%. Not defined anatomically (severe extension)

Pain Score (intensity of pain):

0 = No pain
1 = Patients with mucosal sensitivity
2 = Continuous debilitating pain

TOTAL DISEASE SEVERITY SCORE =

Guy's and St Thomas' NHS
NHS Foundation Trust

KCL Dental Institute
Department of Oral Medicine
DISEASE SEVERITY SCORE

King's College London

LICHEN PLANUS

Name: …………………………………… **Hospital Number** …………………. **Date** ………………

Predominant type (ring type): Reticular / Atrophic / Plaque / Desquamative gingivitis / Ulcerative

Site (possible score values)		Site score	Activity score (0 - 3), Double if site score = 2
Outer lips	(0-1)		
Inner lips	(0-1)		
R Buccal Mucosa	(0 or 1 [<50%] or 2 [>50%])		
L Buccal Mucosa	(0 or 1 [<50%] or 2 [>50%])		
Gingivae	(0-1 each segment)		
Lower R (distal)			
Lower central			
Lower L (distal)			
Upper R (distal)			
Upper central			
Upper L (distal)			
Dorsum tongue	(0 or 1 or 2)		
R ventral tongue	(0-1)		
L ventral tongue	(0-1)		
Floor of mouth	(0 or 1 or 2)		
Hard palate	(0 or 1 or 2)		
Soft palate	(0 or 1 or 2)		
Oropharynx	(0 or 1 or 2)		

Totals: SITE SCORE: ACTIVITY SCORE: PAIN SCORE (0-10):

VAS ____

TOTAL DISEASE SEVERITY SCORE:

Key

Activity Score

0 = no lesion at site

1 = mild erythema (e.g. on gingivae, papillae only or less than 3mm along margins)

2 = marked erythema (e.g. full thickness on gingivae, extensive with atropy or oedema on non-keratinised mucosa)

3 = Ulceration at this site

Site Score

0 if no lesion at site

1 if less than 50% of area affected

2 if greater than 50%. Not defined anatomically

Pain Score

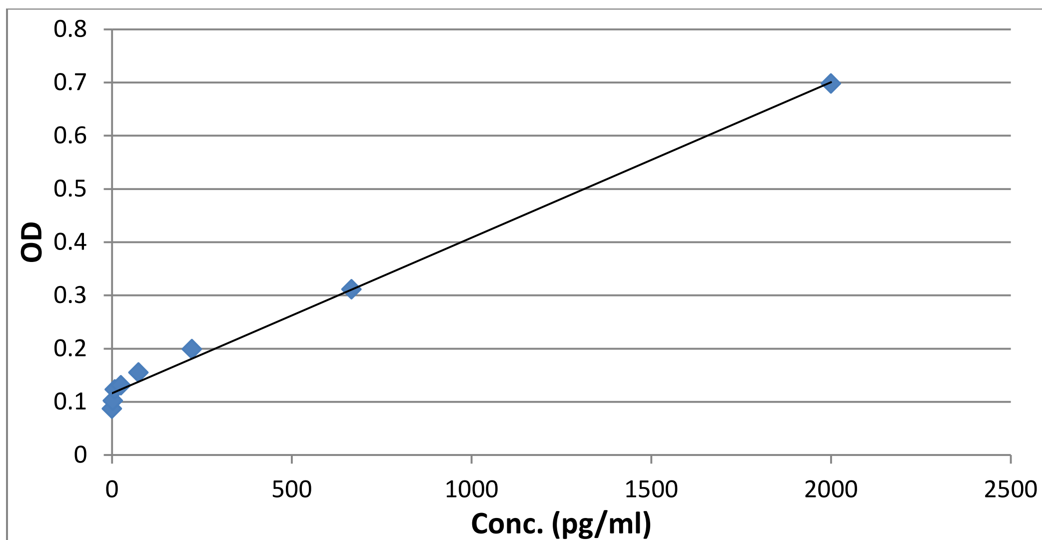
Analogue scale from 0 (no discomfort) to 10 (unbearable pain)

