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ORIGINAL ARTICLE

Immunohistochemistry of Detection HER2 on Breast Cancer in Basra City

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ABSTRACT

Objectives: Breast cancer is the most typical style of cancer in females. The human epidermal growth factor receptor 2 (HER2) is a potential molecular target in breast carcinoma and it is abundantly expressed in this type of cancer and other cancers. The present study target is detection about HER2 in breast cancer patients of Basrah city.

Methods: Fifty patients were registered and diagnosed as breast cancer disease patients and without breast cancer. Immunohistochemistry was carry out to evaluate the distribution of HER2 by using positive charge slide.

Results: The result was screen about HER2 status in female patients with breast cancer in Basra city / Iraq, and the ages female patients with breast cancer ranged were from 20 to 60 years. The HER2 status reported there was no-significant difference in age groups of cancer status, while there was a significant association between stage I stage II and III of HER2 status and was (P= 0.001).

Conclusion: This study review the role of HER2 diverse cancers, and diagnostic by immunohistochemistry assay. Immunohistochemistry is a well-established ancillary technique to facilitate the diagnosis of infectious and neoplastic processes and help to diagnosis and as a proof to appropriate therapy.

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INTRODUCTION

Daily, cells in the body grow, divide, and die in an regular manner, but sometime these cells are divide abnormally and uncontrolled is lead to the so-called cancer . Cancer is a group of disease characterized by abnormal cells reproduction or uncontrolled growth to induce and spread of these cells[1]. These cells are usually form a tumor that can often be felt as a lump or seen on an x-ray. the prevalence of cancer cells and not controlled it can led to death. However cancer is caused by both:

•**External factor** such as tobacco, infectious organism, chemicals and radiation[2,3].

•**Internal factor** as inherited mutation, hormones, immune condition and mutations that occur from metabolism[4].

Breast cancer (BC) is a one of form cancer, is a major public health issues and is the most common type cancer in the women world and sometime occurs in men. Breast cancer starts cells in the breast begin to grow out of the control [1], these cells and usually formed in different parts of the breast. The growth of cells compose a lamp or mass named a tumor . Tumors are either benign or malignant.

•**Malignant (Cancerous**): The cells are grow into spread (metastasize) to distant areas of the body or tissue surrounding (invade).

•**Benign (not cancerous**): These difference with benign tumors, which do not spread to other parts of the body.

Generality breast cancers begin in the ducts that transfer milk to the nipple (duct cancers), some begin in the gland that produce breast milk (lobular cancer), a small numeral of cancers start in else tissues in the breast is named **sarcomas** and **lymphomas,** so the breast cancer is deem the second most popular cancer yet, after lung cancer, when grade by cancer occurrence in both sexes. Around 55% of global burden is currently experienced in developed countries, but happening rate rapidly gowing in development countries[5]. Breast cancer is a complex and intrinsically heterogeneous disease, molecular profile, and clinical behavior which require different treatment[6].

Growth factors are main for the development of the cells[7]. Growth factors are needed for cell to cell connection implicit embryonic tissue creation, apoptosis, cell survival, fate determination, cell migration and tissue specialization. Growth factor receptors transfer signals directly through receptor from extracellular and translocation to nucleus or the activation of intracellular messengers[8]. Breast cells have receptors which that need for hormones to growth, such as the estrogen receptor and progesterone receptor and HER2 receptors, but they are existing a limited numbers, its play a role for growth and survival the cells, this study focused on human epidermal growth factor receptor 2 (HER2). The human epidermal growth factor receptor 2 (HER2) belong to HER family[9], also called the ErbB protein family or epidermal growth factor receptor (EGFR). This family have many roles are regulate cell growth, differentiation and survival by multiple signal transduction pathways and share in cellular proliferation and differentiation[10]. Any insufficient or excessive of ErbB signaling in humans is associated with the development a lot of disease for example: if insufficient in ErbB signaling lead up to neurodegenerative diseases, like multiple sclerosis and Alzheimer's Disease[11] or excessive ErbB signaling is linked with the development of a broad variety of types of solid tumor[12]. Human epidermal growth factor receptors family they live on the outside of some cells and receive signals from the body[13], and consists of four plasma membrane-bound receptor tyrosine kinases[14,15] are HER1(EGFR/ ErbB1), HER1 (ErbB2), HER3 (ErbB3), and HER4 (ErbB 4) as shown in Figure 1.

