Evaluation of Interleukin-6, Matrix Metalloproteinase-8 and Osteoprotegerin levels in both Chronic and Aggressive Periodontitis patients

Thesis submitted in partial fulfillment of the requirements of the Master Degree in Oral Medicine, Periodontology, and Oral Diagnosis
Faculty of Dentistry
Ain Shams University

By

Manal Salem Bazina

Demonstrator, Department of Periodontology, Faculty of Dentistry, Benghazi University. Libya B.D.S. (2005)

Faculty of Dentistry Ain Shams University 2014

Supervised By

Prof.Dr. Hadir Fouad El Dessouky

Professor of Oral Medicine, Periodontology, and Oral Diagnosis Department of Oral Medicine, Periodontology, Oral Diagnosis and Radiology

> Faculty of Dentistry Ain Shams University

Dr. Ahmed Abdel Aziz Hassan

Lecturer of Oral Medicine, Periodontology, and Oral Diagnosis Department of Oral Medicine, Periodontology, Oral Diagnosis and Radiology

> Faculty of Dentistry Ain Shams University

Prof.Dr. Ola Ibrahim Ahmed

Professor of Medical Microbiology & Immunology Department of Medical Microbiology & Immunology Faculty of Medicine Ain Shams University

تقييم مستوى كل من الأنترلوكين- ٦ والماتريكس ميتاللوبروتينيز- ٨ والأستيوبروتجرين في مرضى التهاب الأنسجة الداعمة حول السنية المسترمن والهجومسي

رسالة مقدمة توطئة للحصول علي ماجستير طب القم وعلاج اللثة و التشخيص كلية طب الاسنان وجامعة عين شمس

مقدمة من الطبيبة

منال سالم بازينكة

معيدة بقسم علاج اللثة, كلية طب وجراحة الفم و الأسنان جامعة بنغازي بعدة بقسم علاج الله الأسنان, بنغازي ليبيا

2005

كلية طب الأسنان جامعة عين شمس 2014

تحت أشـــراف

أ.د. هدير فيواد الدسوقى

استاذ بقسم طب الفم, علاج اللثة, التشخيص, والاشعة كلية طب الاسنان - جامعة عين شمس

د. أحمد عبد العريز حسن

مدرس بقسم طب الفم ,علاج اللثة ,التشخيص, والاشعة كلية طب الاسنان _ جامعة عين شمس

استاذ بقسم الميكروبيولوجي و المناعة كلية الطب جامعة عين شمس

AKNOWLEDGMENT

First and for most, thanks to ALLAH who is the most Gracious and most Merciful.

I would like to express my deepest thanks, gratitude and profound respect to **Prof.Dr. Hadir El-Dessouky**, Professor of Oral Medicine, Periodontology and Oral Diagnosis, Faculty of Dentistry, Ain-Shams University. For her endless encouragement, great help, extreme patience, valuable guidance, and immeasurable support, will always be sincerely remembered.

I am also deeply thankful to **Dr. Ahmed Abdel Aziz Hassan** Lecturer of Oral Medicine, Periodontology, and Oral Diagnosis, Faculty of Dentistry, Ain Shams University For his continuous guidance which was of paramount importance for the progress and completion of this work, will always be deeply remembered.

My deepest thanks and sincere gratitude as well as appreciation to **Prof.Dr. Ola Ibrahim Ahmed**, Professor of Medical Microbiology & Immunology Faculty of Medicine Ain shams University for her valuable advice, devoted effort, and unique cooperation.

My sincere gratitude to the chairman of Oral Medicine, Periodontology, Oral Diagnosis and Radiology Department, Ain Shams University and all faculty and staff member for their sincere help and cooperation.

DEDICATION

To the soul of my father who taught me the value of learning.

To the person I admire most, my mother who offered me unconditioned support throughout the course of my life.

To my husband who has been a great source of motivation and inspiration, and to my beautiful angels Nour and Mohamed.

Contents

List of Abbreviations	Ι
List of Tables	III
List of Figures	V
Introduction	1
Review of Literature	8
Aim of the Study	61
Subjects and Methods	62
Results	92
Discussion	105
Summary	126
Conclusion	129
Recommendations	130
References	131
Arabic Summary	