Physician signature……………………………………………………………………………………

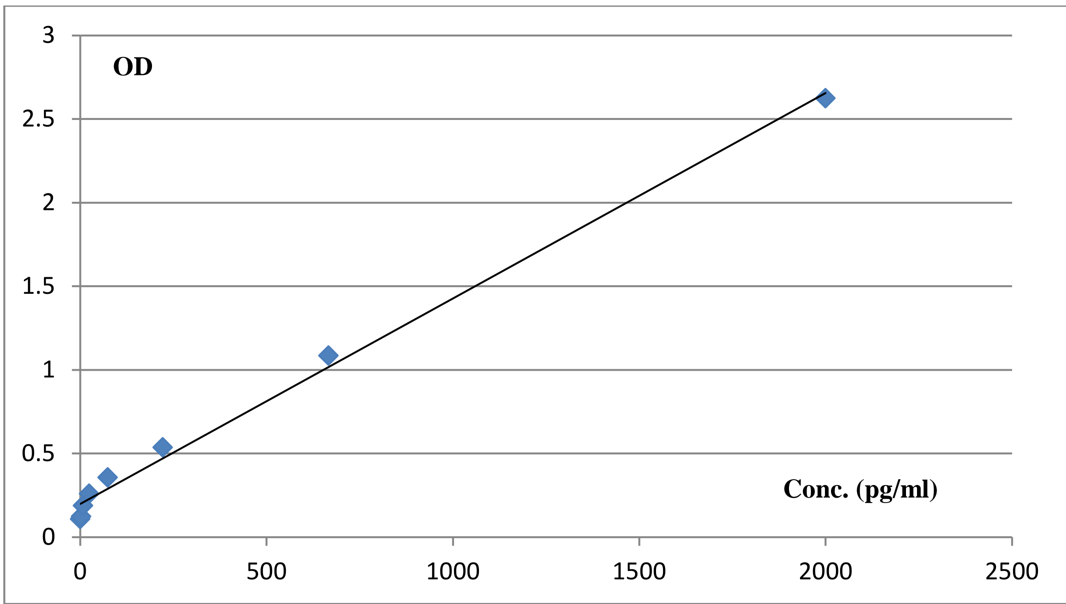
Appendix III

Fas Map and Standard Curve:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0 pg/ml	74.07 pg/ml	sample	sample	control	control	sample	sample	control	control	sample	sample
B	0.0 pg/ml	74.07 pg/ml										
C	2.74 pg/ml	222.2 pg/ml										
D	2.74 pg/ml	222.2 pg/ml										
E	8.23 pg/ml	666.7 pg/ml										
F	8.23 pg/ml	666.7 pg/ml										
G	24.69 pg/ml	2000 pg/ml										
H	24.69 pg/ml	2000 pg/ml										



