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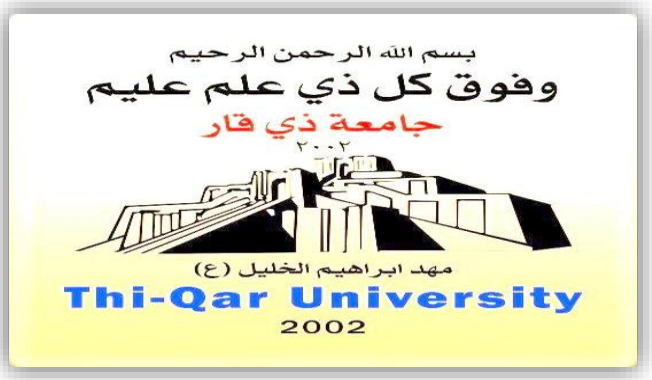

Expression of Estrogen, Progesterone and Human epidermal growth factor Receptors in Breast Cancer in Al-Nasiriya 2014-2015

A Dissertation

Submitted to the college of medicine and committee of postgraduate studies of the University of Thi-qar, in partial fulfillment of the requirements for the degree of Higher Diploma in family-community medicine

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بسم الله الرحمن الرحيم

إِنَّمَا يَخْشَى ٱللَّهَ مِنْ عِبَادِهِ ٱلْعُلَمٰٓاء إِنَّ ٱللَّهَ عَزِيزٌ غَفُورٌ

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we, certify that this thesis was prepared under our supervision at the College of Medicine, Thi-qar University, as a partial fulfillment of the requirement for the degree of Diploma of family medicine.

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In view of available recommendation, I Forward this dissertation for debate by the examining committee.

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We, the examining committee, after reading this dissertation and examining the student in its content, certify that it is adequate for the degree of Higher Diploma in Family- Community Medicine.

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Dedication

To my parents and my family

Acknowledgment

First of all dear GOD thanks for all the blessings which lightened my way in life.

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List of abbreviations

BRCA1	**Breast Cancer1**
BRCA2	**Breast Cancer2**
DCIS	**Ductal Carcinoma In Situ**
ER	**Estrogen Receptor**
FISH	**Fluorescent in situ hybridization**
HER2	**Human epidermal growth factor receptor 2**
IHC	**Immunohistochemistry**
ITC	**Isolated Tumor Cells**
LCIS	**Lobular Carcinoma In Situ**
MRI	**Magnetic Resonance Imaging**
PAI-1	**plasminogen activator inhibitor-1**
PR	**Progesterone Receptor**
SLN	**Sentinel Lymph Node**
TSGs	**tumor suppressor genes**
u-PA	**urokinase plasminogen activator**

ABSTRACT

Background:

Breast cancer is the most common malignancy and is real general well-being issue for ladies through the world and in Iraq**.** Breast cancer is an extremely heterogeneous disease, There are three predictive markers: estrogen receptors, progesterone receptors and Her2-neu receptors have independent prognostic value in breast cancer. Estrogen receptors expression appears in 80-90 % of patients with breast cancer, while Progesterone receptors expression appear in 70-80 % of cases Human epidermal growth factor over expression present in 15-20 % of cases.

Therefore breast cancer is better represented by combined receptor expression than by single receptor status. Immune-histochemical(IHC) markers of the estrogen receptor (ER), Progesterone receptor (PR) and human epidermal growth factor receptor (HER2) can classify breast cancer into four subtypes : type1 (ER+,PR+,HER-2+) ; type2(ER+,PR+,HER2-) ;type3(ER-,PR-,HER2-) and type 4(ER-,PR-,HER2+) .Previous studies had shown evidence of molecular difference which may be responsible for difference in outcome.

Aim of study:

The aim of the study is to evaluate the hormonal receptor status in patients with breast cancer in Nasiriya city and their association with grading and staging of tumor at the time of diagnosis.

Patients and methods:

The type of study is a cross-sectional study carried out in Thi-qar governorate in Nasiriya city in Al Habboby hospital –Oncology center**,** 165 cases of patients who were diagnosed during the period of two years (Junuary2014 – December 2015) with invasive breast cancer were included in this study . The information of each patient were collected and analyzed which include : age of patient, sex, place of residence and tumor related information include grading, staging of tumor, status of receptors(ER, PR, HER2neu receptors).

Results:

The mean age 49±11.1. Most cases were PR+(75.2%) while ER+(72.7%) but most of them were HER2 negative(78.2%). Most patients were in grade II (64.2%) and stage II (50.3%) ,The most common hormonal receptor expression was (ER/PR+ ,HER2-) which accounted for 64.8%.

