CORRELATION BETWEEN DNA PLOIDY AND PS2 IN BREAST CANCER

Thesis

Submitted For Partial Fulfillment Of

Ph.D. Degree In Biochemistry

By

Maha Imam Ahmed

M.Sc. Biochemistry

616.99249 H. I

Supervisors

Prof. Nawal Abdou Zeiada

Professor and Head of

Biochemistry Department

Faculty of Medicine

Ain Shams University

Prof. Ali Khalifa Ali

Professor of Biochemistry

Head of Oncology

Diagnostic Unit

Faculty of Medicine

Ain Shams University

Dr. Thanaa El-Sayed Helal

Ass. Professor of Pathology

Faculty of Medicine

Ain Shams University

Dr. Sanaa Eissa Hamed

Lecturer of Biochemistry

Faculty of Medicine

Ain Shams University

Faculty of Medicine

Ain Shams University

1994

فَالُوالْمِرْرُورُ وَالْمُعَاعَلَمْ اللَّهُ اللَّهُ الْكَالَةُ الْحَامَةُ الْحَمَةُ الْحَامَةُ الْحَمَةُ الْحَامَةُ الْحَامِةُ الْحَامَةُ الْحَامَةُ الْحَامَةُ الْحَامَةُ الْحَامَةُ الْحَامَةُ الْحَامَةُ الْحَامَةُ الْحَامِةُ الْحَامِةُ الْحَامِةُ الْحَامِةُ الْحَامِةُ الْحَامِةُ الْحَامِةُ الْحَامَةُ الْحَامِةُ الْحَ

المنظمة العظمة

سورة البقية آبر ۲۲



Approval Sheet

Prof. Dr. Ahmad S. Fayth-Allah

Prof. Dr. Nawal A. Zeyada

Prof. Dr. Mofida M. Salah

Prof. Dr. Ali Khalifa Ali

Dr. Nawal Brade

ACKNOWLEDGEMENTS

First and foremost thanks to God.

I would like to extend, cordial appreciation and infinite gratitude to Prof. Nawal Abdou Zeiada Professor and Head of Biochemistry Department, Ain Shams Faculty of Medicine, for her motherly guidance and advice. She spared no effort to help me along the entire course of the work.

My words fail me to express my deepest appreciation to Prof. Ali Khalifa, Professor and Head of Oncology Diagnostic Unit, Biochemistry Department, Ain Shams Faculty of Medicine. He has been, as he always is, most helpful to me throughout the whole work. His continual watchfullness, his invaluable counsel and most creative and instructive ideas and thoughts have motivated my energy. His endurance has pushed me ahead, and have endowed me with hope, patience and perseverance.

I am deeply grateful to my tutor Dr. Sanaa Eissa, Lecturer of Biochemistry, Ain Shams Faculty of Medicine. She gave time unselfishly and shared in the objective and critical evaluation of the text. I extend very special thanks to her for sharing ideas and 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.

I am so grateful to Dr. Thanaa Helal, Assistant Professor of Pathology, Ain Shams Faculty of Medicine for her scientific guidance and valuable assistance.

I am also very grateful to Dr. Sohir sayed Ismail, Assistant Professor of Radiotherapy, Ain Shams Faculty of Medicine.

Finally, very special thanks are due to a supportive and considerate family, particularly to my husband to my parents and to both my father and mother-in-low.

CONTENTS

| | | Page |
|---|--|------|
| • | Introduction and aim of work | 1 |
| • | Review of literature | 3 |
| | Breast cancer. | 3 |
| | Biology of breast cancer. | 15 |
| | Prognostic factors in breast cancer. | 18 |
| | Estrogen receptors. | 24 |
| | Progesterone receptors. | 29 |
| | PS2 protein. | 33 |
| | Flowcytometry: | 39 |
| | - Clinical FCM instrumentation. | 39 |
| | - Clinical applications of FCM. | 44 |
| | - DNA content as a genetic marker of | |
| | cancer cells. | 52 |
| | - Cell cycle. | 58 |
| | - Methodology of DNA analysis by FCM. | 62 |
| | - FCM and breast cancer. | 70 |
| • | Patients and Methods | 75 |
| | • Patients. | 75 |
| | •• Estimation of cytosolic total proteins. | 79 |

| | Page |
|--------------------------------|------|
| Estimation of cytosolic ER. | 81 |
| . Estimation of cytosolic PGR. | 87 |
| - Estimation of cytosolic PS2. | 90 |
| • DNA analysis by FCM. | 94 |
| •• Statistical analysis. | 103 |
| • Results | 104 |
| · Disscusion. | 159 |
| • Summary and conclusions. | 185 |
| References. | 186 |
| · Appendex. | |
| • Arabic summary. | |

