MOLECULAR ASPECTS OF BREAST CANCER

Thesis

Submitted For Partial Fulfilment Of Ph.D Degree In Biochemistry

By

RANDA ALI LABIB IBRAHIM

M.D.,M.Sc.Biochemistry

612.015
R.A

59306

Supervisors

Prof. Fawzia Khalil
Professor of Biochemistry
Head of Biochemistry
Department
Faculty of Medicine
Ain Shams University

Prof. Ali Khalifa
Prof.of Biochemistry
Head of Oncology
Diagnostic Unit
Faculty of Medicine
Ain Shams University

Prof. Nahed Swelam
Prof.of Biochemistry
Faculty of Medicine
Ain Shams University

Prof.Abd El-Meguid El-Shinnawy
Prof.of Surgery
Head of Breast Unit
Faculty of Medicine
Ain Shams University

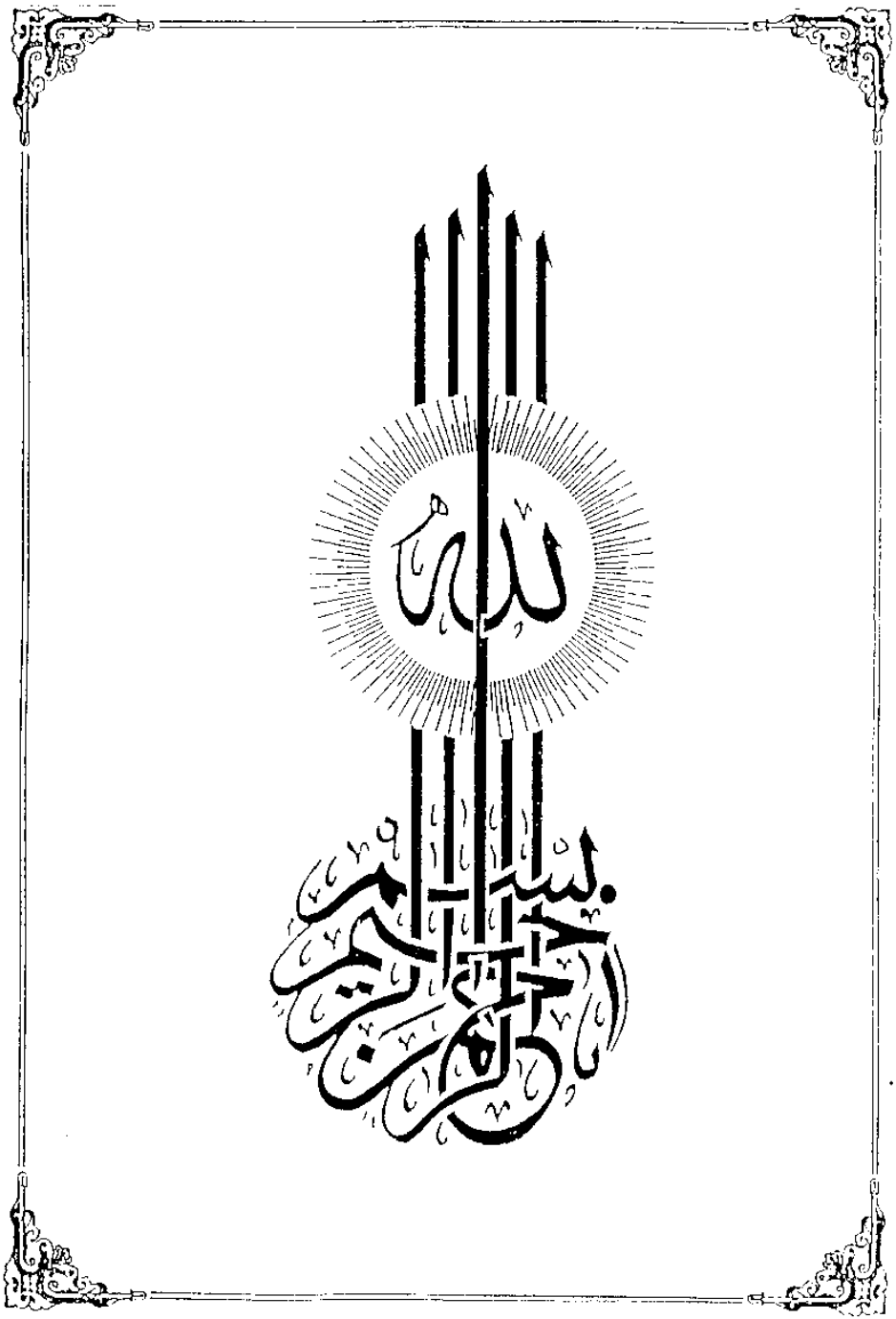
Dr.Sanaa Eissa
Ass.Prof. of Biochemistry
Faculty of Medicine
Ain Shams University