List of Abbreviations

AAP American Academy Of Periodontology

Aa Aggregatibacter actimomycetemcomitans

AgP Aggressive periodontitis

CAL Clinical Attachment Level

CP Chronic periodontitis

CRP C-reactive protein

ECM Extracellular matrix

ELISA enzyme-linked immunosorbent assay

GAgP Generalized aggressive periodontitis

GCF Gingival crevicular fluid

HbA1c Hemoglobin A1C

IFN Interferon

Ig Immunoglobulin

IL-6 Interleukin-6

IL-1Ra IL-1 receptor antagonist

LPS Lipopolysaccharides

MMPs Matrix metalloproteinases

MPO Myeloperoxidase

OPG Osteoprotegerin

OSM Oncoststin M

PBI Papillary Bleeding Index

PD Periodontal disease

PDL Periodontal ligament

Pg Porphromonas gingivalis

PG Prostaglandins

PI Plaque index

PMNs Polymorphonuclear granylocytes

PPD Probing pocket depth

RANKL Receptor activator of nuclear factor kappa B

ligand

SDD Subantimicrobial-dose doxycycline

TGF- β Transforming growth factor- β

Th1 T- helper type 1

TIMP Tissue inhibitors of matrix metalloproteinases

TNF Tumor necrosis factor

TNF- α Tumor necrosis factor- α

T2DM Type 2 diabetes mellitus

UWS Unstimulated whole saliva

LIST OF TABLES

Table 1:	Materials supplied with IL-6 ELISA kit	74
Table 2:	Materials supplied with MMP-8 ELISA kit	82
Table 3:	Materials supplied with OPG ELISA kit	84
Table 4:	Mean, standard deviation (SD) and results	94
	of Friedman test and Newman-Keuls post	
	hoc test for comparison between IL-6	
	levels in the three groups.	
Table 5:	Mean, standard deviation (SD) and	96
	results of Friedman test and New man-	
	Keuls post hoc test for comparison	
	between MMP-8 levels in the three groups.	
Table 6:	Mean, standard deviation (SD) and results	98
	of Friedman test and New man-Keuls post	
	hoc test for comparison between OPG	
	levels in the three groups.	
Table 7:	Mean, standard deviation (SD) values of	101
	clinical parameters and results Friedman	
	test	

Table 8:	Correlation between IL-6 and clinical	103
	parameters in periodontitis patients	
Table 9:	Correlation between MMP-8 and clinical	103
	parameters in periodontitis patients	
Table 10:	Correlation between OPG and clinical	104
	parameters in periodontitis patient	

Table 11: Correlation between IL-6, MMP-8 and 104 OPG in periodontitis patients

LIST OF FIGURES

Figure 1:	Showing periopaper used in the collection	68
	of GCF samples.	
Figure 2:	Showing collection of GCF sample from	68
	distal surface of upper right central	
	incisor.	
Figure 3:	Collection of salivary sample	69
Figure 4:	Showing salivary and gingival crevicular	70
	fluid samples.	
Figure 5:	Showing MMP-8, IL-6 and OPG ELISA	72
	Kit	
Figure 6:	ELISA reader	73
Figure 7:	Showing the content of IL-6 ELISA Kit.	75
Figure 8:	Showing the content of MMP-8 ELISA	83
	Kit.	
Figure 9:	Showing the content of OPG ELISA Kit	84
Figure 10:	Frontal view photograph of an aggressive	88
	periodontitis female patient 25 years old.	
Figure 11:	A panoramic radiograph of the same	88
	patient.	
Figure 12:	Photograph showing 7 mm pocket depth	89
	distal to upper right central incisor.	

- Figure 13: A photograph showing collection of gingival 89 crevicular fluid sample from the same probing site.
- Figure 14: Frontal view photograph of a chronic 90 periodontitis female patient 37 years old.
- Figure 15: A panoramic radiograph of the same patient. 90
- Figure 16: Photograph showing 5 mm pocket depth 91 mesial to upper right first molar.
- Figure 17: A photograph showing collection of gingival 91 crevicular fluid sample from the same probing site.
- Figure 18: Histogram representing mean IL-6 levels in 94 the three groups.
- *Figure 19:* Histogram representing mean MMP-8 levels 97 in the three groups.
- Figure 20: Histogram representing mean OPG levels in 99 the three groups.
- Figure 21: Column chart of clinical parameters mean 101 values in the three groups

INTRODUCTION

Periodontal disease (PD) is a chronic bacterial infection characterized by persistent inflammation, connective tissue breakown and alveolar bone destruction. Contributing inflammatory mediators and tissue destructive molecules have been detected in the gingival tissue, gingival crevicular fluid (GCF) and saliva of patients affected by periodontitis.⁽¹⁾

Chronic periodontitis (CP) is characterized by the occurrence of an irreversible destruction of periodontal supporting tissues. Disease appears to result from a complex interaction between the periodontopathogenic bacteria and the host immunoinflammatory response. Periodontal loss is considered to occur as cycles of acuteactivity episodes that alternate with prolonged periods of quiescence. (2) The disease progression involves a network interacting molecular pathways including proinflammatory mediators, reactive oxygen species, matrix metalloproteinases (MMPs), and their MMP inhibitors and regulators. (3) Type I collagen is the main extracellular matrix component of periodontal tissues, and thus, collagen degradation is regarded as one of the key factors in uncontrolled destructive lesions. (4)

Aggressive periodontitis (AgP) is characterized by a rapid loss of clinical attachment and alveolar bone and normally affects young adults. As opposed to chronic amount of biofilm and calculus periodontitis. the aggressive periodontitis accumulation in subjects is inconsistent with the severity and progression of the periodontal destruction. It is subdivided into localized and generalized form, according to the extent of the periodontal destruction. Diagnosis of aggressive periodontitis requires exclusion of the presence of systemic diseases that may severely impair host defenses and lead to premature tooth loss. (5,6)

Interleukin-6 (IL-6) is an important cytokine involved in the regulation of host response to tissue injury and infection .⁽⁷⁾ It is produced by a variety of cells, such as monocytes, fibroblasts , osteoblasts and vascular endothelial cells in response to inflammatory challenges . It plays an important role in B-cell differentiation and in T-cell proliferation , while IL-6, synergistic with