Human Epidermal Growth Factor Receptor (HER-2/neu) oncoprotein in Breast Cancer

Thesis

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بسم الله الرحمن الرحيم

﴿قَالُوا سُبْحَانَكَ لا
عِلْمَ لَنَا إِلاَّ مَا
عَلَّمْتَنَا إِنَّكَ أَنْتَ
العَلِيمُ الحَكِيمُ﴾

صدق الله العظيم

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Abstract:

Breast cancer is the most common malignancy in women. Human epidermal growth factor receptor (HER-2/neu) is an important prognostic indicator in breast cancer. This study was performed on 49 breast cancer patients, 16 benign breast lesions and 15 age matched normal controls. Serum HER-2/neu was measured by Immunochemiluminescence to correlate with its status by Immunohistochemistry (IHC), pathological grade, estrogen and progesterone receptor status of tumors. Serum HER-2/neu was significantly increased in patients who had +ve IHC when compared to those who were –ve. Serum HER-2/neu should complement IHC, but whether or not it can substitute it, is still a subject of study.

Keywords: breast cancer, HER-2/neu, immunochemiluminescence .

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LIST OF ABBREVIATIONS

ALN	**Axillary lymph nodes**
BCC	**Breast cancer cells**
BM	**Bone marrow**
CA 15.3	**Cancer antigen 15.3**
CEA	**Carcino embryonic antigen**
CISH	**Chromogenic in situ hybridization**
CTCs	**Circulating tumor cells**
DCIS	**Ductal carcinoma in situ**
DFS	**Disease free survival**
DTCs	**Disseminated tumor cells**
ECD	**Extracellular domain**
EGF	**Epidermal growth factor**
EGFR	**Epidermal growth factor receptor**
ER	**Estrogen receptor**
FFTP	**First full term pregnancy**
FGF	**Fibroblast growth factor**
FISH	**Fluorescence in situ hybridization**
HRT	**Human replacement therapy**
IBC	**Inflammatory breast cancer**
IGF-1	**Insulin like growth factor-1**
IGF-2	**Insulin like growth factor-2**
IHC	**Immunohistochemistry**
LN	**Lymph node**
MBC	**Metastatic breast cancer**
PB	**Peripheral blood**
PCR	**Polymerase chain reaction**
PR	**Progesterone receptor**
PTEN	**Phosphatase and tensim homolog deleted on chromosome ten**
q PCR	**Quantitative PCR**
RTKs	**Receptor tyrosine kinases**
TDLU	**Terminal duct lobular unit**
TGF-α	**Transforming growth factor alpha**
TGF- β	**Transforming growth factor beta**

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INTRODUCTION AND AIM OF THE WORK

Breast cancer is the most commonly occurring cancer among women ***(Herbst et al., 2006)***. It is second to lung cancer as a cause of cancer death (15% of cancer deaths) and is the first among cancers for causing early death ***(Mirtunen at al., 2003)***.The death rate for cancer has not significantly declined over the last 50 years ***(Leaf 2004)***. Moreover, the incidence of many cancers, including breast cancer, is increasing. The probability for a woman to develop breast cancer in Western countries is higher than 0.13% ***(Jemal et al., 2005)***.

In Egypt, breast cancer is the commonest cancer among women, representing 18.9% of total cancer cases and constitutes 29% of National Cancer Institute cases ***(Omar et al., 2003)***.

An increasing number of women are subjected to routine annual screening with clinical examination and digital mammograms, which have ultimately resulted in dramatic increase in early detection rate ***(Bieche et al., 2004)***.

For more than 20 years, the only tumor markers used routinely in making treatment decisions in breast cancer have been the estrogen-receptor (ER) and progesterone receptor (PR) status to predict response to hormone therapy ***(Osborne et al., 2005)***.