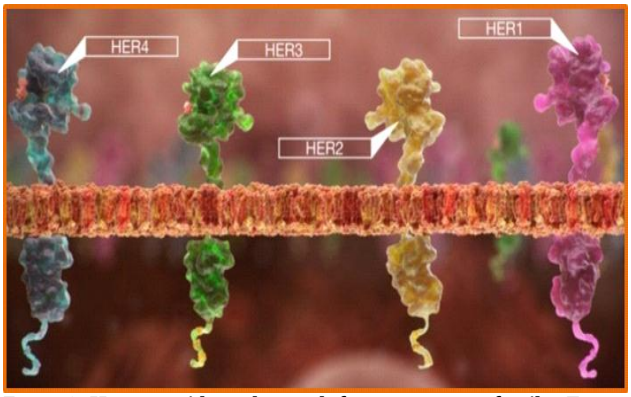


Figure 1. **Human epidermal growth factor receptors family: Four members of HER family including HER1, HER2, HER3, and HER4 are illustrated by purple, green blue and yellow respectively[16].**

The HER2 receptor is a type I transmembrane glycoprotein (1255 amino acid), a 185kD, sit at the long arm of chromosome 17q12 [17]. The human epidermal growth factor receptor type 2 have multiple named [18] are CD 340, $p^{185HER2}$, Erbb2 (rodent) or ERBB2 (human), proto-oncogene Neu, and HER2/neu[19,20], and its encoded by the HER2/neu oncogene located at the long arm of human chromosome 17 [17]. All HER receptors family similar the contents and they are composed of three distinct regions: N – terminal extracellular domain (ECD), single α- helix Transmembrane domain(TM), and intracellular tyrosin kinase domain (Figure 2).

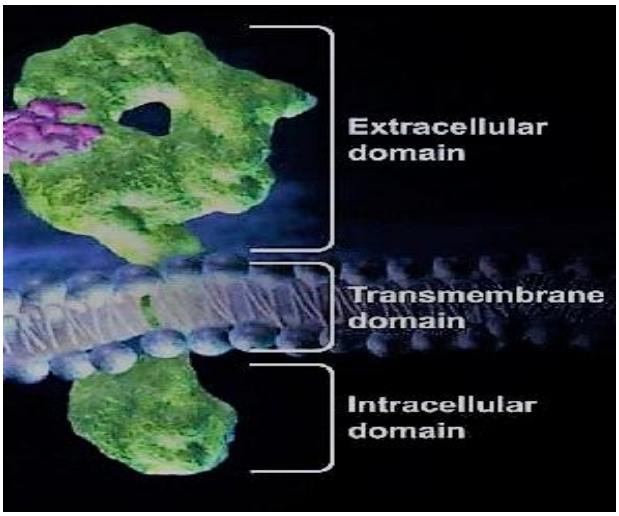


Figure 2 : **The extracellular , Transmembrane and intracellular domain of HER family.**

These receptors are very important in development the breast cancer one of them is (HER2) which amplified and over expressed in different tissues and its main role in these tissues is to ease excessive/uncontrolled cell growth and tumorigenesis[21,17], and becomes more aggressive and more resistant for treatment For example in breast cancer, HER2 over-expression about 20% – 30% [22], gastric cancer[23], salivary duct carcinomas[24], esophageal cancer, ovarian cancer, stomach and adenocarcinoma of the lung[25], Pancreatic cancer and uterine serous endometrial cancer[26].

The human epidermal growth factor receptor 2 (HER2) different from other EGFR family members, is as an "orphan receptor "due to lacking a known ligand[27]. HER2 signaling is a complex network comprised of membrane receptor and their ligands protein kinase and regulating genes that affect various cellular functions. The formation HER2 is either heterodimers or homodimer[28,29], and after activates the intracellular tyrosine kinase, then excite the autophosphorylation of special tyrosine residues. Phosphorylation of tyrosine in transformation adaptor proteins or enzymes to start a succession of signaling cascades and regulate cellular processes[30,31]. The induction of PI3K signaling activities is spur by the heterodimer composed of HER2 and HER3. However, Ras/Raf/MAPK signaling route, its activated by all of the dimers which containing HER2 "HER1/HER2, HER2/HER2, HER2/HER3 and HER2/HER4" [32]. ErbB-1 and ErbB-2 are present in many human cancers and their excessive signaling may be critical factors in the development and malignancy of these tumors[33].

