The Role of Some Biomarkers in Early Detection of Bone Metastasis in Egyptian Breast Cancer Patients

Thesis Submitted by

May Malak M. Tadros

(B.Sc. in Biochemistry, 2008)

In Partial Fulfillment of the Degree of Master of Science in Biochemistry

Supervised by

Prof. Dr. Nadia Y. S. Morcos

Prof. of Biochemistry Biochemistry Department Faculty of Science Ain Shams University

Prof. Dr. Nadia I. Zakhary

Minister of Scientific Research Professor of Medical Biochemistry Cancer Biology Department National Cancer Institute Cairo University

Dr. Mahmoud M. Said Abd El-Hamid

Lecturer of Biochemistry Biochemistry Department Faculty of Science Ain Shams University

Ain Shams University Faculty of Science Biochemistry Department

Declaration

This thesis has not been submitted for a degree at this or any other university

May Malak M. Tadros

Dedication

To my father, my mother, my brother, my fiancé

<u>1</u>

My real friends
Their love, encourage, help and prayers
made studies possible and to them I owe
everything.

Acknowledgement

I would like to thank from all of my heart and from my deep soul *Dr. Nadia Y. S. Morcos*, Professor of Biochemistry, Faculty of Science, Ain Shams University, to whom I am so grateful for her endless help, motherly attitude, creative thinking, valuable suggestions and constant advice during this work. Without her support, the performance of this work would be difficult.

It is really difficult for me to find words that can express my deep feelings and sincere appreciation towards *Dr. Nadia I. Zakhary*, Minster of Scientific Research, Professor of Medical Biochemistry, Cancer Biology Department, National Cancer Institute, Cairo University, for suggesting the point, instructive guidance, tremendous concern and care, and invaluable assistance. It is a great honor to me to work under her supervision.

I am so grateful to *Dr. Mahmoud M. Said Abd El-Hamid*, Lecturer of Biochemistry, Biochemistry Department, Faculty of Science, Ain Shams University for his spiritual and practical guidance, his enthusiastic encouragement and revising every detail, as well as profound reading of the manuscript.

Thanks are also due to *Dr. Ola Mohamed Reda Khorshid*, Assistant Professor of Medical Oncology, National Cancer Institute, Cairo University, for her cooperation and kind help in samples collection.

My deep thanks and regards are also due to the staff members of the Biochemistry Department, Faculty of Science, Ain Shams University and Cancer Biology Department, National Cancer Institute, Cairo University for their support and help.

May Malak

Biography

Name: May Malak Matter Tadros

Date and place of birth: 14/5/1987, Cairo, Egypt

Date of Graduation: 2008

Degree Awarded: B.Sc. in Biochemistry, 2008

Grade: Excellent with Honor Degree

Contents

Abstract			
List of Tables			
List of FiguresList of Abbreviations			
Intro	duction		
Aim	of Work		
	oter I: Review of Literature		
	Introduction		
	The Hallmarks of Cancer		
1.3.	The Breast: Normal Architecture		
1.4.	Benign Breast Tumors		
1.5.	Breast Cancer		
	1.5.1. Types of breast cancer		
	1.5.2. Molecular classification of breast cancers		
	1.5.3. Staging of breast cancer		
	1.5.4. Breast cancer grade		
1.6.	Etiology of Breast Cancer		
1.7.	Connection between Estrogen and Obesity,		
	Inflammation and Postmenopausal Breast Cancer		
1.8.	Breast Cancer at the Molecular Level		
	1.8.1.Breast cancer type 1 susceptibility protein		
	(BRACA1)		
	1.8.2. Human epidermal growth factor receptor 2		
	1.8.3. Estrogen receptor		
1.9.	Breast Cancer Progression and Metastasis		
	1.9.1. The steps of the metastatic cascade		
	1.9.2. Models of metastasis		
1.10.	Breast Cancer Bone Metastasis		
	1.10.1. Bone structure		
	1.10.2. Bone resorption and formation		
	1.10.3. Breast cancer bone metastasis		
	1.10.4. Angiogenesis and osteoclastogenesis		
	1 10 5 The Tumor microenvironmental		

