بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

سورة البقرة : ٣٢

MITOCHONDRIAL DNA MUTATIONS AS A GENETIC MARKER FOR COLORECTAL CANCER

Presented by

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B.SC., Zoology

A thesis submitted
To
Faculty of Science - Cairo University
in partial fulfillment of the requirements for
the Degree of
M.SC. of Science
(Cytology, Histology & Genetics)

Zoology Department

Faculty of Science

Cairo University

(2010)

APPROVAL SHEET FOR SUMISSION

Thesis Title:

"Mitochondrial DNA mutations as a genetic marker for colorectal cancer"

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إلي

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كجزء من متطلبات الحصول على درجة

الماجستير

(علم الخلية والانسجة والوراثة)

قسم علم الحيوان

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جامعة القاهرة

(٢٠١٠)

This thesis is dedicated to my parents, who taught me the value of education. I am deeply indebted to them for their continued support and unwavering faith in me.

Acknowledgements

My eternal gratitude to God almighty for granting me the precious helps to accomplish this work. Wishing that God may accept it as an ongoing charity.

My deep appreciation to Prof. Dr. M. Akmal Abd El-Rahim Elghor, professor of Genetics, Zoology Department, Faculty of Science Cairo University, for supervising this work and for his unrivalled knowledge, effort and patience.

A very special dedication to Prof. Dr. Abd El-Hady Ali Abd El-Wahab, Professor of Biochemistry& Molecular Biology, Cancer Biology Department, National Cancer Institute, Cairo University, for supervising and planning this work and for the great support and suggesting which contributed to the accomplishment of this Thesis.

Thanks to Dr. Ibrahim Abd El-Bar, Consultant of Surgical Oncology, Tanta Cancer Center.

My great thanks and respect to Assistant Prof. Dr. Nadia El gundy, Assistant Professor of Biochemistry& Molecular Biology, Cancer Biology Department, National Cancer Institute, Cairo University, for her encouragement and for review manuscript.

Eternally grateful for my professors; Prof. Dr. Fouad Saleh, the previous Chairman of Zoology Department, Faculty of Science, Cairo University; Prof Dr. Rashika El Ridi Professor of Immunology Faculty of Science, Cairo University, Dr. Medhat El-Halawany, lecturer of Molecular Biology, Faculty of Science, Cairo University, Prof. Dr El Said Salem Faculty of Science, Zoology Department,Tanta University; Prof.Dr Abdel Rahman Zakri, Cancer Biology Department, National Cancer Institute, Cairo University and prof. Dr Mahmoud El-Rouby, Cancer Biology Department, National Cancer Institute, Cairo University, for their kindly, unrivalled knowledge, guidnace, assistance, encouragement and support.

I wish to express my love and eternal gratitude to my parents for their understanding, endless love and for all things.

Eternally grateful also for, my brothers Mohamed Abd El-Hady and Mahmoud Abd El-Hady and also my sisters Safaa Abd El-Hady and Howida Abdelhady, for thier assistance and support, and understanding, without them, I could not have completed this work.

Express my gratitude to all my friends especially, Hanan Kiwan, Noha Ramadan, Ebtsam for their extended support and their helped to me.

I would also like to convey my thanks to the Cairo University for providing the financial means, academic and laboratory facilities. All thanks and appreciation to the National Cancer Institute, Cairo University; Faculty of Science, Cairo University and also Kasr Al Eini Endoscopic Unit, Tropical Medicine Department, Faculty of Medicine.

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Abbreviations

APC :	Adenomatous Polyposis Coli
APS:	Amonium persulfate.
ASTR:	Egyptian Academy of Science and Technology Research
bis:	N,N'-methylenebisacrylamide.
bp:	base pair.
CT:	Computed Tomography
CRC:	Colorectal cancer
CTC:	Computed Tomography Colonography.
DCC:	Deleted in Colorectal Cancer.
DCBE	Double-Contrast Barium Enema.
dGTP:	2'-deoxyguanosine 5'- triphosphate.
D-loop:	displacement loop.
DNA:	Deoxyriboneucleic acid
dNTP:	2'-deoxynucleoside 5'- triphosphate.
E&H:	Eosin & hematoxylin stained
EDTA:	Ethylenediaminetetra-acetic acid
FAP:	Familial Adenomatous Polyposis.
FIT:	Fecal Immunochemical Test
FOBT:	Fecal Occult Blood Test.
g:	gram.
GTP:	Guanosine diphosphate.
HNPCC:	Hereditary Nonpolyposis Colorectal Cancer.
HPR:	homopolymer region.
HPLC:	High performance liquid chromatography.
IBD:	Inflammatory Bowel Diseases.
IRB:	institutional review board
KD:	Kilo Dalton.
LOH:	Loos of Heterozygosity.
M:	Mollar.
mg:	Milligram.
µg:	Microgram.
µl:	Microliter.
mMin:	Minute.
mtDNA:	mitochondrial DNA.
ml:	Milliliter.
mM:	Millimolar
MMR:	Mismatch repair.
MSI:	Microsatellite Instability.
MSNT:	matched surrounding normal tissues.
nDNA:	nuclear DNA.
C :	Grade Celsius.
OD:	Optical Density.
OMM:	Outer Mitochondrial membrane.
OXPHOS:	oxidative phosphorylation system.
PCR:	Polymerase chain reaction.
pH:	The hydrogen ion concentration.
ROS:	Reactive Oxygen Species.
rpm:	Round per minute.
rRNA:	ribosomal RNA.