LIST OF TABLES

| | | Page |
|-----|---|------|
| 1: | DNA ploidy in human cells. | 56 |
| 2: | Clinical and Histopathological Findings of Benign | |
| | and Malignant Groups | 76 |
| 3: | Tumor Size in Relation to Other Clinical and | |
| | Histapathological Findings. | 109 |
| 4: | Lymph Node Status in Relation to Other | |
| | Clinical and Histapathological findings. | 113 |
| 5: | Number of Positive Lymph Nodes in Relation to | |
| | Other Clinical and Histapathological Findings. | 115 |
| 6: | Tumor Grade in Relation to Other Clinical and | |
| | Histapathological Findings. | 116 |
| 7: | Clinical Stage in Relation to Other Clinical and | |
| | Histapathological Findings. | 118 |
| 8: | Tumor Type in Relation to Other Clinical and | |
| | Histapathological Findings. | 119 |
| 9: | Mean Values of Investigated Parameters in Benign | |
| | and Malignant Groups. | 120 |
| 10: | The Positivity Rate of Investigated Parameters in | |
| | Benign and Malignant Groups. | 122 |

| | | Page |
|-----|--|------------|
| 11: | Mean Values and Positivity Rate of ER in Relation | 1 |
| | to other Clinical and Histopathological Findings | |
| | (in malignant group). | 124 |
| 12: | Mean Values and Positivity Rate of PGR in Relation | o n |
| | to other Clinical and Histopathological Findings | |
| | in malignant tumors. | 128 |
| 13: | Mean Values and Positivity Rate of PS2 in Relation | n |
| | to other Clinical and Histopathological Findings | |
| | in Malignant Tumors. | 130 |
| 14: | Relation Between DNA Ploidy and Clinical and | |
| | Histopathological Findings in Malignant Tumors. | 141 |
| 15: | Mean Values of DI and Positivity Rate in Relation | 1 |
| | to other Clinical and Histopathological Findings | |
| | in Malignant Tumors | 144 |
| 16: | Mean % SPF and Positivity rate (Cut: 10%,20%) | |
| | in Relation to other Clinical and Histopatho | ological |
| | Findings in Malignant Tumors . | 150 |
| 17: | Correlation Between the Positivity Rate of | |
| | Different Investigated Parameters in | |
| | Malignant Tumors. | 153 |
| 18: | Correlation coefficients of different | |
| | investigated quantitative parameters in | |
| | the malignant group. | 158 |

LIST OF FIGURES

| | | Page |
|------------|---|------|
| 1-a: | Mechanism of action of steroid hormones . | 27 |
| 1-b: | Model of estrogen receptor action. | 27 |
| 2: | Schematic diagram of a flow cytometer. | 40 |
| 3: | Schematic diagram of a flow cell. | 43 |
| 4 : | Schematic diagram of a photomultiplier | |
| | tube. | 43 |
| 5-a: | DNA aneuploid population. | 55 |
| 5-b: | DNA diploid poulation. | 55 |
| 6: | Mathematical methods for SPF | |
| | approximation. | 57 |
| 7: | Relationship between changes in DNA content | |
| | through the cell cycle and the frequency | |
| | distribution curve of | 59 |
| | DNA content. | |
| 8-a: | Comparison of peak and integral pulses. | 71 |
| 8-b: | Histogram gating to exclude doublets. | 71 |
| 9: | Protein standard curve. | 82 |
| 10: | ER standard curve. | 86 |
| 11: | PGR standard curve. | 91 |
| 12: | PS2 standard curve. | 95 |
| 13: | Correlation between tumor size and clinical | |
| | stage. | 110 |
| 14: | Correlation between tumor size and lymph | |
| | node status. | 111 |
| 15: | Correlation between tumor size and relapse. | 112 |
| 16: | Correlation between lymph node status and | |
| | clinical stage. | 114 |