Biochemistry Department
Faculty of Medicine
Ain Shams University

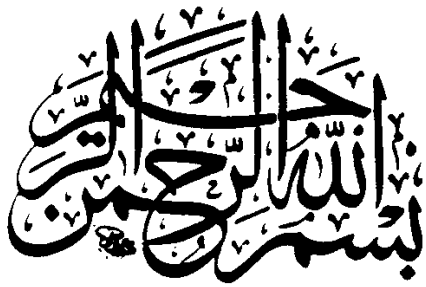
1998

Central Library - Ain Shams University

Central Library - Ain Shams University

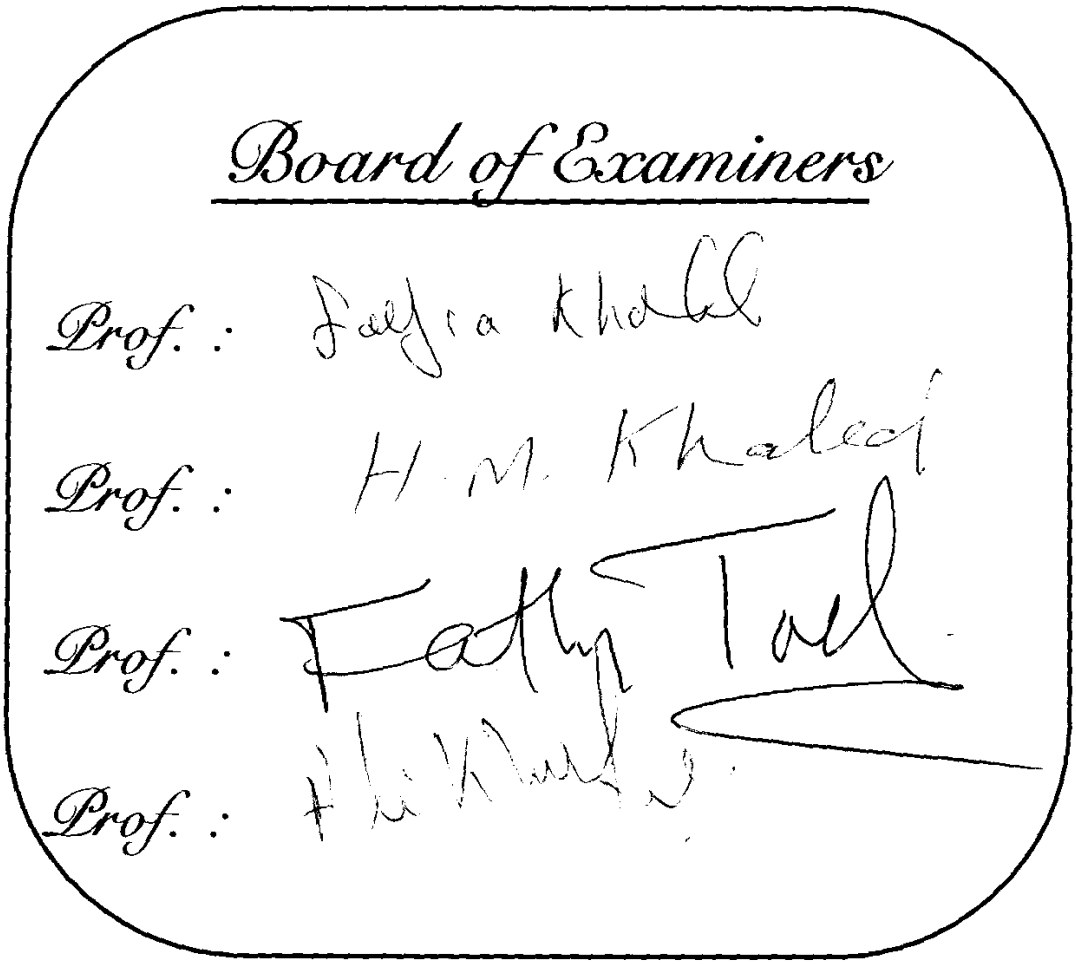


Central Library - Ain Shams University



Central Library - Ain Shams University

Central Library - Ain Shams University



Central Library - Ain Shams University

Central Library - Ain Shams University

Dedicated
To
My Parents, Husband
And
Daughters
(Alaa, Nada & Ola)

Central Library - Ain Shams University

Central Library - Ain Shams University

ACKNOWLEDGEMENT

First and foremost thanks to ***God***

I would like to extend, cordial appreciation and infinite gratitude to ***Prof. Dr. Fawzia Khalil*** *, Professor and Head of Biochemistry Department ,Faculty of Medicine ,Ain Shams University,for her motherly guidance and advice. It would have been impossible to achieve this work without her continuous support along the entire course of the study.*

My words fail me to express my deepest thanks and gratitude to ***Prof. Dr. Ali khalifa*** *, Professor and Head of Oncology Diagnostic Unit , Biochemistry Department, Faculty of Medicine,Ain Shams University , for his great help,valuable criticism and continuous encouragement throughout the whole research . I am very much privileged and honored to have him as my supervisor.*

I am also deeply indebted to ***Prof. Dr. Nahed Swelam ,*** *Professor of Biochemistry , Biochemistry Department , Faculty of Medicine , Ain Shams University, for her valuable cooperation, great interest,encouragement in performing my work, valuable support, sincere directions ,fruitful and profound reading of the manuscript.*

