

**Simultaneous detection of IFN-gamma in whole  
unstimulated saliva and lesional tissues from oral lichen  
planus patients**

**Thesis**

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

*Presented By*

**Nayroz Abdel Fattah Mohamed Tarrad**

**B.D.S  
Cairo University**

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

**2010**

## **Supervisors**

### **Prof. Dr. Mahmoud El-Refai**

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

### **Prof. Dr. Fatheya Zahran**

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

### **Prof. Dr. Olfat Shaker**

Professor of Biochemistry  
Faculty of Medicine  
Cairo University

## ACKNOWLEDGEMENTS

*First of all, thanks to **GOD** who provided me with strength, hope and faith.*

*Furthermore, I would like to express my gratitude to all who gave me the possibility to complete this thesis. Words can not express my deepest appreciation and gratitude to **Professor Dr. Mahmoud El Refai** Chairman of Oral Medicine and Periodontology Department for his endless support, guidance and encouragement throughout all the course of this work. Also I would like to thank him for giving me permission to commence this thesis in the first place.*

*My deepest thanks and appreciation to **Professor Dr. Fatheya Zahran** for her valuable direction, help, support and suggestions from the beginning step of my work. She spared no time or effort in guiding me to accomplish this work.*

*Also, I would like to acknowledge and thank **Professor Dr. Olfat Shaker** for her great efforts and suggestions that helped me a lot in my research.*

*My sincere thanks go to all the staff members for their help and support.*

*Finally, my deepest gratitude goes to my beloved family; for their unflagging love and support throughout my life.*

بسم الله الرحمن الرحيم

رب اشرح لي صدري  
و يسر لي امري

صدق الله العظيم

## **Contents**

◆ Introduction	1
◆ Review of Literature	3
◆ Aim of the Study	48
◆ Subjects and Methods	49
◆ Results	65
◆ Discussion	94
◆ Conclusions	104
◆ Summary	105
◆ References	107
◆ Arabic Summary	

## **LIST OF TABLES**

<b>Table number</b>	<b>Heading</b>	<b>Page</b>
1	IFN- $\gamma$ regulated genes and their function in producing IFN- $\gamma$ effects (Griscelli et al., 1989, Chang et al., 1994, Heufler et al., 1996, Boss, 1997 Waldburger et al., 2000)	39
2	Immunoregulatory functions of IFN- $\gamma$	40
3	The used reagents	57
4	Descriptive data for all patients' groups	67
5	Results of IFN- $\gamma$ levels (pg/ml) in tissues and saliva in all included patients' groups and the control group	72
6	Statistical analysis (ANOVA test) comparing control group with papular, atrophic and erosive lichen planus regarding the tissue level of IFN- $\gamma$	75
7	Statistical analysis (ANOVA test) comparing control group with papular, atrophic and erosive lichen planus regarding the salivary level of IFN- $\gamma$	77
8	Statistical analysis comparing control group with papular lichen planus regarding IFN- $\gamma$ (pg/ml) in tissues	79
9	Statistical analysis comparing control group with atrophic lichen planus regarding IFN- $\gamma$ (pg/ml) in tissues	79
10	Statistical analysis comparing control group with erosive lichen planus regarding IFN- $\gamma$ (pg/ml) in tissues	81
11	Statistical analysis comparing papular with atrophic lichen planus regarding IFN- $\gamma$ (pg/ml) in tissues	81
12	Statistical analysis comparing papular with erosive lichen planus regarding IFN- $\gamma$ (pg/ml) in tissues	82
13	Statistical analysis comparing atrophic with erosive lichen planus regarding IFN- $\gamma$ (pg/ml) in tissues	82
14	Statistical analysis comparing control group with papular lichen planus regarding IFN- $\gamma$ level (pg/ml) in saliva	85
15	Statistical analysis comparing control group with atrophic lichen planus regarding IFN- $\gamma$ level (pg/ml) in saliva	85
16	Statistical analysis comparing control group with erosive	86

	lichen planus regarding IFN- $\gamma$ level (pg\ml) in saliva	
17	Statistical analysis comparing papular with atrophic lichen planus regarding IFN- $\gamma$ level (pg\ml) in saliva	86
18	Statistical analysis comparing papular with erosive lichen planus regarding IFN- $\gamma$ level (pg\ml) in saliva	87
19	Statistical analysis comparing atrophic with erosive lichen planus regarding IFN- $\gamma$ level (pg\ml) in saliva	87
20	Statistical analysis showing correlation of IFN- $\gamma$ level between saliva and tissues	91