| | | Page |
|-----|---|------|
| 17: | Correlation between tumor size and tumor | J |
| | grade. | 117 |
| 18: | Mean values of investigated parameters | in |
| | benign and malignant groups. | 121 |
| 19: | Positivity rate of investigared parameters | |
| | in benign and malignant groups. | 123 |
| 20: | Correlation between ER status and tumor size. | 125 |
| 21: | Correlation between ER status and | |
| | clinical stage. | 126 |
| 22: | Correlation between ER and recurrence | |
| | in ANP tumors. | 127 |
| 23: | Correlation between PGR and recurrence | |
| | in ANN tumors. | 129 |
| 24: | Correlation between PS2 and tumor size. | 131 |
| 25: | Correlation between PS2 and relapse in | |
| | ANN tumors. | 132 |
| 26: | Correlation between PS2 and relapse in ANP | |
| | tumors. | 133 |
| 27: | Ploidy pattern in benign and malignant | |
| | tumors. | 134 |
| 28: | Ploidy pattern in malignant tumors. | 135 |
| 29: | DNA diploid pattern. | 136 |
| 30: | DNA aneuploid pattern. | 137 |
| 31: | DNA multiploid pattern. | 138 |
| 32: | DNA hypertetraploid pattern. | 139 |
| 33: | DNA tetraploid pattern. | 140 |
| 34: | DI frequency distribution curve in | |
| | malignant tumors. | 142 |
| 35: | DI frequency distribution curve in beni | |
| | tumors. | 143 |
| 36: | Correlation between DI and lymph nod | |
| | status. | 145 |
| 37: | Correlation between DI and histologic grade. | |

| | | Page |
|-------------|--|------|
| 38: | Correlation between DI and relapse in ANP | |
| | tumors. | 147 |
| 39: | Correlation between DNA ploidy and lymph | |
| | node status. | 148 |
| 40 : | Correlation between DNA and No of positive | |
| | lymphnodes. | 149 |
| 41: | Correlation between SPF and tumor size. | 151 |
| 42: | Correlation between ER and PGR. | 152 |
| 43: | Correlation between SPF and ploidy status. | 154 |
| 44: | Correlation between ER and PGR. | 155 |
| 45: | Correlation between ER and PS2. | 156 |
| 46: | Correlation between PGR and PS2. | 157 |

LIST OF ABBREVIATIONS

AJCC : American joint committe for cancer.

ANN : Axillary node negative.

ANP : Axillary node positive.

AT : Adenine thymine.

BCEI : Breast cancer estrogen induced gene.

Cath.D : Cathepsin D.

C-erb-B2 : Oncogene from erythroblastosis virus.

CV : Coeffecient of variation.

DCIS : Ductal carcinoma in situ.

DFS : Disease free survival.

DI : DNA index.

DNA : Deoxy ribonucleic acid.

EGFR : Epidermal growth factor receptor.

EIA : Enzyme-immunoassay.

ER+ : Estrogen receptor positive.

FCM: Flowcytometry.

GC: Guanine cytosine.

HER2 : Human epidermal growth factor

receptor related gene.

HIV: Human immundefeciency virus.

HSP: Heat shock protein.

ICA: Immunocytochemical assay.

IDC: Invasive duct carcinoma.

IGFs : Insulin growth factors.

ILC: Invasive lobular carcinomas.

LCIS: Lobular carcinoma in situ.

LPR : Lysing permealizing reagant.

MAb : Monoclonal antibodies.

neu : Neuroblastoma oncogene.

nm 23 : nonmetastatic 23 gene.

OS : Overall survival.

p53 : Protein 53.

PCNA: Proliferating cell nuclear antigen.

PDGF: Platelet derived growth factor.

PGR+ : Progesterone receptor positive.

PMTs : Photomultiplier tubes.

PS2 : Protein S2.

RB: Retinoblastoma gene.

RBCs : Red blood cells.

RIA: Radioimmunoassay.

RNA: Ribonucleic acid.

SPF: Synthetic phase fraction.

 $TGF-\alpha$: Transforming growth factor-alpha.

TGF-β: Transforming growth factor-beta.

TNM: T (tumor), N (lumph node), M (distant

metastases).

+,+ve : Positive.

-,-ve : Negative.