I would like to express my thanks to ***Prof.Dr Abd El-Megid El-Shenawy****, Professor of surgery, Faculty of Medicine,Ain Shams University,for giving me this chance to carry out my work under his supervision. My deepest thanks for his generous help in diagnosis and selection of patients for the present study.*

I have the greatest pleasure in acknowledging ***Dr. Sanaa Eissa,****Assistant Professor of Biochemistry, Faculty of Medicine,Ain Shams University,for her willing assistance,guidence and encouragement during the course of this study. I am indebted to her for teaching me the proper way of thinking,unselfishly giving time and sharing in the objective and critical evaluation of the text.I extend very special thanks to her for making recommendations to improve the work and for cooperating throughout the period of preparation. She carefully read and reviewed every part of the manuscript making many excellent suggestions and corrections.*

Central Library - Ain Shams University

I am very much obliged to the kindness and great help of ***Dr. Samer Kamal****, Lecturer of Biochemistry , Faculty of Medicine , Ain Shams University , for her scientific guidance , constructive effort and valuable assistance in this work.*

I am so grateful to ***Dr.Lila Saeda*** *,Lecturer of Pathology , Faculty of Medicine ,Banha University for her kind assistance and for giving me the chance to learn recent techniques applied in this work..*

Finally, I would like to express my deepest gratitude and indebtedness to all staff members and colleagues in the Biochemistry Department, Faculty of Medicine, Ain Shams University. I offer my thanks and gratitude to those who generously devoted much effort and time in helping me to accomplish this work.

