PCR BASED ASSAY OF MITOCHONDRIAL DNA MUTATIONS IN ORAL LEUKOPLAKIA: CORRELATION WITH COX-2 EXPRESSION

Thesis

Submitted to the Faculty of Dentistry, Ain Shams University,

In Partial Fulfillment of the Requirements for **Doctor Degree of Philosophy in Oral Pathology(PhD)**

Ву

Nermeen Sami Afifi

Assistant Lecturer of Oral Pathology
B.D.S, Ain Shams University (2002)
M.D.S, Ain Shams University (2010)
Faculty of Dentistry, Ain Shams University

Faculty of Dentistry
Ain Shams University
2015

Supervisors

Dr. Ehab Saeed Abdel-Hamid

Professor of Oral Pathology and Vice Dean of Post Graduate Affairs Faculty of Dentistry, Ain Shams University

Dr. Houry Moustafa Baghdadi

Professor of Oral Pathology
Faculty of Dentistry, Ain Shams University

127,18 787,18 (20) 175 16-12 76-12 8,02 Ci

والمناع المناع ا

ではいいいかいからいからいからいからいからいっという

To Mom and Dad, who always picked me up on time and inspire me by love, encouragement and support....

To my beloved husband, Ehab, and my dear sons, Mohannad and Eyad Thank you for your support and patience....

To all who will read this one day and find it useful,
Thank you for your time and trust....

Acknowledgment

First of all, deep thanks for God who gave me everything I have and guided me so that I am able to accomplish this thesis.

Immeasurable appreciation and deepest gratitude for the help and support are extended to the following persons who in one way or another have contributed in making this study possible.

Grateful thanks for **Dr. Ehab Saeed Abdel-Hamid**, Professor of Oral Pathology and Vice Dean of Post Graduate Affairs, Faculty of Dentistry, Ain Shams University, who has given his valuable time, provision and correction to this thesis and continuous advice whenever needed.

Deepest thanks for **Dr. Houry Moustafa Baghdadi**, Professor of Oral Pathology, Faculty of Dentistry, Ain Shams University, who gave her love, care, valuable comments and suggestions to accomplish this work.

I would like also to thank **Dr. Dina Sabry**, Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, for her efforts and valuable assistance through the practical work in this study.

To all staff members of oral pathology department, Faculty of Dentistry, Ain Shams University, thank you for your love and support.

Table of Contents

List of Abbreviations	i
List of Figures	iii
List of Tables	vi
Introduction	1
Review of Literature	4
Aims of the Study	28
Material and Methods	29
I- Case Selection.	29
II-Histopathological Examination.	29
III- Immunohistochemical Staining	31
A- Reagents used	31
B- Immunostaining Procedures	32
IV-Immunohistochemical Evaluation	32
V- DNA Extraction	38
VI- D-loop Amplification	40
VII-Detection of the Amplified DNA and D-loop Semiquantitation	42
VIII- DNA Sequencing	44
IX- Statistical Analysis	45

Results	46
I.Histopathological Results	46
II.Immunohistochemical Results	52
III. PCR Results and Mitochondrial D-loop Semiquantitation	60
IV.Mitochondrial D-Loop Sequencing Results	61
V.Statistical	
Results	63
COX-2 immunoexpression	63
Mitochondrial D-loop semiquantitation	68
Discussion	74
Conclusions	88
Recommendations	89
Summary	90
References	92
Arabic Summary	112

List of Abbreviations

ANOVA: Analysis of Variance

ATP: Adenosine Tri-Phosphate

Bcl-2: B-cell lymphoma-2

BLAST: Basic Local Alignment Search Tool

COX: Cyclo-oxygenase

Cyt-c: cytochrome -c

DAB: Diamino Benzidine

D-loop: Displacement Loop

DNA: Deoxyribonucleic Acid

dNTP: deoxyribonucleotide triphosphate

DPX: Distrene, Plasticiser, Xylene

EB: Ethidium Bromide

EBV: Epstein Barr Virus

EDTA: Ethylene Diamine Tetra Acetic Acid

H&E: Hematoxylin and Eosin

HBV: Hepatitis B Virus

HIF: Hypoxia Inducible Factor

HIV: Human Immunodeficiency Virus

HNSCC: Head and Neck Squamous Cell Carcinoma

HPV: Human Papilloma Virus

HSV: Herpes Simplex Virus

MDB: Membrane Desalting Buffer

MOMP: Mitochondrial Outer Membrane Permeabilization

MtDNA: Mitochondrial DNA

MW: Molecular Weight

NADH: reduced Nicotinamide Adenine Dinucleotide

NSAIDs: Non-Steroidal Anti-Inflammatory Drugs

OED: Oral Epithelial Dysplasia

OSCC: Oral Squamous Cell Carcinoma

PBS: Phosphate Buffer Saline

PCR: Polymerase Chain Reaction

PGs: Prostaglandins

pH: Power of Hydrogen

PKB: Protein Kinase B

PMDs: Potentially Malignant Disorders

PTEN: Phosphatase and Tensin homolog

PTP: Permeability Transition Pore

ROS: Reactive Oxygen Species

rpm: revolution per minute

SPSS: Statistical Package for Social Science

TAE: Tris-Acetate EDTA

Taq: Thermus aquaticus

TCA: Tricarboxylic Acid

tRNA: transfer Ribonucleic Acid

UV: Ultra Violet

WHO: World Health Organization

List of Figures

Fig. 1:	The mitochondrial oxidative phosphorylation system	16
Fig. 2:	Human mitochondrial genome	17
Fig. 3:	Harmful effects of mitochondrial ROS	20
Fig. 4	Mitochondrial DNA mutations in different types of cancers	24
Fig. 5:	Plate showing the various steps for the immunohistochemical evaluation	37
Fig. 6:	Plate showing the steps of DNA extraction	41
Fig. 7:	Photomicrograph of the control group showing normal covering epithelium (H&E, orig. mag. x20)	47
Fig. 8:	Higher magnification of the previous photomicrograph showing epithelial cells free from cytological and archtectural features of the dysplasia (H&E, orig. mag. x40)	47
Fig. 9	Photomicrograph of non dysplastic leukoplakia showing acanthosis, hyperkeratosis and basilar hyperplasia (H&E, orig. mag. x20)	48
Fig. 10:	Higher magnification of the previous photomicrograph showing basilar hyperplasia (H&E, orig. mag. x40)	48
Fig. 11:	Photomicrograph of leukoplakia with mild dysplasia showing nuclear hyperchromatism as well as cellular and nuclear pleomorphism (H&E, orig. mag. x20)	49
Fig. 12:	Higher magnification of the previous photomicrograph showing basilar hyperplasia, nuclear hyperchromatism, cellular and nuclear pleomorphism and prominent basal mitosis (H&E, orig. mag. x40)	
	mug. Λτυ/	49