Amplification of HER-2 Gene in Benign and Malignant Breast Lesions in a Sample of Iraqi Women

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Abstract

Background: Breast cancer is the most common cancer worldwide. From several markers of this malignancy, HER-2 is considered to have a particular importance because it associates with the treatment and prognosis of the disease.

Objective: To investigate the amplification of HER-2 gene in benign and malignant lesions of breast in a sample of Iraqi women.

Patients and Methods: A total of 24 excisional breast biopsies were obtained from women with breast lesions. Biopsies were preserved in 10% formalin and undergone paraffin embedding according to the standard protocol. Four µm thick sections were prepared and placed on positively charged slide and stained with fluorescent in situ hybridization. The stained slides were examined with fluorescence microscope to detect HER-2 gene amplification.

Results: Fourteen women were found to have benign lesions, while 10 were with malignant lesions. All benign lesions revealed two copies of the gene while seven of malignant cases showed positive results for HER-2 amplification (i.e more than 5 copies of the gene).

Conclusion: These results support the idea that amplification of HER-2 could be considered as an indicator for tissue transformation into malignant lesion .

Key words: HER-2, Breast Cancer, Fluorescent in Situ Hybridization.

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Introduction

Human epidermal growth factor receptors (HER) are group of 4 members (HER1-4) which has crucial roles in normal body functions though regulation of cell growth, differentiation and survival [1]. Of these members, HER-2 (also known as Her-2/neu, c-erb2 and p185) has the strongest catalytic kinase activity [2]. Although there is no known ligand for this receptor, it performs many functions through dimerization with the other family members. Furthermore, in cases of overexpression, spontaneous dimerization

of HER-2 occurs in ligand-independent manner.In either homo- or heterodimerization, the result is the autophosphorylation of tyrosine residue in the cytoplasmic portion of HER-2 receptor. This in turns induces several signal pathways, the most important of which are phosphatidylinositol-4,5-biphosphate 3-kinase/Protein kinase B (PI3K/AKT) and rapidly accelerated fibrosarcom/ mitogen-activated protein kinase (Raf/MAPK) axes [3].

These and other signals are responsible for growth and survival of different cells. However, the over expression and amplification of HER2 gene induces constitutive growth and anti -apoptotic activity [4] which predispose to different tumors. In fact, about 15-30% of women with breast cancer (BCa) and 9-32% with ovarian cancers are positive for HER-2 amplification [5][6]. Even though, the role of this gene in cancer initiation is a debate issue. Some in vitro studies argued that overexpression of HER-2 is a cause rather than a sequel of cancers [7]. On the other hand, it was frequently found that overexpression of this protein in benign lesions is not necessarily associated with increased risk of developing malignant tumors [8].

Thus, this study aimed to investigate HER-2 gene status in a sample of Iraqi women with benign and malignant BCa.

Patients and Methods

A total of 24 excisional breast biopsies were obtained from women attending Oncology Department in Al-Imamain AL-Kadhumain Medical City during the period from January to October, 2016. The age range of those women were (18-47 years, mean 38.9±4.17 years). All biopsies were preserved in 10% formalin and undergone paraffin embedding according to the standard protocol. Two 4-µm thick sections were prepared from each biopsy. One section on histological slide for hematoxylin and eosin (H&E) staining and the other on positively charged slide (Fisher Scientific/ Germany) for fluorescent in situ hybridization (FISH).

Fluorescence In Situ Hybridization Analysis: Fluorescence in situ hybridization was performed using her-2/neu specific probe (Poseidon Repeat-free FISH probes, Kereatech company/Holland, Cat No. KBI-10701) according to manufacturer's instruction as follows:

The positively charged slide mounted with paraffin sections were warmed for 6 hours at 56 ℃, after which they undergone deparaffinization by soaking in xylene for 10 minutes. Dehydration was achieved by soaking in ascending gradients of ethanol for 3 minute each. After washing with dH2O for 3 minutes, the slide were placed in pretreatment solution A at 98 ℃ for 15 minutes. The sections were then covered with 200 µL pepsin solution (KBI-60007 Tissue Digestion Kit I, Kereatech/Holland) and incubated for 15 minutes at room temperature (RT), then washed with dH2O for 1 minute and in 2x saline-sodium citrate (SSC) for 5 minutes at RT. Finally, the slides were dehydrated by soaking in ascending gradients of ethanol for 1 minute each.

Ten µL of probe per 22x22 mm of field was applied, after which the slides were covered with cover slip and sealed with rubber cement. The sample and the probe were denatured on a hot plate at 80 ℃ for 5 minutes, and then incubated overnight at 37 ℃ in a humidified chamber. The rubber cement was removed and the slides were washed with different washing buffers and dehydrated again with ascending gradients of ethanol for 1 minute each.

Fifteen µL of 4',6-diamidino-2-phenylindole (DAPI) counterstain were applied for each slide followed by covering with cover slip and then examination using

an Eclipse TE300 fluorescence microscope (Nikon, Japan).