Standard curve for Serum Fas

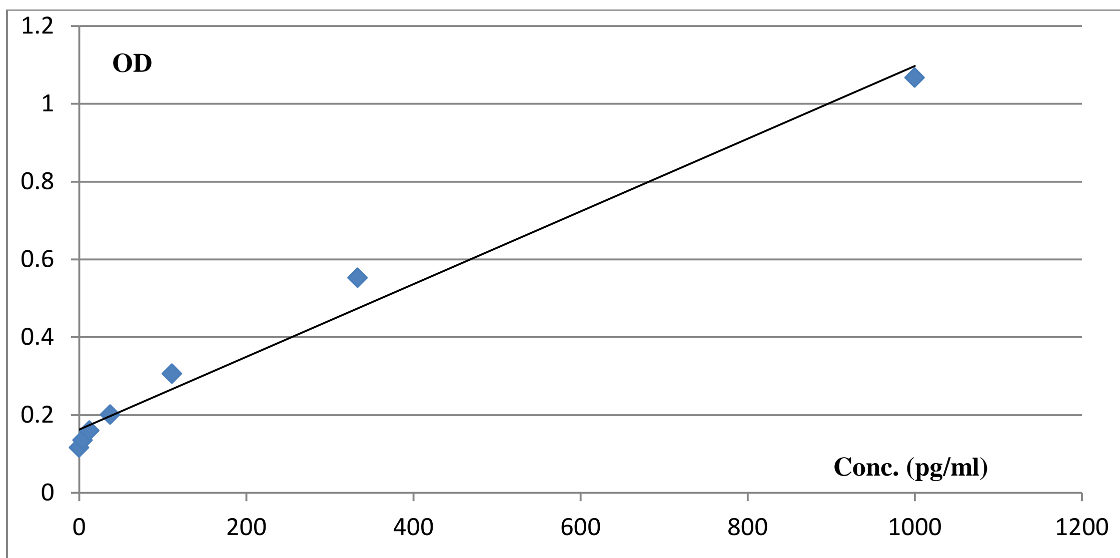


Standard curve for Saliva Fas

Appendix IV

Fas Ligand Map and Standard Curve:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0 pg/ml	37.04 pg/ml	sample	sample	control	control	sample	sample	control	control	sample	sample
B	0.0 pg/ml	37.04 pg/ml										
C	1.37 pg/ml	111.1 pg/ml										
D	1.37 pg/ml	111.1 pg/ml										
E	4.12 pg/ml	333.3 pg/ml										
F	4.12 pg/ml	333.3 pg/ml										
G	12.35 pg/ml	1000 pg/ml										
H	12.35 pg/ml	1000 pg/ml										

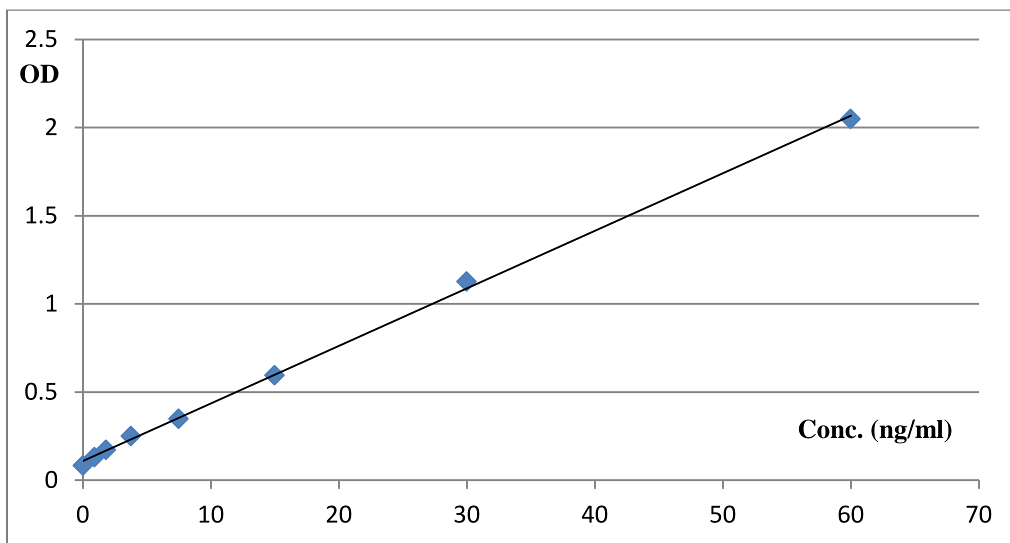


Standard curve for Serum Fas L. Conc. (pg/ml)

Appendix V

Bcl-2 Map and Standard Curve:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0	7.5 ng/ml	sample	sample	control	control	sample	sample	control	control	sample	sample
B	0.0 ng/ml	7.5 ng/ml										
C	0.9 ng/ml	15 ng/ml										
D	0.9 ng/ml	15 ng/ml										
E	1.8 ng/ml	30 ng/ml										
F	1.8 ng/ml	30 ng/ml										
G	3.75 ng/ml	60 ng/ml										
H	3.75 ng/ml	60 ng/ml										