Central Library - Ain Shams University

CONTENTS

	Page
• List of Tables.	i
• List of Figures.	ii
• List of Abbreviations.	v
• Introduction & Aim of Work.	1
• Review of Literature.	4
-Epidemiology of breast cancer.	4
-Risk factors of breast cancer.	4
-Classification of breast cancer.	6
-Pathological classification of breast cancer.	8
-Histological classification of breast cancer.	10
-Grading of breast carcinoma.	14
-Benign breast lesions.	15
-Molecular genetics of cancer.	17
-Prognostic factors in breast cancer.	21
-Apoptosis (Programmed cell death).	23
-Apoptosis in pathogensis of disease.	29
-The molecular regulation of apoptosis.	35
* The Bcl-2.	37
* The p53.	45
* The FAS death factor.	47
* ICE family of cysteine proteases.	50
-BCL-2 in human breast cancers.	51
-Apoptosis and oncogenesis.	55
-Apoptosis as a goal of cancer therapy.	58
-Steroid hormone receptors.	60
-Flow cytometry (FCM).	65
*Application of FCM.	68
*FCM and DNA parameters.	69
*Methodology of DNA analysis by FCM.	75
*Flow cytometry and breast cancer.	76
• Material & Methods.	79
-Patients.	79
-Tissue samples.	79
-DNA analysis by FCM.	81

Central Library - Ain Shams University

Page
-Subcellular fractionation. 87
-Preparation of cell lysate. 88
-Estimation of protein concentration in the different fractions. 90
-Nuclear extraction. 93
-Estimation of mutant p53 in nuclear extract. 94
-Estimation of ER in cytosolic fraction. 99
-Dot immunoblotting for Bcl-2 protein. 104
-SDS-polyacrylamide gel electrophoresis. 107
-Western blotting. 111
-Quantitative estimation of bcl-2 protein in lysate fraction. 123
-Detection of Bcl-2 gene translocation by PCR. 128
-Immunohistochemical localization of BCL-2 protein. 136
-Statistical analysis. 140
• Results. 142
• Disscusion. 194
• Summary & Conclusions. 211
• References. 215
• Appendix.
• Arabic summary.

Central Library - Ain Shams University

LIST OF TABLES

		Page
Table(1)	DNA ploidy in human cells.	73
Table(2)	Clinical and histopathological findings of benign and malignant groups.	143
Table(3)	DNA ploidy in benign and malignant breast tumors.	144
Table(4)	DNA ploidy in relation to clinicopathological factors in breast carcinoma.	149
Table(5)	Synthetic phase fraction (SPF) in benign and malignant breast tumors.	151
Table(6)	Synthetic Phase Fraction(SPF) in relation to clinicopathological factors in breast carcinoma.	154
Table(7)	Sensitivity ,specificity and predictive values at different cutoff values for bcl-2 for discrimination between benign and malignant breast tumors.	157
Table(8)	Bcl-2 expression in benign and malignant breast tumors.	158
Table(9)	Bcl-2 expression in relation to clinicopathological factors in breast carcinoma.	161
Table(10)	ER expression in benign and malignant breast tumors.	173
Table(11)	ER expression in relation to clinicopathological factors in breast carcinoma.	176
Table(12)	Sensitivity ,specificity and predictive values at different cutoff values for mutant p53 for discrimination between benign and malignant breast tumors.	179
Table(13)	Mutant p53 expression in benign and malignant breast tumors.	180
Table(14)	Mutant p53 expression in relation to clinicopathological factors in breast carcinoma.	183
Table(15)	Correlation of bcl-2 with investigated molecular genetic parameters in malignant breast tumors.	186
Table(16)	Correlation of DNA ploidy with mean rank of investigated molecular genetic parameters in malignant breast tumors.	190
Table(17)	DNA ploidy in relation to different investigated molecular genetic parameters (positivity rate) in breast carcinoma.	192

Central Library - Ain Shams University

Central Library - Ain Shams University