HER2 is expressed in different tissues and its main role in these tissues is to ease excessive/uncontrolled cell growth and tumor genesis[34]. HER2 is a protein in humans is encoded by the *ERBB2* gene which located on the long arm of chromosome 17q12 [35], *HER2* gene is amplification (over-expression) because HER2 receptors results transmitting excessive signals for cell proliferation to the nucleus that result in increased mRNA and a functional HER2 receptor[36]. The continuation deregulated growth cells is lead up to fortified signaling connections between the HER2-activated signaling pathways and effectors cell (proliferative, apoptotic, and metabolic). The aim of this study was screen about HER2 status in female patients with breast cancer in Basra city/Iraq by using immunohistochemistry assay.

MATERIALS AND METHODS

Sample Collection: Fifty patients were registered and diagnosed as cancer disease patients and without breast cancer (in two hospitals are Al- Sader Teaching and Al-Mawany), during the period January 2015 to December 2016, all the patients were female, aged was between 20 – 60 years. Control Group were twenty-five healthy controls, were included in the present study matched with patients for age, disease stage, they were without breast cancer (benign), while the model group were twenty-five patients is a model group (breast cancer), they were included in the present study matched with patients for age, disease stage, and they are with breast cancer (malignant).

Immunohistochemistry: The specimen (biopsy) was placed in 10% formalin for fixation tissue, and then the tissue was processed into a different concentration of alcohol (Chem lab, Belgium); finally the specimen was cut by microtome (Laica, China) and placed the tissue on the slide named positive charge slide (SAIL BRAND, CHINA).

Section Preparation

The Procedure as the following:

Fixation: The first day, the fresh tissue which is suspected as cancer was placed in a container containing 10% formalin. The sample was sent to histopathology unite. The suitable piece of tissue was taken and placed in a capsule, then put the capsule in formalin 10 % for 24 hours for fixation.

Dehydration: The second day, the sample was processed from low to high concentration of Alcohol as shown in Table 1.

Table1: **The different concentration of ethanol alcohol.**

Concentration	Times
70 %	One hr.
80%	One hr.
90%	One hr.
99% (absolute ethanol)	24 hr.

Clearance: The third day, the capsule was removed from alcohol, and then the sample transferred into xylol (Thomas Baker, India) for 2 hours. The tissue was transferred to high temperature 50 ℃ to dissolve wax (ALEXANDRIA WAX). The wax was poured in the mould. The tissue was placed in the mould, and then it was left to cold wax.

Cutting: Fourth day, the tissue was cut by microtome the thickness was 5 micrometer. The tissue was placed in a water bath with a temperature of 50 ℃, and then the tissue was placed on a positive charge slide. Then the slide was placed in xylol container at temperature 50 ℃.

Immunohistochemistry

The immunohistochemistry was used for detection about HER2 receptor on breast cancer cells. Procedure as the following**:** The slides were placed in the incubator at 37 ℃ for 24 hours. Next day, the slides stayed in the incubator for one hour at temperature 60 ℃ (for removal the paraffin wax). The slides were placed in xylol at 50℃ for 5 min. The slides were passed from high concentration to low concentration of alcohol as following in Table 2.

Table 2: **The different concentration for sample processing.**

Concentration	Times
99%	5 min.
99%	5 min.
99%	5 min.
95%	5 min.
95%	5 min.
70%	5 min.
D.W	5 min.
D.W	5 min.

The slides were placed in retrieval antigen (pH = 9.0), and then they were placed in the microwave oven radiation for 10 min.

Staining: Procedure as the following:

•The tissue section slide was incubate for 5 – 10 min in 0.1 – 1% hydrogen peroxide diluted in PBS to quench endogenous peroxidase activity. The slides were washed in PBS twice for 5 min.