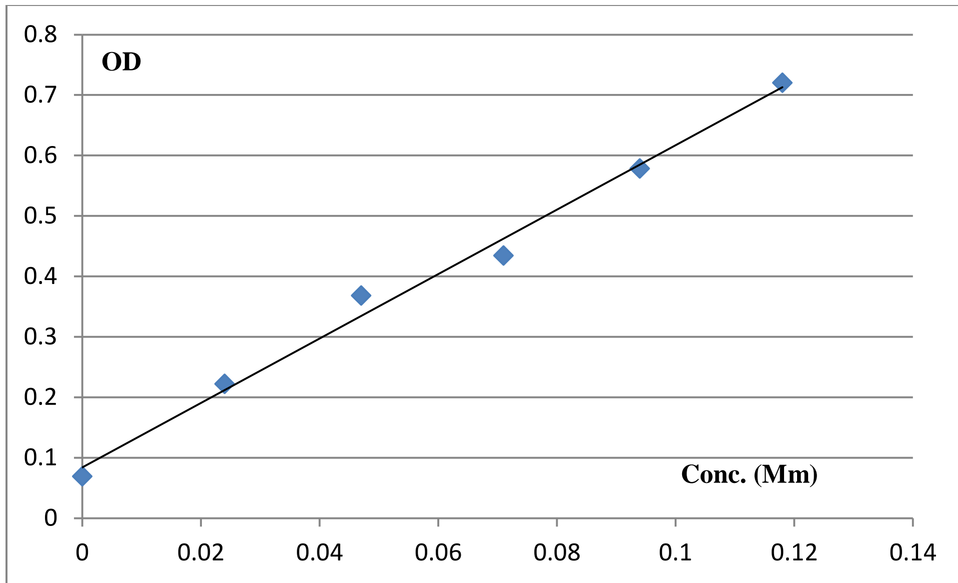


Standard Curve of Serum Bcl-2

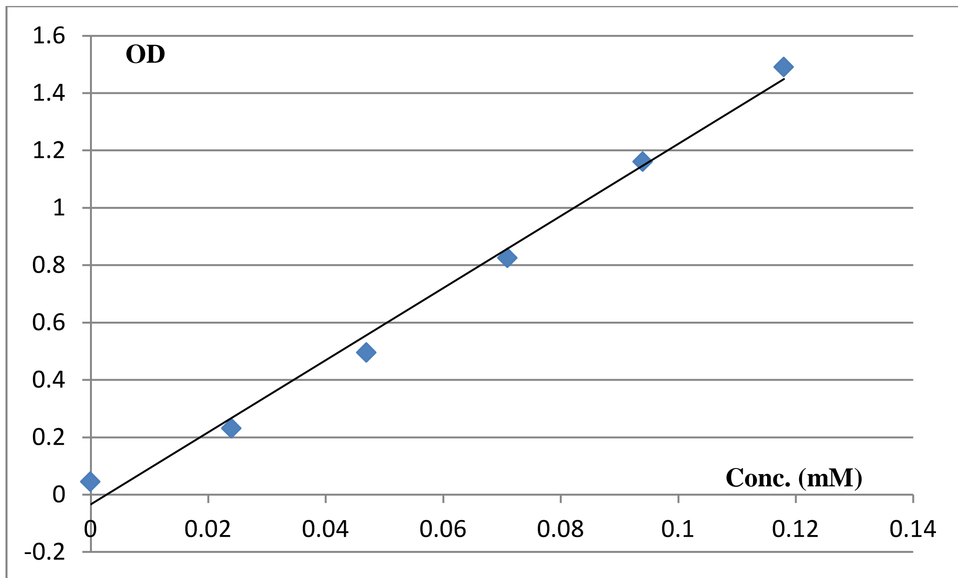
Appendix VI

Nitric Oxide Map and Standard Curves:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0mM S1	sample	sample	control	control	sample	sample	control	control	sample	sample	
B	0.024mM S2											
C	0.047mM S3											
D	0.071mM S4											
E	0.094mM S5											
F	0.118mM S6											
G												
H												