The FISH signals were interpreted according to the American Society of Clinical Oncology and the College of the American Pathologists (ASCO/CAP) for single probe in 2013 [9] as follows:

HER-2 positive: average HER-2 copy number ≥6.0 per cell.

HER-2 equivocal: average HER-2 copy number ≥4.0 and< 6.0 per cell.

HER-2 negative: average HER-2 copy number < 4.0 per cell.

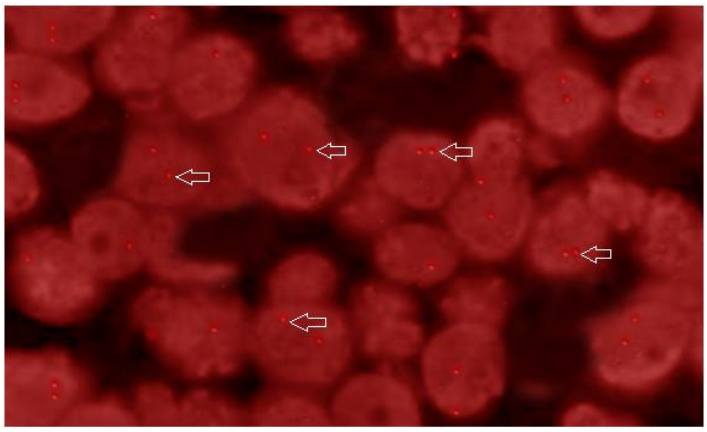
Statistical Analysis

Chi-square test was used to compare the difference in HER-2 amplification between younger women (< 40 years old) and older women (40 years or older) using statistical package for social sciences software.

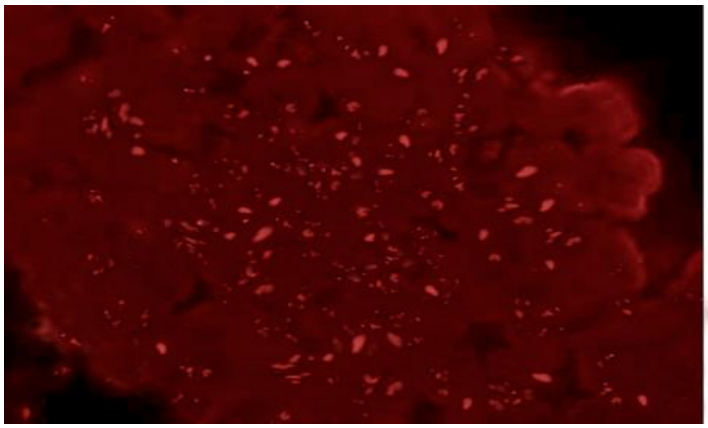
A P-value of 0.05 or lesser was considered significant.

Results

Out of the examined 24 excisional breast biopsies, the histopathological examination of H&E-stained section revealed benign breast lesions in 14 sections, while 10 sections were found to have malignant lesions. FISH signals for HER-2 gene were detected in all tissue sections. All benign lesions revealed two copies of the gene (figure 1). Seven out of 10 malignant cases showed positive results for HER-2 amplification (i.e more than 5 copies of the gene) (figure 2). The other three cases revealed two copies of the gene.



Figure(1): Fluorescence in situ hybridization for HER-2 gene in cancerous breast tissue. Very few signals are present in each cells (arrows) indicating negative result for HER-2 amplification.



Figure(2): Fluorescence in situ hybridization for HER-2 gene in cancerous breast tissue. Numerous signals are present in each cells indicating amplification of HER-2 gene.

Regarding age classes, 11 out of 14 women with benign BCa (87.57%) were 40 years old or older compared with 3(21.43%) who were under this age. For women with malignant BCa, the majority (5 out of 7 HER-2 positive (71.43%)) were young women versus 2(28.57%) over 40 years old, while all the rest 3 HER-2 negative women were older than 40 years with significant difference (P=0.038).

Discussion

With the advances in molecular techniques, HER-2 becomes one of the most important targeted markers for breast tumors. In fact, cases of BCa can have up to 25-50 copies of this gene, and up to 40-100 folds' increase in the expression of HER2 protein with more than million of HER-2 receptors will be expressed on the malignant cell instead of few of them in normal status [10].

The current study revealed no gene amplification of HER-2 in women with benign breast lesion. This result is not in accordance with many previous studies. Stark et al. [11] conducted a nested case-control study at the Myo Clinic/USA for detection of HER-2 amplification in different breast lesions. They found that 9.5% of benign breast lesions versus 18% in malignant lesions were positive for HER-2 amplification. Recently, Daoud et al. [12] studied the expression of HER-2/neu protein in Egyptian women with benign and malignant BCa. They noticed overexpression of this protein in 31% of benign cases. Surprisingly, Xu et al. [13] reported a very high percentage of HER-2 gene amplification in the USA women either in malignant (ductal carcinoma in situ, 21 out of 22 cases) or in benign (atypical ductal hyperplasia 7 out of 13 cases). The discrepancy in the results could be attributed to several factors among which the different ethnicity, different types of malignant and benign lesions and the technique which is used for exploring amplification or expression.