Regarding to association of hormonal receptor expression with grading and staging of tumor appears that higher grade tumor (II) was observed (76.93%) in type IV (ER/PR-, Her2+) ,there was significant association between hormonal receptor expression and staging of tumor.

Conclusion:

Breast cancer has hormonal receptor character ER,PR and Her2/neu receptors. Majority of cases presented with grade II and stage II at time of diagnosis.

Negative hormonal receptor expression was more likely to be associated with advanced stage of breast cancer. Efforts should be directed at standardization of current methods and development of more reliable testing for early detection of the disease.

Chapter One

Introduction

&

Review of literature

1.1. Introduction:

Breast cancer is the most common malignancy that affects women in developed countries and some developing countries .In the US, it is the most common cancer in women; and the second cause of cancer death. In 2007 it accounted for 26% of cancer cases and 15% of cancer death, which translates 176,296 new cases and 40,515 deaths. [(1)] in 2001, almost 240,000 Women. diagnosed with breast cancer , and over 40,000 died from the disease. [(2)] Breast cancer was the most common tumor seen in Europe in 2006, with 429,900 new cases, representing 13.5% of all new cancers. [(3)] In Iraq according to the ministry of Health /Iraqi cancer registry 2011, breast cancer occupies the first cancer registered and 18.96% of total cancer cases.[(4)]

Breast cancer is an extremely heterogeneous disease caused by interactions of both inherited and environmental risk factors which lead to progressive accumulation of genetic and epigenetic changes in breast cancer cells. Although epidemiological evidence support the existence of certain risk factors (e.g., age, obesity, alcohol intake, estrogen exposure).The family history of breast cancer remains the strongest risk factor for the disease. Familial forms occupy approximately 20% of all breast cancers and appear to have a distinctive pathogenesis dependent on particular susceptibility gene involved.[(5,6)]

Although the genes responsible for most familial breast cancers have been identified, approximately half of familial cancers are caused by germline mutation in tumor suppressor genes (TSGs); most of which had functions implicated in preserving genome reliability. These genes include [(1)] BRCA1 and BRCA2.[(7)]

Breast cancer is the most widely recognized threatening tumor of woman regularly get from the inward coating of milk conducts (ductal carcinoma) or from the Lobules(lobular carcinoma) that supply the channels with milk.[(8)]

Estrogen and progesterone are two hormones that are required for normal breast function and development, but their unregulated stimulation by extrinsic estrogen such as xenoestrogens can de regulate

the cell-cycle and result in breast cell proliferation, inducing carcinogenicity.[9-11] Also, required for normal breast growth is human epidermal growth factor receptor 2 (Her2), a proto-oncogene can mutate into its oncogenic state causing breast carcinogenesis .There are three predictive markers: estrogen receptors, progesterone receptors and Her2-neu receptors have independent prognostic value in breast cancer. ER expression appear in 80-90% of patients with breast malignancy ,While PR expression appear in 70-80% of cases.[12] Her2-neu over expression present in 15-20% of cases.[13,14]

Therefore breast cancer is better represented by combined receptor expression than by single receptor status.[15,16]

Estrogen and progesterone receptors expressions are the greatest important and useful predictive factors currently available. Patients with breast cancer whose malignancy totally lacking in ER and PR do not benefit from hormonal treatment. Current assays for ER and PR are performed by using IHC techniques, which have the advantages of not being confounded by endogenous estrogen, can be linked with histological findings to eliminate the likelihood that the assessment was done on noncancerous slide and do not have tumor size as a limiting factor. it is still controversial whether laboratories can correctly report the percentage of positive ER and PR staining.[17] ER/PR status also has some prognostic value; Patients with ER/PR positive tumors also have improved disease-free survival in relation to patients with ER/PR negative tumors with similar stage at 5 years, but this difference is less apparent at 10 years.[18]

HER-2 status is the major predictive factor that determine the benefit from trastuzumab (Herceptin). There is some evidence suggests that HER-2 status is predictive for benefit from anthracycline-based chemotherapy, although this relationship is not certain, particularly with the availability of trastuzumab.[19] Measurement of HER-2 can be performed by either IHC or fluorescent in situ hybridization.

1.1.A. Aim of study:

To determine the hormonal status of receptors in breast cancer patients in Nasiriya city and their association with grading and staging of tumor at time of diagnosis.