•The section was incubated for one-hour n 1.5% of blocking serum in PBS (mixing bottle 1).

• The section was incubated with primary antibodies (10 µl primary antibody mix with 500 µl antibody dilution in PBS) for 30 min at room temperature or overnight at 4 ℃, and the section was washed three change of PBS for 5min.

•The section was incubated for 30 min with AB enzyme reagent (AB mixing bottle), the section was washed three change of PBS for 5min each.

• The section was incubation in 1-3 drops peroxidase substrate (substrate mixing bottle) for 30 seconds to 10 min., or until desired stain intensity develops.

•The section was washed in deionized H_2O for 5 min.

•The hematoxylin (Dako, USA) was put on the section for 5-10 second and current washed with some changes of deionized H2O, The section was washed with tap water.

•The section was put 1-2 drops of permanent mounting medium (Dako, USA) and cover with a glass coverslip. It was observed by light microscopy.

Statistical Analysis: A standard statistical software package (SPSS) used in the analysis. Descriptive statistics were calculated for all variables, and the data were formulated as counts and presented as mean ± standard deviation and percentages. P values less than 0.05 were consider.

RESULTS

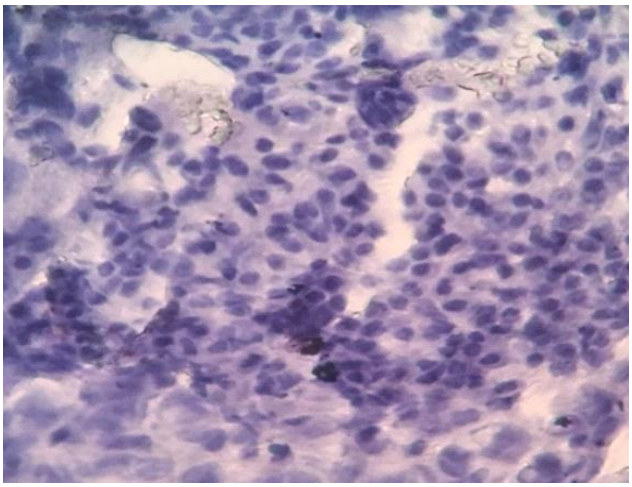
This study was screening about HER2 status in female patients with breast cancer in Basra city / Iraq, and included 50 female patients (25 female patients were breast cancer used as a cancer case, and 25 female patients were without breast cancer used as a control case), and the ages female of patients with breast cancer ranged were from 20 to 60 years (mean age was 45±9.2), 14 (56%) patients out of 25 cases were ≥ 50 years, while 11 (44%) patients out of 25 cases were younger than 50 years. There was non-significant difference in patients' age groups which have HER2 as shown in Table 1. The data showed 1+ immunostaining, 3 (12%), while showed 2+ immunostaining 7 (28%), and 3+ immunostaining was 15 (60%) expression for HER2. The results reported 2+ and 1+ are an equal percentage, and that meaning no significant between them, while the result show score 3+ more than scores (1+ and 2+) and that mean there are a significant and was (P= 0.04) as shown in Table 1. Four (16%) patients of 25 patients with breast cancer had stage I, while 6 (24%) patients of 25 patients had stage II and 16 (60%) patients out of 25 patients had stage III, stage III was more than stage II and stage I, and there was a significant association between stage (I stage II) and III of HER2 status and significant was (P= 0.001) as shown in Table 1.

Table 3: **Association between HER2 positive status and age groups, stage I, II, III, and immunostaining (score 1+, 2+ and 3+).**