## **LIST OF FIGURES**

<b>Figure number</b>	<b>Heading</b>	<b>Page</b>
1	3D structure of IFN- $\gamma$ (Courtesy of Protein Data Bank <a href="http://www.rcsb.org/pdb/">http://www.rcsb.org/pdb/</a> )	27
2	IFN- $\gamma$ signaling pathway	28
3	IFN- $\gamma$ secreting cells and its effect on various cells (Courtesy of, <a href="http://pathmicro.med.sc.edu">http://pathmicro.med.sc.edu</a> )	41
4	Clinical photograph showing a case of oral lichen planus; reticular (papular) pattern (male).	51
5	Clinical photograph showing a case of oral lichen planus; reticular and erosive pattern (male).	51
6	Clinical photograph showing a case of oral lichen planus; atrophic pattern (female).	52
7	Photomicrograph of H&E stained biopsy specimen of one of the included lichen planus cases	55
8	Photomicrograph of H&E stained biopsy specimen of another one of the included lichen planus cases	55
9	ELISA kit box for detection of human IFN- $\gamma$ in saliva and tissue	58
10	Constituents of human IFN- $\gamma$ ELISA kit for quantitative detection of human salivary and tissue IFN- $\gamma$	58
11	ELISA steps	59
12	A photograph showing the centrifuge	59
13	ELISA reader	64
14	Standard curve showing the mean absorbance for each standard against the concentration	64
15	Distribution of the papular group according to gender.	68
16	Distribution of the atrophic group according to gender	68



17	Distribution of the erosive group according to gender.	68
18	Histogram showing gender distribution compared among different groups	69
19	Comparison between all studied groups regarding age	69
20	Comparison of IFN- $\gamma$ level in tissues and saliva among all included groups	73
21	Distribution chart for IFN- $\gamma$ level in tissues for the 40 included individuals	89
22	Distribution chart for IFN- $\gamma$ level in saliva for the 40 included individuals	89
23	Positive correlation between salivary and tissue IFN- $\gamma$ levels in control group	92
24	Positive correlation between salivary and tissue IFN- $\gamma$ levels in erosive group	92
25	Positive correlation between salivary and tissue IFN- $\gamma$ levels in atrophic group	93
26	Positive correlation between salivary and tissue IFN- $\gamma$ levels in papular group	93

# **INTRODUCTION**

Oral lichen planus (OLP) is a chronic mucocutaneous inflammatory disease that appears in about 1–2% of the general population, very frequently showing oral manifestations (**Pindborg et al., 1997; Miller et al., 2001**) and is characterized by a clinical course with periodic remissions and reactivations (**Eisen, 1993; Lozada-Nur and Miranda, 1997; Scully et al., 1998; Chainani-Wu et al., 2001**).

As T cell-mediated autoimmunity is considered to be involved in the pathogenesis of this disease (**Sugerman et al., 2002**), the roles of a line of T cell associated-cytokines and chemokines have been investigated in the past decades.

Cytokines are the major immunomodulators, determining the pathophysiologic outcome of infectious disease and systemic inflammatory responses, with either inhibitory or stimulatory effects on cellular growth, differentiation and function (**Tracey and Cerami, 1994**).

Among these cytokines ,interferon (IFN)-gamma and interleukin (IL)-4 have been studied more extensively because IFN-gamma and IL-4 are regarded as the characteristic cytokines of T helper 1) Th1) cells and T helper 2 (Th2), respectively (**Sugerman et al.; 2002, Neurath et al.; 2002**).

To date, the results on both cytokines in OLP have been inconsistent. **Khan et al. (2003)** found that IFN-gamma expression increased strongly in

## INTRODUCTION

---

OLP lesions which were secreted by lesional T cells in vitro culture, but **Yamamoto et al., (1991)** observed that IFN-gamma was significantly decreased in the peripheral blood of patients with OLP.

IFN- $\gamma$  exerts profound effects on inflammation as it upregulates major histocompatibility class II molecules on most cells (**Steiniger et al., 1988, Chang and Flavell, 1995**), activates macrophages and enhances expression of adhesion molecules on endothelial cells (**Issekutz, 1995**).

IFN- $\gamma$  has also, in an animal model, been found to preferentially mediate lymphocyte extravasation compared with neutrophils into inflammatory lesions (**Colditz and Watson, 1992**).

Great numbers of unstimulated cells producing IFN- $\gamma$  were detected in OLP lesions (**Yamamoto and Osaki, 1995**).

Currently, the protein composition of human saliva has been initially investigated by proteome techniques, and a large number of unrevealed proteins in saliva were identified and further demonstrated as possible biomarkers in oral diseases, such as dental caries, periodontitis, and oral squamous cell carcinomas. However, few studies have utilized simultaneous detection of cytokines in local tissues and saliva to determine whether salivary cytokines could reflect the facts of local lesions (**Vitorino et al., 2004; Ghafouri et al., 2004; Tao et al., 2008**).

Consequently it was found of interest to study the simultaneous prevalence of IFN gamma in tissues and saliva of OLP.

## **REVIEW OF LITERATURE**

Lichen planus (LP) is a chronic inflammatory disorder of cutaneous and mucosal tissues that is considered by some authors to be an autoimmune disease of unknown aetiology in which epithelial cells are recognized as foreign due to changes in cell surface antigenicity (**Edwards & Kelsch, 2002**).

