ORIGINAL ARTICLE

Prevalence of HER2 Gene Expression and Its Correlation with Histopathological Parameters in Breast Cancer Patients

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ABSTRACT

Background: There were conventional reportes about overexpressionrate HER2(Human epidermal growth factor receptor) in breast cancer.
Aim: To determine HER2 gene over expression using immunohistochemical method in Breast cancer.
Methods: This descriptive analytical research was conducted on 74 breast cancer samples admitted in hospitals in Isfahan Iran. Each sample was fixed in formalin and tissue processing and sectioning were performed . The slides were stained by H/E. All samples were diagnosed by two expert pathologists. Determination of HER2gene expression was done after antigen retrieval with primary specific rabbit monoclonal HER2antibody by immunohistochemistry. Data was analyzed with SPSS16 using with chi- squre tests.
Results: Out of 74 specimens of breast cancer 39(52.7 %) specimens detected of HER2gene over expression but 35(47.3%) samples malignancy were not showed HER2 gene expression. There was significant (P<0.026)relationships between high stage tumor with over expression of HER2 gene. Among 39 specimens HER2 positive 6 samples (8.1%) were in grade one, 18 samples (24.3%) were in grade two and 15(20.3%) samples had grade three. There was significant (p=0.029). relationships between HER2 gene expression with grade tumor
Conclusion: This study showed that patients HER2 who were over 50 years old had HER2positive more those under fifty. Patients in high stage had more HER2 over expression and poor predictive value compared to low stage .
Keywords: Breast cancer, Immunohistochemistry,HER2

INTRODUCTION

One of the most common cancers that threaten women worldwide is breast cancer. It is going to be a serious health issue in both developed and developing countries[1]. In fact, after lung cancer, it is the second leading death cancer amongst American women[2] and is increasing with a high pace in Asian countries[3] because of different factors such as nutrition, obesity, urbanization, decreasing fertility,genetic factors, and over expression or suppression of some growth factors genes[3,4,5]. Unfortunately, about 11% of patients with breast cancer passed away[6]. According to data, the incidence of primary breast cancer in Iran is approximately 22.6 per 100,000 women[4]. Based on DNA analysis, breast cancer is divided into various sub-groups. Hence, knowing and evaluating the genes that are involved in this disease is essential for selecting an appropriate treatment[7]. low expression of some genes, which play a key role in inhibiting and suppressing tumors, including P53, PTEN, and P16 will accelerate the growth and progression of cancer[7,8,9]. On the other hand, the excessive expression of genes such as AURKA and Human epidermal growth factor receptor 2 (HER2) usually increase malignant cells within a short period[6,10].

HER receptor family has four members that are epidermal growth factor receptors including EGFR/HER1/ErbB1, HER2/ErbB2, HER3/ErbB3, and HER4/ErbB4. Aside from HER4, other receptors will overexpress due to breast cancer. It has been proven that targeting HER2 in patients with breast cancer has positive effects. There are many controversies about the rate of HER2 expression during breast cancer[11]. Krishnamurti et al have reported that the overexpression of HER2 in breast cancer accounts for 15% to 20%[12] while Nami et al has showed that 20% to 30% of patients have positive HER2[11]. Moreover, only 15% to 24% of patients with positive HER2 were recorded by Perns et al[13]. Understandably, an in-depth knowledge of expression of different genes is vital for the prevention and treatment of breast cancer. Since there is no adequate information about the role of HER2 in breast cancer, we aimed to investigate its expression in tumors of patients who are in different ages and stages of cancer by immunohistochemistry techniques.

METHOD AND MATERIAL

This descriptive and analytical survey was performed on 74 patients with breast cancer who referred to Isfahan hospitals during the years 2016 to 2019.The protocol of this study was reviewed and confirmed by Ethics Committee at Sabzevar University of Medical Sciences. (IR.MEDSAB.REC.1398.083). The samples that were taken from them, were fixed in formalin and after preparing sections IHC staining was done[5]. The samples were examined by two pathologists. The tumor samples were divided into 1 to 3 grades based on changes on mitosis, polymorphism, and the presence or absences of tumors[14].
Evaluation of HER2 Marker Expression: IHC staining was done for all the patients to determine HER2 expression level. At first tissue sections were deparaffinized after that for the purpose of endogenous biotin blocking and detection of HER2 IHC, the sections were heated to 100 ºC

for 20 minutes. In a bid to suppress the activity of endogenousperoxidase, the specimens were soaked into 3% H2O2 solution for 30 minutes and then were washed three times with Saline phosphate[15,16]. Specimens were incubated with anti-HER2 antibody (clone 4B5, Ventana) and the antibody binding site was visualized using a 3,3′ diaminobenzidine IHC kit. HER2 DISH was applied for the detection of both HER2 gene and CEN17 targets. A protease digestion was happed followed by a second heat on HER2 IHC tissue sections. A cocktail of 2,4-dinitrophenol (DNP)-labeled HER2 probe and digoxigenin (DIG)-labeled CEN17 probe was hybridized on HER2 IHC stained tissue section. The samples were washed three times then HER2 and CEN17 probe hybridization sites were visualized by SISH DNP detection kit and the red ISH DIG detection Kit, respectively[17]. considering how much the samples were colored, they were divided into two groups of positive and negative. In this regard, the slices that less than 5% of malignant cells were colored put in the negative group and the ones that most of their cells were colored put into the positive group. According to the value of the coloring, the positive group were divided into 3 subgroups including score 1 (pale color), score 2 (medium color), and score 3 (deep color)[17].