Standard curve of serum Nitric Oxide

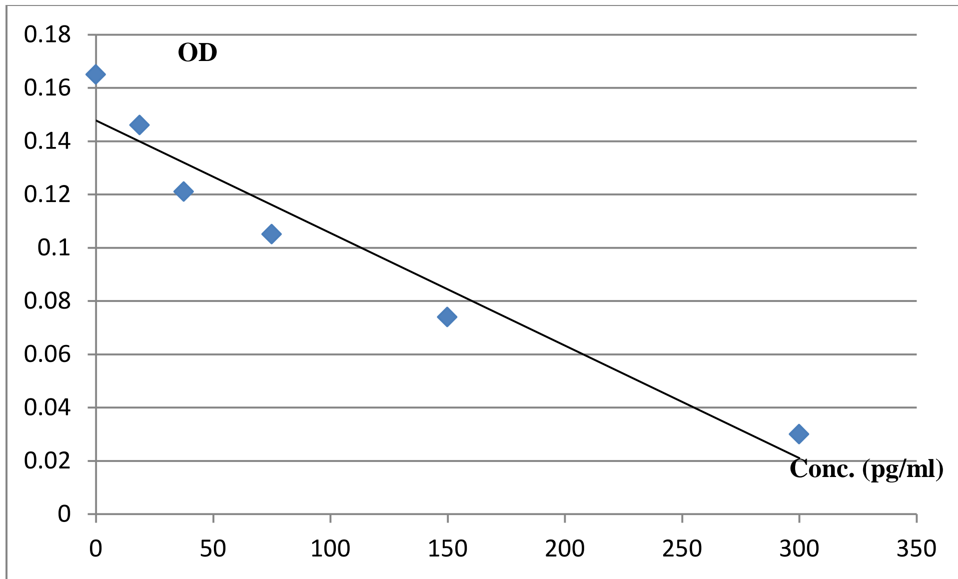


Standard curve of saliva Nitric Oxide

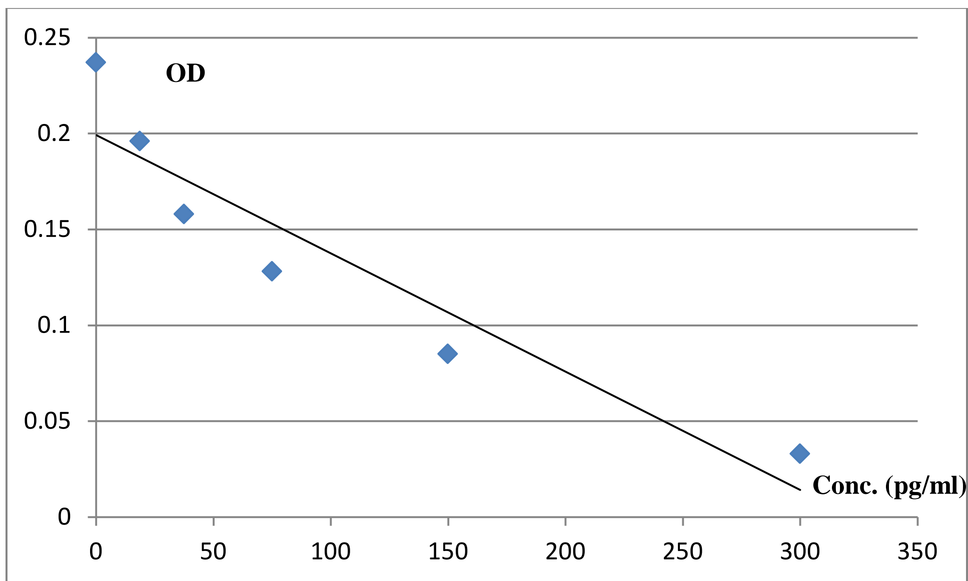
Appendix VII

GSSG Map and Standard Curves:

