

DETECTION OF FAS LIGAND AS A MARKER OF
APOPTOTIC CELLS IN SALIVA AND SERUM
OF PATIENTS WITH ORAL LICHEN PLANUS

THESIS

Submitted in Partial Fulfillment for the Requirement
of Master Degree in
Oral Medicine and Periodontology

Presented By:

Soher Nagi Mohammed Nagi Jayash

B.D.S (Ibb University)

Oral Medicine and Periodontology Department
Faculty of Oral and Dental Medicine
Cairo University

2012

Supervisors

Prof. Dr. Soheir Mohamed Gaafar

Professor of Oral Medicine, Oral Diagnosis and
Periodontology
Faculty of Oral and Dental Medicine
Cairo University

Prof. Dr. Olfat Gamil Shaker

Professor of Medical Biochemistry
and Molecular Biology
Faculty of Medicine
Cairo University

Dr. Shahira Gamal El Ashiry

Assistant professor of Oral Medicine, Oral
Diagnosis and Periodontology
Faculty of Oral and Dental Medicine
Cairo University

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَىٰ

صدق الله العظيم

Dedication



To my light when all was dark,

My Mother & Father...

To my hope when there was no hope,

My sisters & brothers....



ACKNOWLEDGMENT

Before everything and after all things I would like to express prayerful thanks to Allah the Almighty for everything.


I would like to express my deepest appreciation to my supervisors ***Prof. Dr. Soheir Gaafar***, Professor of Oral Medicine, Oral Diagnosis and Periodontology, Faculty of Oral and Dental Medicine, Cairo University for her outstanding encouragement, continuous advice and powerful support throughout this work. I am very fortunate to be one of her students.

My great thanks and appreciation go to the ***Prof. Dr. Olfat Shaker***, Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University for her continuous advice, help encouragement and supervision during this study.

Words are not enough to thank ***Dr. Shahira El Ashiry***, Assistant professor of Oral Medicine, Oral Diagnosis and Periodontology, Faculty of Oral and Dental Medicine, Cairo University for her enthusiastic encouragement, guidance, advice and support –without delay- in each stage of the work.

I am always thankful to my ***colleagues*** and ***friends*** for their continuous unlimited support.

Last but not least, my true love goes to all my ***family***, without whom I would never been able to accomplish this work.



List of figures

Fig no	Title	Page
1	Figure showing two principal pathways of apoptosis.	20
2	A clinical photograph of a male patient (43 years) showing atrophic OLP on the left buccal mucosa.	44
3	A clinical photograph of a female patient (37 years) showing erosive/ulcerative type of OLP on the right buccal mucosa (black arrow).	44
4	A clinical photograph of female patient (43 years) showing reticular OLP on the right buccal mucosa. The atrophic ulcerative pattern is dominant posteriorly (black arrow).	45
5	A clinical photograph showing measurement of OLP ulcer on the left buccal mucosa utilizing periodontal probe.	46
6	A clinical photograph showing ELISA.	47
7	A clinical photograph showing the contents of ELISA kit.	48
8	Diagram showing the principle of the test.	49
9	Diagram showing reagents.	50
10	Preparation of sFas Ligand calibrator dilutions.	54
11	Representative calibration curve for sFasL ELISA.	55
12	Histogram showing mean age values in the two groups.	59
13	Histogram showing sex distributions in the two groups.	60

Fig no	Title	Page
14	Histogram showing distribution of oral lichen planus.	61
15	Histogram showing distribution of intra-oral and extra-oral lesions.	62
16	Histogram showing the mean level of salivary sFasL (pg/ml) in control and patient's groups.	64
17	Histogram showing the mean level of serum sFasL (pg/ml) in control and patient's groups.	65
18	Histogram showing the mean level sFasL (pg/ml) in serum and saliva of subgroup A.	67
19	Histogram showing the mean level sFasL (pg/ml) in serum and saliva of subgroup B.	68
20	Histogram showing the mean level of serum sFasL(pg/ml) in the three studied groups.	69
21	Histogram showing the mean level of salivary sFasL (pg/ml) in the three groups.	70
22	Histogram showing the mean level of serum sFasL (pg/ml) in cases with intra- and intra & extra-oral lichen planus.	71
23	Histogram showing the mean level of salivary sFasL (pg/ml) in cases with intra- and intra & extra-oral lichen planus.	72
24	Histogram showing the mean level of sFasL (pg/ml) in serum and saliva of patients.	73

Fig no	Title	Page
25	Histogram showing the mean level of sFasL (pg/ml) in serum and saliva of control group.	74
26	Histogram showing the correlation between clinical and pain scores in patient's group.	76

List of tables

Table no	Title	Page
1	Clinical score.	45
2A	The descriptive data of the two studied groups.	57
2B	The descriptive data of control group.	58
3	Age distribution of the two studied groups.	58
4	Sex distribution of the two studied groups.	59
5	Distribution of OLP intraorally.	61
6	Prevalence of extra- and intra-oral involvements of OLP patients in males and females.	62
7	Comparison of the mean level of sFasL (pg/ml) in saliva between control and patient's groups.	63
8	Comparison of the mean level of sFasL (pg/ml) in serum between control and patient's groups.	65
9	Comparison between sFasL levels (pg/ml) in serum and saliva of subgroup A.	66
10	Comparison between sFasL levels (pg/ml) in serum and saliva of subgroup B.	67
11	Comparison of the mean level of serum sFasL (pg/ml) in three groups.	68
12	Comparison of the mean level of salivary FasL (pg/ml) in the three studied groups.	69