Data were analyzed using the statistical analysis software SPSS vs.22 in order to describe the data generally, statistical indexes like, mean, median standard deviation and ranges were used. For the main data analysis Chi-squared test was used to determine the relation between HER2 gene amplification and the score each carcinoma sample gained in IHC. $P < 0.05$ was regarded as statistically significant.

RESULTS

General Findings of HER2 with Promenopausal and After Menopause Age: In this study, the minimum age of malignant samples was 30 and the maximum age was 88 years. The mean age of women with breast cancer and positive HER2 was 53.41±14.82 (from 39 patients with positive HER2, 29 of them were older than 50 years (menopause) and 10 were under 50 years (pre-menopause). while the average age of cancer patients with negative HER2 was 52.74±12.65 (from 35 patients with negative HER2, 17 of them were under 50 years and 18 were over 50 years). According to the data, there is a significant association between age and HER2 gene expression (P<0.035).

Findings of HER2 According to Cancer Stage and Histological Findings: In control samples (healthy) the expression of HER2 did not happened. However, amongst 74 patients, the expression of HER2 were considerably high in 39 of them (52.7%) and this gene expression did not detect in 35 of them (47.3%).

7 specimens of 39 positive HER2 samples were in 0 stage of cancer (in situ carcinoma). 4 specimens were in stage I (invasive carcinoma without involving lymph nodes). 8 of them were in stage II (invasive carcinoma with involved lymph nodes). 11 samples were from the patients in stage III (invasive carcinoma with involved breast central lymph node) and 9 of them were in stage IV (invasive carcinoma with involved adjusted tissues) (table1). Hence, there is a notable correlation between tumor stage and HER2 expression(Fig 2, P< 0.05). As well as tumor stage, cancer grade has significant association with HER2 marker expression. In this way, 6 patients with high expression of HER2 were in grade I, 18 were in grade II, and 15 were in grade III (Fig 3).

There was a significant relationship between the expression of HER2 marker and stages oftumor. For example, the malignant tissues that were in stages III and IV had higher HER2 gene expression compared to the rest and I stages (P<0.05) (Fig2).

A significant correlation was observed between the expression of HER2 gene marker and gridscancer (P <0.029). Actually,the malignancies in high grades showed more cytoplasmic accumulation that was represented for high HER2 expression (Fig 3).

Table 1: Evaluation of relationship between the expression ofHER2 marker and stages oftumor. The results showed frequency of positive and negative HER2.

HER2/ Stage	Frequency of HER2 - Positive (%)	Frequency of HER2 - Negative (%)	P value
Stage 0	9.5	10.8	P>0.05
Stage 1	5.4	8.1	P= 0.05
Stage 2	10.8	20.3	P<0.028
Stage 3	14.8	6.7	P<0.035
Stage 4	12.2	1.4	P<0.026
Total Frequency	52.7	47.3	P<0.026

Figure1: Microscopic pictures of immunohistochemistry samples in order to investigate HER2 gene in breast cancer; A) Score 0; B) Score +1; C) Score +2; D) Score +3. Score 0 (negative result is assigned to those cases showing no detectable stained cells or stained cells are observed in less than 10% of tumor cells). Score +1 is assigned to colorfulness with less than 10% weakly stained tumor cells membrane, score +2 includes cases with weakly to partial stained in more than 10% of tumor cells membrane, and +3 is for cases with more than 30% complete and strongly stained tumor cells membrane amined by the method IHCe in the different Stages. Diagram showed Significant difference between different Stages. (* P<0.5)