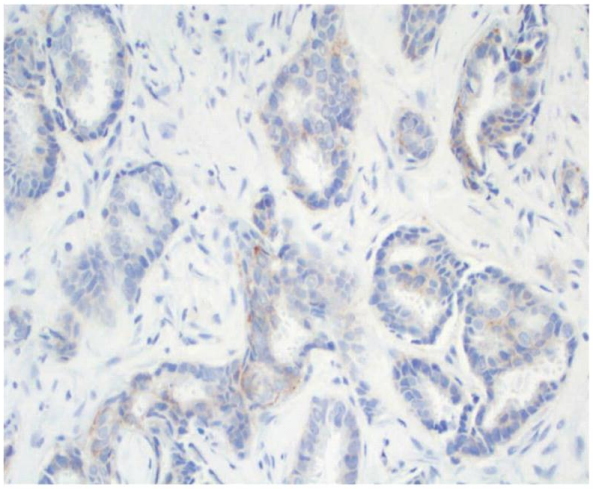
Clinical parameters		Count	Percentage%	P-value
Age	< 50 year	11	56 %	No significant (NS) between age groups HER2 cases was (P=0.89)
	≥ 50 year	14	44 %	
	Total	25	100 %	
HER2 receptor	I	4	16 %	Significant (S) between stage III and stages (II and I) and was (P = 0.001)*.
	II	6	24 %	
	III	16	60 %	
	Total	25	100%	
Immunostaining	Score 1+	3	12%	Significant (S) between score 3+ and scores (2+ and 1+) and was (P = 0.04).
	Score 2+	7	28%	
	Score 3+	15	60%	

*P value = Significant P < 0.05, NS= No significant

In Figure 4 show results detection about HER2 on breast cancer by using immunohistochemistry, and depended on the Food and Drug Administration (FDA) is suggests that HER2 immunohistochemistry scores of (zero) is no staining that means negative, also (1+) weak or incomplete membrane staining in any ratio of tumour cells must as shown in (Figure 4 A and B) were negative, while in Figure 4 D was regarded as HER2 positive (3+) scores that mean uniform intense membrane staining of > 30% of invasive tumour cells, and this indicate patients are eligible for anti-HER2 therapies [37,38], but in (Figure 4 C) HER2 equivocal invasive breast cancer are those with HER2 (2+) score[39] that is a complete membrane staining, no uniform or weak in intensity in at least 10% of the cells or intense complete membrane staining in 30% or less of tumour cells, and should be confirmed by fluorescence in situ hybridization (FISH) to verify their HER2 expression more accurately.



A



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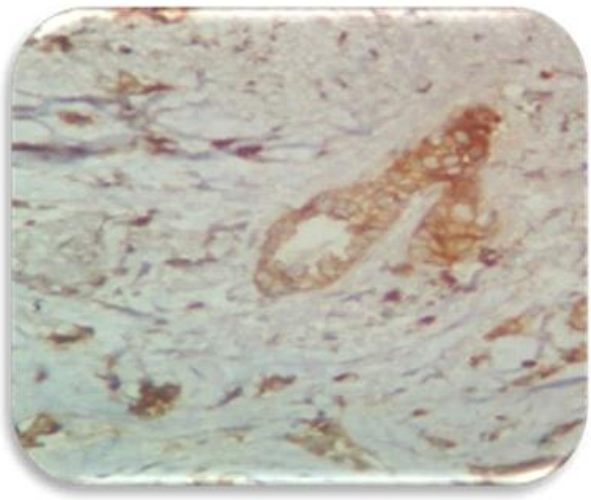
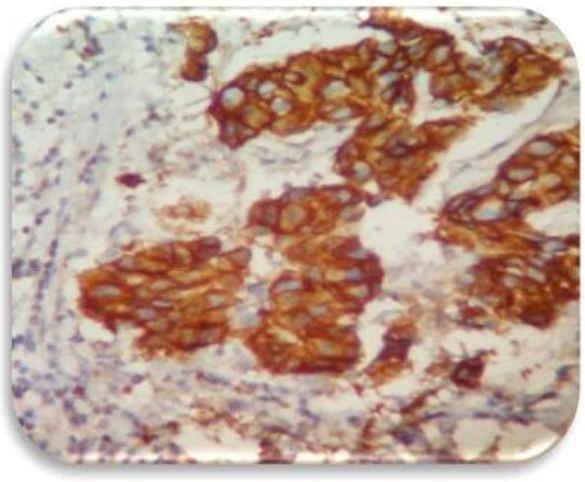


C



D

Figure 4 (A,B,C and D). **Detection about HER2 on breast cancer by using immunohistochemistry, and depended on the Food and Drug Administration (FDA).**