The prevalence of lichen planus is unknown, but it is estimated to occur in one to two percent of the general adult population. Estimates of the prevalence vary among different populations, but the condition does not appear to exhibit a racial predilection and it is the most common non-infectious oral mucosal disease in patients referred to Oral Medicine and Oral Pathology clinics (**Axéll and Rundqvist, 1987; Boyd and Neldner, 1991; Bowers et al., 2000**).

An epidemiological study demonstrated that women suffer from oral lichen planus (OLP) more frequently (75%) than men (25%), and that the disease is more frequent in people over 40 years of age, although younger adults and children may be affected (**Vincent et al., 1990; Chainani-Wu et al., 2001; Eisen, 2002**).

Lichen planus often occurs on cutaneous surfaces and also may involve the oral mucosa, the genital mucosa, the nails and the scalp. Moreover, these areas may be exclusively involved. On the other hand laryngeal,

oesophageal and conjunctival involvement is uncommon (**Katta, 2000; Ismail et al., 2007**).

As a sole manifestation of LP, oral lesions make up to 15-35% of the patient's group, but up to 65% of patients with classical cutaneous LP have concomitant oral disease (**Boyd and Neldner, 1991**).

The classic appearance of skin lesions includes violaceous polygonal flat-topped papules and plaques. Close examination reveals a reticulated pattern of white scales known as Wickham's striae. Early cutaneous lesions appear as scattered erythematous papules. The flexor surfaces of extremities, particularly the wrists, are common locations for lichen planus (**Katta, 2000**).

Cutaneous lesions may occur in areas exposed to trauma, such as lacerations and this tendency is known as an isomorphic response or Koebner's phenomenon. Lesions often resolve with intense hyperpigmentation (**Katta, 2000**).

Some physicians describe lichen planus with the six "Ps"; pruritic, polygonal, planar (flat-topped), purple papules and plaques. While some patients may be asymptomatic, most of them experience intense pruritus, a hallmark of lichen planus (**Katta, 2000**).

Other variants of LP do exist and exhibit various morphologies. Patients with hypertrophic lichen planus present with thick hyperkeratotic plaques, commonly found on the anterior surface of the legs. In vesiculobullous

lichen planus, patients exhibit blisters within the plaques, while the actinic type of lichen planus occurs in sun-exposed areas of skin (**Katta, 2000**).

Nail involvement results in pitting, pterygium formation, and permanent nail loss. Scalp involvement results in scarring alopecia (**Sugerman et al., 2000b**).

The oral form of lichen planus seems more common, chronic, and recalcitrant than the cutaneous type, persisting up to more than 20 years without spontaneous remission (**Scully et al., 2000**).

The clinical presentation of OLP varies. In many patients, the onset of OLP is insidious, and patients are unaware of their oral condition. Some patients report roughness of the lining of the mouth, sensitivity of the oral mucosa to hot or spicy food, painful oral mucosa, red or white patches on the oral mucosa, or oral ulcerations (**Eisen, 1999**).

Six clinical forms of OLP have been described which are white forms namely reticular, papular, plaque-like and the red forms namely the erosive (ulcerated), atrophic (erythematous), and bullous (**Andreasen, 1968; Pindborg et al., 1997**).

The most common type is the reticular pattern which presents as fine white striae known as Wickham's striae. The striae are typically bilateral and symmetrical. The buccal mucosa is the most commonly affected, although any site can be affected. Patients with reticular lesions are often asymptomatic (**Eisen, 2002; Ingafou et al., 2006**).

Atrophic OLP presents as a diffuse red lesion. The lesions may appear as a mixture of clinical subtypes. For example, white and gray streaks may form linear or reticular pattern on erythematous background. Alternatively, a central area of shallow ulceration (erosion) may have a yellowish surface (fibrinous exudates) surrounded by an area of erythema (**Silverman et al., 1985**).

Erosive OLP presents as irregular erosion or ulceration covered with a fibrinous plaque or pseudomembrane. The periphery of the lesion is usually surrounded by reticular or finely radiating keratotic striae. Atrophic (erythematous) or erosive (ulcerated) OLP are often associated with burning sensation and pain (**Eisen, 2002**).

Plaque type OLP appears as homogenous white patches which resemble leukoplakia. However, the presence of white striations and histologic confirmation will allow for the definitive diagnosis of OLP to be made. This type commonly affects the dorsum of the tongue and buccal mucosa and is more common among tobacco smokers, it may range from a slightly elevated and smooth to a slightly irregular form (**Thorn et al., 1988**).

Bullous OLP is the least common type. The bullae range from few millimeters to several centimeters in diameter. They tend to rupture leaving ulcerated and painful surfaces. The periphery of the lesion is usually surrounded by reticular or finely radiating keratotic striae (**Zegarelli, 1993**).

The papular type can coexist with any of the previously described types (**Bricker, 1994**).