1.11.	Biomarkers	58
	1.11.1. Tumor markers in breast cancer	
	1.11.2. Inflammation: monocytes, macrophages	61
	and white blood cells	64
	1.11.3. Angiogenesis markers: vascular	
	endothelial growth factor (VEGF)	67
	1.11.4. <i>Zinc</i>	70
	1.11.5. Biochemical bone markers	74
1.12.		74
1.13.		
Char	ter II: Subjects and Methods	80
2.1.	Subjects	80
	2.1.1. <i>Diagnosis</i>	82
	2.1.2. <i>Plan of the work</i>	83
2.2.	Methods	83
	2.2.1. Blood collection & sampling	84
	2.2.2. Biochemical analysis	102
2.3.	Statistical Analysis & Equation Used	104
Char	oter III: Results	139
Chap	oter IV: Discussion	151
Conclusion & Recommendation		152
Summary		156
	rences	
	ic Abstract	
Arah	ic Summary	

The Role of Some Biomarkers in Early Detection of Bone Metastasis in Egyptian Breast Cancer Patients

May Malak M. Tadros Faculty of Science, Ain Shams University

Abstract

The present study was undertaken to identify postoperative simple biochemical markers for prediction of bone metastases in Egyptian breast cancer patients. Seventy eight cases with breast cancer (BC) after mastectomy were included. From these patients, 46 had no bone metastasis (NBM) and 32 with radiologically confirmed bone metastasis (BM). Patients with NBM were further observed for all year by bone scan in order to monitor development of bone metastasis (New BM). Nine healthy women with no history of breast disease or metabolic bone disease were included for reference ranges. Parameters included full blood picture, blood tumor markers (carcinoembryonic antigen [CEA] and cancer antigen [CA 15.3]), breast tissue receptor markers (estrogen receptor [ER], progesterone receptor [PR] and human epidermal growth factor receptor 2 [HER-2]), together with blood biochemical markers (tartrate-resistant acid phosphatase [TRAP5b], vascular endothelial growth factor [VEGF], alkaline phosphatase [ALP] and zinc). Analyses significantly elevated CEA, ALP, showed and the inflammation markers: ALP/monocytes% and platelet²/(monocytes%+segmented neutrophils%) (P2ms) at the time of primary diagnosis in patients with BM, compared to those without BM. Elevated CA 15.3, P2ms, VEGF and lower monocytes% were independently associated with the development of New BM (4 patients). The increase in TRAP activity was related to progesterone receptor expression in breast cancer tissues. In conclusion, this study provides evidence that circulating markers of cancer; CA 15.3, vascularization (VEFG/monocytes %) and inflammation (P2ms) markers have the highest prognostic value for predicting development of BM within one year in breast carcinoma patients.

List of Tables

No.	Title	Page
1.1.	Types of breast cancers.	11
1.2.	Definition of breast cancer stages according to the National Cancer Institute.	15
1.3.	Established risk factors for breast cancer.	22
1.4.	Characteristics of <i>BRCA1</i> -and <i>BRCA2</i> -mutation associated breast cancers.	28
1.5.		68
1.6.	Markers of bone resorption and formation.	70
2.1.	TNM classification of breast cancer patients.	82
3.1.	Baseline characteristics of the 78 breast cancer patients included in the study.	105
3.2.	Blood picture of the three groups of breast cancer patients.	106
3.3.	Other metastasis status of breast cancer patients with and without bone metastasis at diagnosis.	107
3.3a.	Level of some biochemical markers at the time of diagnosis in breast cancer patients, showing the healthy reference ranges.	108
3.3b.	Level of some biochemical markers at the time of diagnosis of breast cancer patients who remained free, developed or had bone metastases after al year follow up.	109
3.4a.	Levels of biochemical markers in breast cancer patients (Initial groups).	117
3.4b.	Levels of calculated biochemical markers at the time of diagnosis of breast cancer patients who remained free, developed or had bone metastases after al year follow up.	118

Diagnostic value of biochemical markers 3.5. distinguish breast carcinoma patients with bone (All) from those without bone 129 metastases involvement (Free BM). Crosstabulation showing the reliability of markers 3.6. 131 for bone metastasis. 3.7. Crosstabulation showing the reliability of markers 132 in predicting bone metastasis within one year. Crosstabulation showing reliability 3.8. the calculated markers in predicting bone metastasis 133 within one year. showing 3.9. Crosstabulation the reliability calculated markers in predicting bone metastasis 134 after one year.