Discussion

Breast cancer specimens should initially undergo HER2 testing by a validated immunohistochemistry (IHC) assay for HER2 protein expression. Immunohistochemistry (IHC) is a good technique used to distinguish the location and distribution of target antigens in cells or tissues[40,41,42] by staining with specific antibodies[43], and this a technique used for distinguish cellular or tissue constituents (antigens) by means of antigen-antibody interactions, the place of antibody binding being identified by direct labeling of the antibody, or by using secondary labeling method [1], it's an important role in the histopathological diagnosis of many tumors[44], and diseases[45]. Many breast cancer specialists think that the fluorescence in situ hybridization test (FISH) is more accurate and exact than IHC. However, it is expensive and takes longer to get the results; therefore, the IHC test is done first. Grading of immunohistochemistry assays is based on a 0, 1+, 2+, and 3+ scoring system. According to the package inserts of immunohistochemistry assays, tumor specimens that demonstrate strong complete membrane staining in >10% of tumor cells are classified as 3+ on immunohistochemistry and constitute an unequivocal positive results. Immunohistochemistry of HER2 results was generally divided into four scale scores the range (from 0 to 3+) depending on the percentage of positive tumor cells and staining intensity[46]. Immunohistochemistry is a simple to carry out than fluorescence in situ hybridization and is lesser expensive about 20% of the cost. Human epidermal growth factor receptor 2/neu is a proto-oncogene located on the long arm of chromosome 17. Many adult tissues, including breast, endometrium, prostate, and ovary, normally express low levels of the protein encoded for this gene. Amplified levels of this gene and its protein product have been found in between 20% and 30% of invasive breast carcinomas[47]. Determination the variable HER2 expression also has become an important help in the determination of which patients will be candidates for the new anti-HER2/neu drug, trastuzumab (Herceptin), which has been reported to be of benefit patients with breast cancers that overexpress HER2/neu breast cancer. HER2 status should be examination in all patients with breast cancer. The aim of diagnostic immunohistochemical studies have been to explore and certify diagnoses by identifying the pathway of differentiation of a given tumour[48]. This finding confirms that HER2/HER3 dimerization is central for HER2 signaling. Amplification or overexpression of HER2 take place in approximately 10–30% of gastric/gastro esophageal cancers and 20–30% of breast cancers and serves as a predictive biomarker and prognostic. HER2 overexpression also been seen in other cancers like bladder, ovary, lung, endometrium, colon, neck and head. Breast cancer remains most common cancer diagnosed in women, in spite of significant improvements in treatment, and the second leading to cause of cancer-related deaths[49]. A serial of researches were orientated to find a complex molecular heterogeneity which causes malignancy. One such discovery was *HER2* gene, which is encoded for HER2 receptors present on the cell surface and that belongs to a tyrosine kinase family. This family plays important

role in survival, differentiation and growth regulating cell by multiple signal transduction pathways and participate in cellular proliferation and differentiation[10], but when HER2 is overexpressed, it causes rapid progression and poor prognosis of the disease[11]. Overexpression of HER2 in breast cancer cause increased homodimerization (HER2:HER2) and heterodimerization (e.g., HER2:HER3), which initiates a strong pro-tumorigenic signaling cascade[50] when it is present in high concentrations, such as in cancer[27]. HER2 are present in many solid tumors, including lung, head and neck, breast, kidney, colon, ovary, prostate brain, and bladder cancers, and in salivary duct carcinomas[43]. So, today HER-2 is a very influential factor in the diagnosis and treatment of metastatic cancer, because it is one of the main mediators of key pathways involved in carcinogenesis, invasive behavior, and cell growth, and this detection of HER2 led to development and agreement of the first treatment for HER2 as a targeted therapy, by using monoclonal antibodies. Overexpression of the human epidermal growth factor receptor-2 (HER2) gene , this linked with a rise risk of recurrence after surgery, rapid tumour growth, weak response to conventional chemotherapy and shortened survival[37].

Conclusions

This study review the role of HER2 diverse cancers, and diagnostic by immunohistochemistry assay. Immunohistochemistry is a well-established ancillary technique to facilitate the diagnosis of infectious and neoplastic processes and help to diagnosis and as a proof to appropriate therapy.

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