sDNA:	stool DNA test.
SSCP:	Single Strand Conformation Polymorphism
TAE:	Tris acetate EDTA
Taq:	Thermus aquaticus
TBE:	Tris borate EDTA.
TBp:	TATA Box Binding Protein.
TCF:	T-Cell- Factor.
TE:	Tris EDTA.
TEMED:	N,N, N', N'-tetramethylethylenediamine.
tRNAs:	transfer RNAs.
U:	Unit(s).
UC:	Ulcerative Colitis.
UV:	Ultraviolet.
W:	Watt.
WHO:	World Health Organization.
WRCF:	World Cancer Research Fund.

ABSTRACT

Student Name: Hala Ahmed Mohamed Abd El-Hady

Title of the thesis: **"Mitochondrial DNA Mutations As A Genetic Marker for Colorectal Cancer"**

Degree: M.SC. (Cytology, Histology & Genetics)

In this study we aim to evaluate the mitochondrial DNA (mtDNA) mutations as a genetic marker for early detection of colorectal cancer (CRC) in Egyptian patients, to reduce the incidence and mortality rate of CRC in Egypt. Six different regions of mtDNA (ND1, ND5, COI, tRNAser & 2 regions in the D-Loop) were chosen for mutation analysis using PCR-SSCP silver staining technique followed by automated sequencing. Biobsy tissue samples from 20 patients with adenomatous polyps (AP), 20 with ulcerative colitis (UC) and 40 with CRC were collected together with blood sample to be used as controls. High frequency of mutations was detected (45% in AP, 35% in UC and 35% in CRC). Our study suggests that Egyptian CRC patients have a number of mtDNA mutations that have not been previously reported and that mutations in mtDNA of precancerous lesions (AP, UC) may contribute to the transformation events leading to CRC and can be considered as a genetic marker (Biomarker) for early detection of CRC in Egypt, but we need more samples to confirm this assumption.

Keywords: Colorectal cancer, Mitochondrial DNA, Mutations, Biomarker, Genetic marker, Early detection.

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1. Introduction

Colorectal cancer is one of the most common human malignancies, with more than 300,000 cases both in the United States and in the European Union each year where the vast majority of cases occurs over age 60 years (Lievre *et al.,* 2005; *Greenle et al.,* 2000). In contrast, this disease is uncommon in developing countries (Magrath and Litvak, 1993), including Egypt where it is only the fifth most common cause of cancer deaths (Soliman *et al.,* 1999*).* However, the percentage of young-onset colorectal cancer cases in Egyptian is strikingly high with more than one third of cases occurring under age of 40 years, and the age-adjusted mortality rates in young Egyptians are likewise high. In addition, rectal cancer is frequent (Soliman *et al.,* 1997; 1999).

The molecular pathology of colorectal carcinoma in Egypt differs from Western patients, and between younger and older Egyptians (Soliman *et al.,* 2001). The rectal predominance in Egyptian cases occurred in both younger and older patients and was unrelated to urban\rural residence, which associated with very different environmental exposure *(*Soliman *et al.,* 2001).

Westernization of Egypt is occurring as the country develops and is affecting young people first because they are likely to change lifestyle than older people. The remarkable differences in molecular pathology as compared to western patients could result from inherent differences in sensitivity and molecular responses to Western lifestyle in Egyptians (Rockhill and Giovannucci, 1999*).*

Early stage CRC does not usually have symptoms; therefore, screening is necessary to detect CRC in its early stages then reduce incidence and mortality can be achieved. Over the last few years, a variety of approaches have been developed to improve results of conventional cancer screening by detecting molecular markers in different populations (Rozen *et al.,2005*).

Each human cell contains two genomes, nuclear genome (nDNA) and mitochondrial genome (mitochondrial DNA (mtDNA)) (Chatterjee *et al.,* 2006). Although much knowledge has been collected concerning alterations in cancer cell nuclear DNA (nDNA), less attention has been paid to mutations within mitochondrial DNA (mtDNA) (Lievre *et al,* 2006). Over the last few years, a variety of approaches have been developed to improve results of conventional cancer screening by detecting molecular markers in different populations (Verma and Kumar, 2007). In the recent years, much attention has been paid to genetic changes in mitochondrial DNA (mtDNA) (Mandavilli *et al.,* 2002).