	1	2	3	4	5	6	7	8	9	10	11	12
A	300 pg/ml	18.75 pg/ml	sample	sample	control	control	sample	sample	control	control	sample	sample
B	300 pg/ml	18.75 pg/ml										
C	150 pg/ml	0.0 pg/ml										
D	150 pg/ml	0.0 pg/ml										
E	75 pg/ml											
F	75 pg/m											
G	37.5 pg/ml											
H	37.5 pg/ml											



Standard Curve of Serum GSSG



Standard Curve of Saliva GSSG

الخلاصة

الخلفية:

الحزاز المسطح الفموي Oral Lichen Planus هو مرض التهابي مزمن يتميز بالموت المبرمج للخلايا القاعدية لظهارة الفم عن طريق الخلايا التائية السامة $CD8^{+}$ حيث تم الافتراض ان هناك الية تساعد هذه الخلايا على الهروب من الموت المبرمج و استمرار التسلل. من ناحية أخرى, فأن الاجهاد التأكسدي الناتج عن عدم التوازن بين المؤكسدات و مضادات الأكسدة يؤدي إلى تلف الجزيئات البيولوجية وتقلبات في عملية التمثيل الغذائي والتي قد تلعب دورا رئيسيا في ظهور وتطور العديد من التهابات الفم.

أهداف الدراسة:

الهدف من هذه الدراسة هو معرفة القدرة التشخيصية للعاب بالمقارنة مع مصل الدم فيما يتعلق بمؤشرات فعاليات الأستماتة من خلال دراسة soluble Fas و soluble Fas ligand و Bcl-2 ومؤشرات الاجهاد التأكسدي Malondialdehyde و Nitric Oxide و مضادات الاكسدة Ceruloplasmin و Uric acid و Oxidized Glutathione في مرضى الحزاز المسطح الفموي مقارنة بالمجموعة الضابطة والتحقيق في ما إذا كان هناك علاقة بين النتائج السريرية و النتائج المخبرية.

طريقة العمل:

شارك في هذه الدراسة ثمانية و أربعون مريضا خضعوا للفحص النسيجي لتأكيد التشخيص و تم تقسيمهم الى مجموعتين اعتمادا على الوصف السريري للحالة , واحد و عشرون مريض يمثلون الحزاز المسطح الفموي الشبكي Reticular Oral Lichen Planus و سبعة و عشرون يمثلون الحزاز المسطح الفموي التآكلي Erosive Oral Lichen Planus بالاضافة الى المجموعة الضابطة و التي تتكون من اثنين و ثلاثين شخصا من الافراد الاصحاء المطابقين من حيث العمر. تم جمع عينات مصل الدم و اللعاب لغرض اجراء التحاليل. وقد تم حساب شدة المرض بالاعتماد على ورقة حساب الشدة.

النتائج:

اظهرت الدراسة أن الافات لمعظم المرضى كانت تصيب منطقة تشريحية واحدة و أن الغشاء المخاطي الشدقي هو الأكثر تضررا بنسبة (91.7%), وأظهرت آفات التآكل أعراض الألم و التي تتراوح في شدتها من الحساسية العالية للألم الى الالم المنهك المستمر مع آفات واسعة بشكل كبير.

أشارت هذه الدراسة عدم وجود اختلاف معنوي ($p>0.05$) فيما يتعلق بمؤشرات فعاليات الأستماتة soluble Fas و soluble Fas ligand و Bcl-2 في مصل الدم لمرضى الحزاز المسطح الفموي مقارنة بالمجموعة الضابطة. كذلك اظهرت الدراسة عدم وجود اختلاف معنوي ($p>0.05$) في متوسط soluble Fas في اللعاب لمرضى الحزاز المسطح الفموي مقارنة بالمجموعة الضابطة, بينما لم يتم الكشف عن وجود soluble Fas ligand و Bcl-2 في اللعاب للمرضى و المجموعة الضابطة.