1.2. Review of literature.

1.2.1. Epidemiology of breast cancer:

Breast cancer is the most common malignancy that affects women in developed countries and some developing countries In the US, it is the most common cancer in women; and the second cause of cancer death. In 2007 it accounted for 26% of cancer cases and 15% of cancer death, which translates 176,296 new cases and 40,515 deaths. [(1)] in 2001, almost 240,000 Women. diagnosed with breast cancer , and over 40,000 died from the disease. [(2)] Breast cancer was the most common tumor Seen in Europe in 2006, with 429,900 new cases, representing 13.5% of all new cancers. [(3)] In Iraq according to the ministry of Health /Iraqi cancer registry 2011, breast cancer occupies the first registered 18.96% of total cancer cases .[(4)]

Since 1990, mortality from breast malignancy in the United States and industrialized countries has been diminished at the level of approximately 2.2% per year. In the United States, this decline has been attributed both to advance in adjuvant therapy and to increase using of mammographic screening in approximately equal measure.[(20,21)] The incidence of breast malignancy increase with age; doubling about every 10 years until the menopause, the rate of increase slows naturally.[(22,23)]

1.2.2.Expression of hormonal receptors

Estrogen and progesterone are two hormones that are required for normal breast function and development, but their unregulated stimulation by extrinsic estrogen such as xenoestrogens can de-regulate the cell cycle and result in breast cell Proliferation, inducing carcinogenicity.[9-11] Estrogen receptors (ERs) are activated by ligands (e.g., estrogen, xenoestrogens), and with the help of many cofactors and growth factors can regulate estrogen responsive genes.[24,25] Also, required for normal breast growth is human epidermal growth factor receptor 2 (Her2), a proto-oncogene, which can mutate into its oncogenic state causing breast carcinogenesis.

The Her2 proto-oncogene which is present in two copies in the normal breast tissue, but in its mutated form there is an increase in the gene copy numbers, also known as Her2 gene amplification or over activation. In its mutated (amplified/overactive) form, it becomes an oncogene (i.e., cancer-causing gene) inducing carcinogenicity of the breast tissue. These tumors present an aggressive phenotype encompassing high tumor proliferation rates, metastasis, and mortality.[26,27]

Estrogen receptor (ER) cross communicates with the Her2 receptors at the cellular surface for normal function of the cell, these signaling processes further activate Her2 gene within the nucleus of the cell (Her2 gene expression) and the phosphorylation of the nuclear ER.[28-30]

Biologically, estrogen signaling can occur by distinct pathways: genomic or non genomic. In the genomic pathway, the ligand activated ER binds to the DNA, which further activates protein kinase (i.e., mitogen activated protein kinase [MAPK]) and modulates genes that regulate cellular functions. On the other hand, the non genomic activity occurs within minutes after the formation of ligand (i.e., estrogen and xenoestrogens) receptor complex. In the non genomic pathway, ligand-activated ER with the help of coactivators activates Her2 that increases the phosphorylation of MAPK and modulates the nuclear ER. This Her2-dependant kinase activity of the nuclear ER is an important and essential component of normal

regulation and function of nuclear ER. However, unregulated stimulation of ER causes an increase in Her2 expression, which then increases expression of coactivators, the MAPK kinase activity, and phosphorylation of nuclear ER.[28,31]

Furthermore, breast cancer cells have been present to be phenotypically different (e.g., ER+, ER-, Her2+, and Her2-)making breast cancer a heterogeneous disease. It has also been observed that for ER positive breast cancers, specifically those with increased Her2 gene copies, the ERs activate Her2 signaling and vice-versa [31]. In Her2 and ER-positive (i.e., Her2+/ER+) breast cancer cells; either Her2 or ER can function as the promoter of cellular proliferation and survival.[32] In fact, women with an ER-negative status had worse survival outcomes, and were resistant to therapy, the use of oral contraceptive pills is associated with increased risk of ER + and ER-.[33]

HER2, an epidermal growth factor receptor, that locates at chromosome 17q11.2-12, encoding a tyrosine kinase that is composed of three separate regions: an extracellular region (a ligand-binding domain), a transmembrane domain and an intracellular region (a tyrosine kinase domain). Ligand binding leads to receptor dimerization and activation of intrinsic tyrosine kinase activity .Activation of its receptors start downstream signaling pathways which regulate various cellular functions; including cell expansion, apoptosis, angiogenesis and motility.

In spite of the fact that it is not communicated on the cell surface of numerous normal tissues [34] HER2 receptor has turned into vital role for cancer therapy with trastuzumab (Herceptin®). Trastuzumab, a refined monoclonal antibody has active therapy of patients with metastatic breast Malignancy. Studies have found that trastuzumab is especially successful in the treatment of HER2-positive metastatic breast tumor.