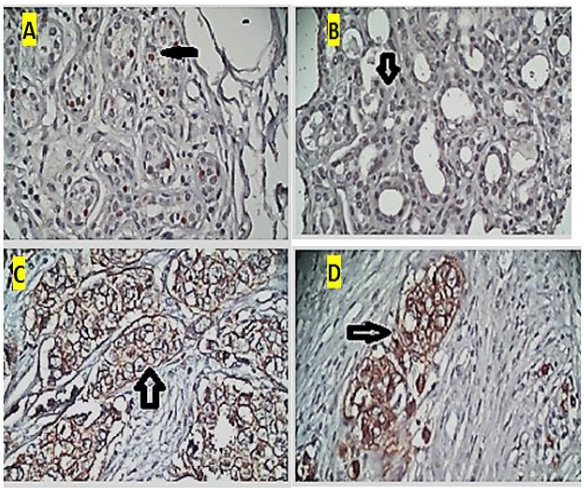
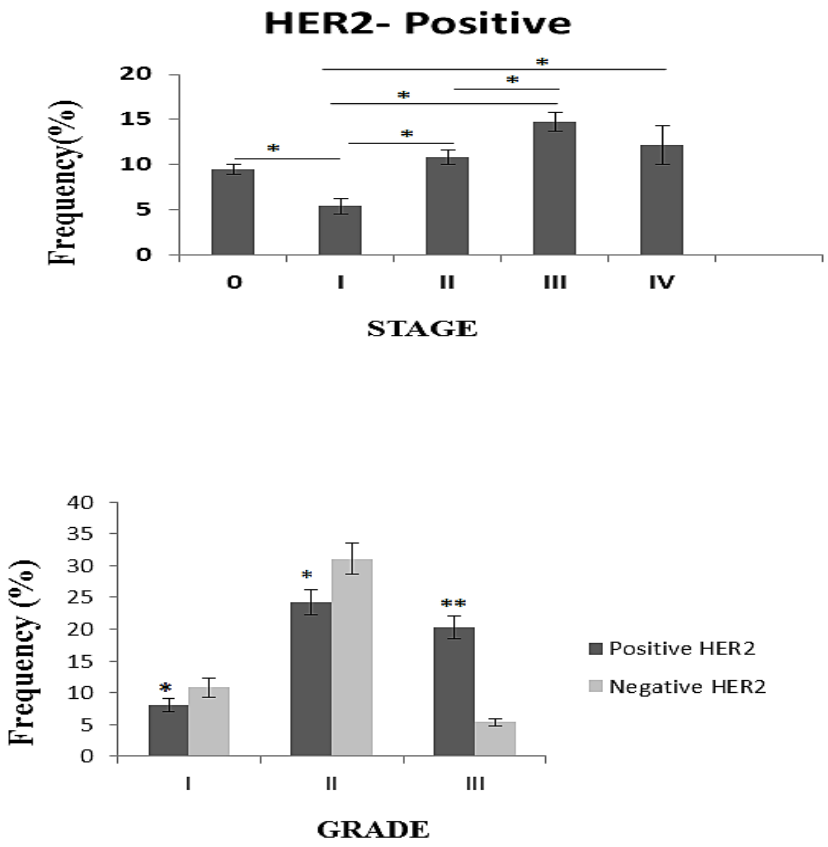


Figure 2: The frequency of HER2-Positive in samples ex

Figure 3: The frequency of grade tumor in samples examined by the method IHC



DISCUSSION

In this study, the expression of HER2 marker gene in the patients who were in the stages of 3 and 4 was significantly higher than the ones who were in the stages of 0, 1, or 2.ourfindings are in line with the results of other studies. Petrou p et al showed that overexpression of HER2 is associated with the progression and development cancer[18]. In the study of Tong ZJ, 106 breast cancer patients were examined and 48 patients had positive HER2 while the others had negative HER2 marker. whereas, they showed that the expression of HER2 is considerably high in patients who were in last grade of their diseases[19]. Nathanson has reported that overexpression of HER2 and metastasis of lymph nodes have a direct connection[20]. Üreyen O et al did not report any significant association with expression of HER2 and histopathological indices. Their results are in contrast with our findings because Üreyen O studied the samples with positive and negative axillary lymph nodes metastasis while we examined the breast tissue. Besides, the number of their patients were low (64 patients) compared to our study (74 patients)[21].

According to our findings, age plays a critical role in the expression of HER2 gene marker. We showed that the women who are over 50 years (postmenopausal) often have positive HER2 while the ones who are younger (premenopausal) the expression of HER2 is dramatically low. However, more researches are needed to investigate the impact and mechanisms of age on HER2 gene. HER2 is regarded as a proto-oncogene and has a profound effect on the proliferation, differentiation, and angiogenesis, so it is likely that the expression of HER2 markers in invasive cell tissues enhances the angiogenesis in the tumors and creates an appropriate condition for the growth of malignant cells that will lead to high pace of growth and development of tumors in other tissues[22,23].

CONCLUSION

In the present study, the HER2 gene expression in patients who were over 50 years as well as malignancies at the advanced stages of the disease was observed more, so overexpression of HER2 gene could be considered as a sign of poor prognosis.

Ethics Confirmation and Consent for Participation: Hereby, it is confirmed that the Journal's guidelines on the affairs related to the ethical publication have been carefully

read, and it is affirmed that the present research followed the defined directions.

Consent for Publication: All co-authors showed their consent for the publication of thefinal draft of this article.

Statement of Output Sharing: Keep in contact with the author for data.

Conflict of Interest: There was no conflict of interest to declare.

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