List of Figures

No.	Title	Page
1.1.	Incidence rate of BC around the world.	2
1.2.	Estimated age-standardized incidence and mortality rates: women in Egypt.	2
1.3.	Hallmarks of cancer.	4
1.4.	A simplified diagram of breast anatomy.	5
1.5.	A simplified diagram of breast anatomy showing benign tumors.	6
1.6.	Major types of breast cancer.	10
1.7.	The major stages of breast cancer.	16
1.8.	Risk factors for breast cancer.	19
1.9.	Different mechanisms of estrogen dependence for hormone-related breast cancer in pre- and postmenopausal women.	24
1.10.	Adipocyte is a non-trivial, dynamic partner of breast cancer cells.	25
1.11.	Suggested roles of BRCA1 and BRCA2 in DNA repair.	27
1.12.	Potential consequences of HER2 dysregulation.	30
1.13.	HER proteins.	30
1.14.	Scheme illustrating the action of the three main groups operating in breast cancer ER, ErbB family, and kinases.	32
1.15.	Cross-talk between signal transduction and	
	endocrine pathways.	33
1.16.	Four different pathways of ER action.	33
1.17.	The molecular mechanism of gene regulation by sex steroid hormones.	35

1.18.	Cancer progression.	37
1.19.	The steps in the metastatic cascade (cellular level).	38
1.20.	Models proposed to explain the biological complexities of metastasis.	39
1.20.	Models of metastasis.	40
1.21.	Bone structure.	43
1.22.	The normal bone remodeling.	45
1.23.	The steps involved in tumor-cell metastasis from a primary site to the skeleton.	47
1.24.	Metastatic breast cancer cells in the bone at the molecular level.	48
1.25.	Schematic of tumor-bone marrow microenvironment interactions.	48
1.26.	Stromal cells involved in metastatic cascade.	52
1.27.	Contribution of EMT to cancer progression.	53
1.28.	EMT promotes metastasis by enhancing local invasion.	54
1.29.	Tumor dormancy as a component of cancer progression.	56
1.30.	Crosstalk between the microenvironment and cellular dormancy.	57
1.31.	Monocytes/macrophages support malignant progression of cancer cells.	62
1.32.	Pro-tumor functions of tumor-associated macrophages (TAM).	63
1.33.	Spatio-temporal dynamics of VEGF-A distribution in the blood.	65

1.34.	Oxygen radicals and oxygen insufficiency	
	(hypoxia) co-operatively promote tumor angiogenesis.	66
1.35.	Roles of Zn transporter family members (ZIP and ZnT) in intracellular signaling and cell functions.	69
1.36.	Two prominent hypothetical models for the function of TRACP in osteoclast (OC) bone resorption.	73
1.37.	Therapeutic targeting of the hallmarks of cancer.	76
1.38.	Therapeutic tactics.	77
2.1.	Plan of the work.	81
2.2.	Calibration curve of TRACP5b.	88
2.3.	Calibration curve of VEGF.	93
3.1.	Box-plots of CA 15.3 level in patients with breast cancer.	110
3.2.	Box-plots of CEA level in patients with breast cancer.	111
3.3.	Box-plots of ALP level in patients with breast cancer without, or with bone metastasis.	112
3.4.	Box-plots of TRAP level in patients with breast cancer without, or with bone metastasis.	113
3.5.	Box-plots of VEGF level in patients with breast cancer without, or with bone metastasis.	114
3.6.	Box-plots of zinc level in patients with breast cancer without, or with bone metastasis.	115
3.7.	Box-plots of monocyte % (monocytes/WBCs %) level in patients.	119