Table no	Title	Page
13	Comparison between serum sFasL (pg/ml) in cases with intra- and intra & extra-oral lichen planus.	70
14	Comparison between salivary sFasL (pg/ml) in cases with intra- and intra & extra-oral lichen planus.	71
15	Correlation between sFasL in serum and saliva (pg/ml) of patients.	72
16	Correlation between sFasL in serum and saliva of patients.	73
17	The correlation between serum and salivary sFasL in subgroups A and B.	74
18	Correlation between clinical and pain scores in patient's group.	75
19	The correlation between different variables in lichen planus group.	76
20	The correlation between different variables in control group.	77

Abbreviations

- AIDS: acquired immune deficiency syndrome.
- AIF: apoptosis-inducing factor.
- Apaf-1: apoptotic protease activating factor.
- APCs: antigen presenting cells.
- ATP: Adenosine Tri-Phosphate.
- BAD: Bcl-2-associated death promoter.
- BAK: Bcl-2 homologous antagonist killer.
- BAX: Bcl-2-associated X protein.
- Bcl: B-cell lymphoma.
- BclXL :B-cell lymphoma-extra large.
- BID: BH3 interacting-domain death agonist.
- CCR: chemokine receptor.
- CD: cluster of differentiation.
- cGVHD: chronic graft versus host disease.
- CTL: cytotoxic T lymphocyte.
- DcR: decoy receptor.
- DCs: dendritic cells.
- DD: death domain.
- DIF: direct immunofluorescence.
- DISC: death inducing signaling complex.
- DFF: DNA fragmentation factor.
- DM: diabetes mellitus.
- DNA: deoxyribonucleic acid.
- ELISA: enzyme-linked immunosorbent assay.

- FADD: Fas-associated death domain.
- FasL: Fas ligand.
- GVHD: graft-versus-host disease.
- HCV: hepatitis C virus.
- HIV: human immunodeficiency virus.
- HLA: human leukocyte antigen.
- HRP: horseradish peroxidase.
- HSP: heat shock protein.
- HSV: Herpes simplex virus.
- IAP: inhibitor of apoptosis protein.
- ICAM-1: intercellular adhesion molecule.
- IFN γ : interferon – gamma.
- Ig: immunoglobulin.
- IIF: indirect immunofluorescence.
- IL-12: interleukin -12.
- LCs: Langerhans cells.
- LP: lichen planus.
- LPS: lipopolysaccharides.
- MAC: mitochondrial apoptosis-induced channel.
- MHC: major histocompatibility complex.
- MIF: macrophage migration inhibitory factor.
- MMPs: matrix metalloproteinases.
- NF- κ B: nuclear factor–kappa B
- OLDR: oral lichenoid drug reaction.
- OLL: oral lichenoid lesion.
- OLP: oral lichen planus.
- RANTES: regulated upon activation, normal T-cell expressed, and secreted).

- ROS: reactive oxygen species.
- SCC: squamous cell carcinoma.
- sFas: soluble Fas.
- sFasL: soluble Fas ligand.
- SMAC: second mitochondria-derived activator of caspases.
- TGF- β 1: transforming growth factor beta 1.
- TNF- α : tumor necrosis factor-alpha.
- TNFR: tumour necrosis factor-receptor.
- TRADD: TNFR associated death domain.
- TRAIL: TNF-related apoptosis-inducing ligand.
- TRAILR: TNF-related apoptosis-inducing ligand receptor.
- VAS: visual analog scale.
- VZV: varicella-zoster virus: (human herpesvirus 3)
- WHO: world health organization.
- WUS: whole unstimulated saliva.

Contents

<i>Review of Literature</i>	1
<i>Aim of the Study</i>	41
<i>Subjects and Methods</i>	42
<i>Results</i>	57
<i>Discussion</i>	78
<i>Conclusions</i>	88
<i>Summary</i>	89
<i>References</i>	91
<i>Arabic Summary</i>	

Introduction & Review of Literature

Lichen planus (LP) is a common mucocutaneous inflammatory disorder, occurs at sites of stratified squamous epithelia. LP affects 0.5–2% of the population, with notable variation by geography and diagnostic criteria (**Bethanee and Schlosser, 2010**).

It was first described clinically by the British physician, **Erasmus Wilson**, in (1896) and histologically by **Dubreuilh** in (1906). The frequency varies on the basis of the population studied, with a particularly high rate of disease noted on the Indian subcontinent.

LP is a disease of middle-aged people, although childhood-onset has also been well described. It is more frequently seen in females more than males in a ratio of 3:2 (**Bermejo et al., 2006**). LP is a self-limited condition that, according to one epidemiologic study, may resolve after 1 month to 7 years. A range of topical and systemic medications have been shown to improve the symptoms associated with LP and to hasten its resolution (**Lehman et al., 2009**).

Patients with oral lichen planus (OLP) may develop lesions that affect the skin, skin appendages, or other mucosa (**Scully and Carrozzo, 2008**). Typically, the lesions that affect the skin are seen on the flexor surfaces of the forearms and are erythematous to violaceous, flat-topped, pruritic, polygonal papules that have a network of fine lines (Wickham's striae) on the surface, and develop within several months of the appearance of oral lichen planus (OLP) (**Scully and Carrozzo, 2008**).

Hypertrophic LP is marked by the development of hyperkeratotic, flat-topped plaques, typically affecting the anterior lower legs. Findings of bullous LP include vesicles and bullae, thus necessitating that other