The current study revealed that young women are significantly more prone to HER-2 positive BCa than older women. These results agree with many previous studies in that BCa in young women is associated with unfavorable tumor characteristics including HER-2 positive [14][15][16]. A comprehensive, large scale genomic analysis including 700 women with BCa revealed higher rates of HER-2 over expression in younger than older women [17]. However, a current study by Lee *et al.* [18] did not show this trend may be due to the specific subtype of BCa that used in this study.

Despite the fact that older ages are well-known risk factor for BCa, this malignancy is the leading cancer-related death in young women [19], and large number of evidence suggested that BCa in young women always associated with worse outcomes including high mortality and recurrence rate compared with older women [18]. Thus, the relatively high percentage of young women who are HER-2 positive in the current study supports the idea that BCa is more aggressive in younger women.

Taken together, the current data support that idea that amplification of HER-2 may be considered as an indicator for tissue transformation into malignant lesion, and this amplification is a consequence rather than a cause of cancer.

References

[1] Gutierrez C, Schiff R. HER2: biology, detection, and clinical implications. Arch Pathol Lab Med 2011;135(1):55-62.

[2] Iqbal N, Iqbal N. Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. Mol Biol Int. 2014;2014.

[3] Ayyappan S, Prabhakar D, Sharma N. Epidermal growth factor receptor (EGFR)-targeted therapies in

esophagogastric cance. Int J Cancer Res Treat 2013;33(10):4139-4155.
[4] Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. Nat Rev Clin Oncol 2012;9(1):16-32.
[5] Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nature reviews Mol Cell Biol 2001;2(2):127-37.
[6] Pils D, Pinter A, Reibenwein J, Alfanz A, Horak P, Schmid BC, et al. In ovarian cancer the prognostic influence of HER2/neu is not dependent on the CXCR4/SDF-1 signalling pathway. Brit J Cancer 2007;96(3):485-91.
[7] Roa I, de Toro G, Schalper K, de Aretxabala X, Churi C, Javle M. Overexpression of the HER2/neu Gene: A New Therapeutic Possibility for Patients With Advanced Gallbladder Cancer. Gastrointestinal Cancer Res 2014;7(2):42-48.
[8] Varella-Garcia M, Mitsudomi T, Yatabe Y et al. EGFR and HER2 Genomic Gain in Recurrent Non-small Cell Lung Cancer After Surgery: Impact on Outcome to Treatment with Gefitinib and Association with EGFR and KRAS Mutations in a Japanese Cohort. J Thoracic Oncol 2009 (3):318-25.
[9] Wolff AC, Hammond MEH, Hicks DG et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 2013;31(31):3997-4013.
[10] Kallioniemi OP, Kallioniemi A, Kurisu W et al. ERBB2 amplification in breast cancer analyzed by fluorescence in situ hybridization. Proceedings of the Nat Acad Sci USA1992;89(12):5321-5325.
[11] Stark A, Hulka BS, Joens S et al. HER-2/neu Amplification in Benign Breast Disease and the Risk of Subsequent Breast Cancer. J Clin Oncol2000;18(2):267-274.
[12] Daoud SA, Ismail WM, Abdelhamid MS, Nabil TM. Possible Prognostic Role of HER2/Neu in Ductal Carcinoma In Situ and Atypical Ductal Proliferative Lesions of the Breast. Asian Pacific J Cancer Preven 2016;17(8):3733-3736.
[13] Xu R, Perle MA, Inghirami G, Chan W, Delgado Y, Feiner H. Amplification of Her-2/neu gene in Her-2/neu-overexpressing and -nonexpressing breast carcinomas and their synchronous benign, premalignant, and metastatic lesions detected by FISH in archival material. Modern patholog. J USA Canad Acad Pathol Inc 2002;15(2):116-124.
[14] Colleoni M, Rotmensz N, Robertson C et al.Very young women (<35 years) with operable breast cancer: features of disease at presentation," Ann Oncol 2002;13(2): 273–279.
[15] Anders CK, Hsu DS, Broadwater G et al.Young age at diagnosis correlates with worse prognosis and denies a subset of breast cancers with shared patters of gene expression. J Clin Oncol 2008;26(20):3324–3330.
[16] Sidoni A, Cavaliere A, Bellezza G, Scheibel M, Bucciarelli E.Breast cancer in young women: clinicopathological features and biological specificity. Breast 200;12(4):247–250.
[17] Anders C, Hsu D, Broadwater G, et al. Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. J Clin Oncol. 2008;26:3324–3330.
[18] Lee MK, Varzi LA, Chung DU et al. The effect of young age in hormone receptor positive breast cancer. BioMed Res Int 2015;ID 325715.
[19] American Cancer Society, Cancer Facts & Figures 2014, American Cancer Society, Atlanta, Ga, USA, 2012.DOI:https://doi.org/10.1093/ndt/gfv379.