فيما يتعلق بمؤشرات الاجهاد التأكسدي, كان متوسط Malondialdehyde في مصل (4.725 µmol/l) و لعاب (0.972 µmol/l) المرضى الذين يعانون من الحزاز المسطح الفموي أعلى و بشكل ملحوظ ($p<0.001$) و ($p<0.05$) مقارنة بالمجموعة الضابطة (1.626µmol/l) و(0.732 µmol/l) على التوالي ، في حين لايوجد فرق معنوي ($p>0.05$) فيما يتعلق بمستوى Nitric Oxide , اما بالنسبة لمضادات الأكسدة، فقد اشارت الدراسة ان متوسط Ceruloplasmin في مصل (0.408 g/l) و لعاب (0.014 g/l) مرضى الحزاز المسطح الفموي كانت مرتفعة وبشكل ملحوظ ($p<0.001$) و($p<0.01$) مقارنة بالمجموعة الضابطة (0.311 g/l) و (0.009 g/l) على التوالي, بينما لايوجد فرق معنوي($p>0.05$) فيما يتعلق بمستوى Uric Acid و Oxidized Glutathione.

في مرضى الحزاز المسطح الفموي, وجد ان جميع المتغيرات في مصل الدم و اللعاب لم تظهر ارتباطا وثيقا ما عدا فيما يتعلق ب Nitric Oxide ($r=0.449, p<0.001$).

وجدت هذه الدراسة أنه لا يوجد اختلاف معنوي في مستويات المصل و اللعاب لجميع المتغيرات بين مجموعتي المرضى الشبكي و التآكلي ، باستثناء soluble Fas و Nitric Oxide والتي أظهرت أن متوسط soluble Fas و Nitric Oxide في لعاب مرضى الشكل التآكلي كان أعلى وبشكل ملحوظ ($p<0.01$) و ($p<0.05$) من مرضى الشكل شبكي.

اظهرت الدراسة عدم وجود اختلاف معنوي للمتغيرات في مصل الدم و اللعاب فيما يتعلق بشدة المرض لمجموعتي المرضى الشبكي و التآكلي ما عدا فيما يتعلق ب Ceruloplasmin في

مصل مرضى الحزاز المسطح الفموي الشبكي حيث ازداد بشكل ملحوظ ($p<0.05$) تبعا لشدة الاعراض السريرية.

الاستنتاجات:

ان حالات مرضى الحزاز المسطح الفموي ترافق بزيادة الاجهاد التأكسدي و مضادات الاكسدة و المتمثلة بزيادة Malondialdehyde و Ceruloplasmin . المؤشرات لفعاليات الأستماتة soluble Fas و soluble Fas ligand و Bcl-2 في مصل الدم و اللعاب لا يمكن اعتبارها كمرشدات للموت المبرمج للخلايا المتعلق بالمرض. أظهرت الدراسة أن اللعاب لا يعبر دائما عن البيئة الداخلية للجسم, كما اوضحت أن مستوى soluble Fas و Nitric Oxide في لعاب يمكن ان يستخدم كمؤشر لفعالية المرض. اظهرت الدراسة ان شدة الاعراض السريرية والتي تضم عدد المناطق التشريحية المصابة و سعة الانتشار للافة و شدة الالم ليس لها تأثير معنوي على مؤشرات الأستماتة و الاجهاد التأكسدي و معظم مضادات الاكسدة في مصل الدم و اللعاب.

التقييم السريري و المؤشرات الحيوية للحالة التأكسدية و نشاط الاستماتة في مصل الدم و لعاب مرضى الحزاز المسطح الفموي

أطروحة مقدمة
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كجزء من متطلبات نيل درجة دكتوراه فلسفة
في طب الفم

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