Overexpression of HER2 had been recognized in human breast cancers. Although similar HER2 receptor expression between primary breast cancer and metastatic lymph nodes has also been reported [34].Therapeutic efficiency is mainly reliant on the receptor expression.[35]

1.2.3. Risk Factors:-

Most common risk factors of breast cancer were distinguished by epidemiologic studies. It is designed for that women who are more than 35years old without a previous diagnosis of LCIS or DCIS and devoid of a family history suggestive of solitary gene mutation[36] include the following risk factors.

1. **Age at Menarche:** Females who achieve menarche when younger than 11 years of age have a twenty percent increased risk compared to females who reach menarche when more than 14 years of age. Late menopause also build hazard.[2]
2. **First Live Birth:** Females with a first full-term pregnancy at younger than 20 years of age have 50 % the risk of nulliparous women or female over the 35 years old at their first birth. It is speculated that the pregnancy results in terminal separation of epithelial cells, removing them from the potential group of cancer precursors. However, the biologic premise of such separation has not been resolved.[37]
3. **First-Degree Relatives with Breast Cancer:** The danger of breast cancer increment with the number of influenced first-degree relatives (mother, daughter or sister). However, most cases of diseases happen in women without such a history, as only 13% of ladies with breast tumor have one influenced first-degree relative, and only 1% have two or more.[38]
4. **Atypical hyperplasia in breast Biopsy:** Expanded the risk of disease is connected with prior breast biopsies showing atypical hyperplasia. This model does not alter for expansion in danger connected with proliferative breast changes without atypia.[18]

5. **Race:** In spite of the fact that the tumor is lower in women of African-American ancestry, women in this group present at a more advanced stage and have an expanded the death rate compared with white women.(39)

Additional danger variables are recognized, but have not been joined into the model attributes to their irregularity, troubles in evaluating the risk, or lack of definitive studies.

-Estrogen Exposure : The Postmenopausal hormone replacement treatment slightly expands the danger of breast cancer in current users but might not increase the risk of death.(40) Estrogen and progesterone together expand the danger more than does estrogen alone.

Invasive lobular carcinomas and other estrogen receptor (ER)-positive carcinomas are reported to be increased in this group. Oral contraceptives are unlikely to increase the risk of breast cancer.(41)and can diminish the danger of other malignancies such as ovarian carcinoma. Diminish endogenous estrogens by oophorectomy diminish the risk of creating breast cancer by up to 75%.

-Radiation Exposure: Women who have been exposed to therapeutic radiation or radiation after atom bomb exposure have a higher danger of breast malignancy. Risk increment with younger age and higher radiation doses. Women in their teens and twenties (but not at older ages) undergoing mantle radiation for Hodgkin disease h2ve a 20% to 30% risk of creating breast cancer 10 to 30 years after treatment.(41) Advanced mammographic screening use low measurements of radiation and unlikely to have an effect on the danger of breast cancer.

-Carcinoma of the Contralateral Breast or Endometrium: Expanded the danger of disease is connected with carcinoma of the contralateral breast or endometrium, probably attributed to the share hormonal risk variables for these tumors.(18)

-Geographic Influence: Breast malignancy incidence rates of the tumor in the United States and Europe are four to seven times higher than those in different nations. The danger of breast malignancy increase in migrants to the United States during several generations.

The particular components have not been distinguished but have received considerable attention in the attempt to identify modifiable danger variables. Diet, breast feeding; physical exercise, and natural poisons have been investigated.[18]

- **Diet:** Various items in diet, in particular dietary fat, have been suggested to increase risk, but large studies have failed to find a strong correlation. Some studies have shown a reduced risk with increased β-carotene intake.[2]
- **Obesity:** There is decreased risk of disease in obese younger than 40 years owing to the association with an ovulatory cycles and lower progesterone levels late in the cycle. There is increased the risk in postmenopausal obese women, which is due to synthesis of estrogens in fat depots.[43]
- **Exercise:** Studies have been inconsistent, but some have shown a diminished danger of breast cancer in premenopausal women who exercise.[2]
- **Breast-Feeding:** The women who have longer breast-feeding, the greater is the reduction in the risk of breast cancer.[44]The lower incidence of breast cancer in developing countries may be largely explained by the more frequent and longer nursing of infants.[45]
- **Environmental Toxins:** There is concern that natural poisons for example organ chlorine pesticides could be have estrogenic effects on humans.[18]
- **Tobacco:** Cigarette smoking is not been associated with breast cancer but is connected with the development of periductal mastitis or a subareolar abscess.[18]