Serum tumor markers have the potential of being incorporated into diagnostic and therapeutic practice in breast cancer. Potential usages of the markers include screening, differentiation of benign from malignant disease, histological differentiation, and defining prognosis. These goals have generated considerable interests in identifying predictive tumor markers over the past three decades ***(Chapman et al., 2007)***.

HER-2/neu is a transmembrane tyrosine kinase growth receptor protein that mediates the growth, differentiation, and survival of cells ***(Yarden et al., 2001,*** and ***Gschwind et al., 2004)***. It is encoded by a proto-oncogene located on chromosome 17q21. It is a member in the epidermal growth factor receptor (EGFR) family ***(Carlsson et al., 2004)***. The overexpression rate of HER-2/neu oncoprotein has been identified in 10% to 40% of human breast cancers ***(Rubin et al., 2001)***. The role of HER-2/neu as an important predictor of patient outcome and response to various therapies in breast cancer has been clearly established ***(DiGiovanna et al., 2005)***.

Patients with HER-2/neu overexpressing breast tumors have an increased incidence of metastasis and a poorer survival rate when compared with patients whose tumors express HER-2/neu at normal levels. It is also associated with resistance to endocrine therapy and adjuvant chemotherapy in those cases ***(Konecny et al., 2003)***.

Moreover, it is an entry criterion in the assessment of patients with advanced breast cancer who may benefit from the therapy with anti-HER-2/neu antibody Trastuzumab/Herceptin® (Genetech, San Francisco, CA) a humanized murine monoclonal antibody which has been shown to be

effective as an adjuvant therapy in patients overexpressing HER-2/neu ***(Viani et al., 2007)***.

Elevated serum HER-2/neu levels are associated with HER-2/neu overexpression and amplification in breast cancer tissue. Nevertheless, discordant results between serum and tissue can be obtained in a small subset of patients ***(Baselga, 2002)***.

Therefore, assessment of HER-2/neu status is crucial for management of breast cancer patients, and several methods have been proposed. No single assay has been universally accepted as the "gold standard" for HER-2/neu status. Methods such as immunohistochemistry (IHC), Western blotting, and ELISA can be used to measure HER-2/neu protein concentrations in the tumor or in serum. Southern blotting, fluorescence *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH), and quantitative PCR (qPCR) can detect gene amplification in the tumor ***(Kakar et al., 2000)***.

Measurement of circulating HER-2/neu extracellular domain in serum (serum HER-2/neu) has been shown to be useful for assessing the prognosis and for predicting the response to trastuzumab ***(Köstler et al., 2004)***.

AIM OF THE WORK

The objective of the present work is to study serum HER-2/neu level in malignant breast cancer, and benign breast lesions versus normal controls, so as to be correlated with different risk factors of breast cancer. Moreover, correlation between serum HER-2/neu oncoprotein and HER-2/neu status in tissue, determined by immunohistochemistry (IHC), histopathological grade, as well as estrogen and progesterone receptors status of the surgically removed breast lesions, will also be done in Egyptian breast cancer patients.

CHAPTER I

BREAST CANCER

Epidemiology of breast cancer

Breast cancer is the most commonly occurring cancer among women ***(Herbst et al., 2006)***. It is second to lung cancer as a cause of cancer death (15% of cancer deaths) and is the first among cancers for causing early death ***(Mirtunen at al., 2003)***.The death rate for cancer has not significantly declined over the last 50 years ***(Leaf 2004)***. Moreover, the incidence of many cancers, including breast cancer, is increasing. The probability for a woman to develop breast cancer in Western countries is higher than 0.13% ***(Jemal et al., 2005)***.

In Egypt, breast cancer is the commonest cancer among women, representing 18.9% of total cancer cases and constitutes 29% of National Cancer Institute cases. Breast cancer in Egyptian patients has a younger age distribution with the majority of cases occurring at 30-60 years of age. The median age is 46 years; one decade younger than the corresponding age in Europe and North America ***(Omar et al., 2003)***.

In most cases, death results from the dissemination of cancer cells and their proliferation at secondary sites, underlining the importance of controlling and preventing these events. An increasing number of women are subjected to routine annual screening with clinical examination and digital mammograms, which have ultimately resulted in dramatic increase in early detection rate ***(Bieche et al., 2004)***.

An overview of mammary gland development

General structure of the mammary gland

The mammary gland is composed of an organized ductal network. Embedded within the stroma, the branching duct system leads from the collecting ducts via the segmental and subsegmental ducts to the terminal duct lobular units (TDLUs). Two cell types compose the epithelium of the duct and lobule system, namely luminal (secretory) cells and myoepithelial cells. The myoepithelial cell layer is found between the luminal epithelial cell layer and the basement membrane. The acini that compose the TDLU are spheroid structures with a central lumen surrounded by a layer of polarized epithelial cells ***(Stingl et al., 2005*** and ***Nelson et al., 2005)***.Figure 1

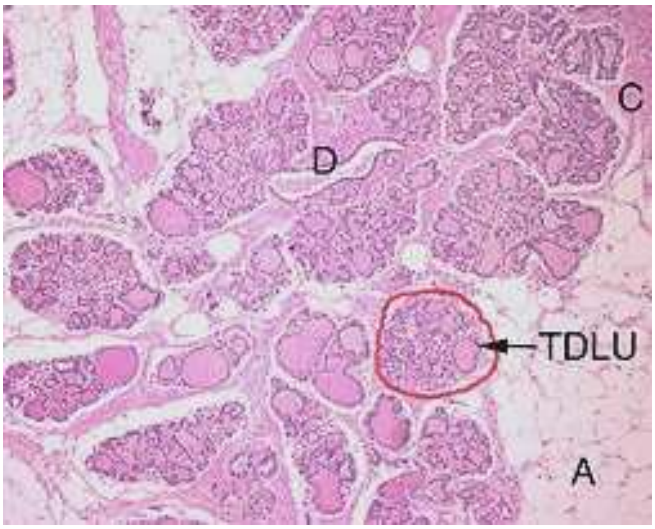

Figure 1: Histology of the mammary gland

Mammary development and cancer

The mammary gland is a cellular ecosystem in which each cell type is a subject to constant turnover. This is particularly the case for the

epithelial cells, which are subjected to various hormones and growth factors stimulation throughout their life, with correlative changes in morphology and metabolism. Failure of normal mechanisms of proliferation and/or failure of apoptosis result in breast cancer. Most of breast tumors are epithelial in origin, and therefore the large majority of malignant breast tumors are classified as carcinomas ***(Hondermarck, 2003)***.

Breast cancer has been hypothesized to develop through a linear histological progression from hyperplasia and *in situ* carcinoma to invasive cancer ***(Arpino et al., 2005)***. It has been suggested that this process is accompanied by increasing genomic instability, among other hallmarks of cancer ***(Hanahan et al., 2000)***. The genomic events identified in hyperplasias and *in situ* carcinomas may be causative for the development of premalignant lesions, thus triggering or disrupting the downstream events that lead to disease progression ***(Mastracci et al., 2006)***.

Almost all mammary carcinomas develop within the TDLU ***(Stingl et al., 2005)***. The epithelial cells that line the acini have polarity markers e.g. epithelial (E)-cadherin and αβ-integrins. Blocking E-cadherin causes selective disorganization of the luminal cells. Following epithelial cell polarization, apoptotic signals counter the proliferative signals produced by the cells residing in the luminal space, allowing for hollowing of the acini in a process known as luminal morphogenesis ***(Debnath et al., 2003*** and ***Debnath et al., 2005)***.

Mammary gland microenvironment

The stroma within the mammary gland is composed of adipocytes, fibroblasts, inflammatory cells, blood vessels, and extracellular matrix. Many stromal factors that are essential to mammary gland development have also been found to be associated with cancer. For example, the