

## Original Research Article

# Prevalence of HER-2/ neu receptor amplification and its effects over prognosis of the patients with breast cancer

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## ABSTRACT

**Background:** The diagnosis and prognosis of patients with breast cancer is routinely carried out with biopsy of growth by H&E staining but it is not commonly practice with various immunomarkers including HER2/neu. However HER2/neu association in breast tumour patients with prognosis has not been studied much, so this study is aim to evaluate the frequency of HER2 (human epidermal growth factor receptor) amplification and its effects over prognosis among the patients with breast cancer.

**Methods:** After ethical approval, retrospective observational study was conducted from October 2014 to September 2017. All operated patients with biopsy proven breast cancer, the patients having any stage of disease, with sufficient data present in hospital record and patients who received neo-adjuvant chemo-therapy/radio-therapy were included. Patients unfit for surgery due to co-morbidities like cardio renal diseases and patients having insufficient hospital record or who missed follow-ups were excluded. SPSS version 20.0 was used for data analysis for data analysis with qualitative data presented as frequency and percentages.

**Results:** A Total 120 patients, 48(40%) had HER-2/ neu positive. Among the HER2 +ve patients, 17(35.4%) had local recurrence within 03 years while 21(43.7%) cases had distant recurrence. The disease free survival rate in 03 years was observed in 22(45.83%) out of 48 HER2 +ve cases.

**Conclusions:** Study reveals 40% patients had HER-2/neu positive expression and was associated with poor outcomes and disease free survival time period in comparison to patient with HER-2/ neu negative.

**Keywords:** Breast Carcinoma, Human epidermal growth factor receptor, Immunohistochemistry

## INTRODUCTION

HER-2 receptor is a member of epidermal growth factor family, encodes a transmembrane tyrosine kinase receptor localized to chromosome 17q, amplification or over expression of this gene has been shown to play an important role in certain aggressive types of breast cancers.<sup>1</sup> The Her-2 /neu protein is a component of a four

member family of closely related growth factor receptors, including HER-1(erb-B1), HER-2(erb-B2), HER-3(erb-B3) and HER-4(erb-B4).<sup>2</sup> In addition to its association with disease outcome in gastrointestinal, pulmonary, genitourinary and other neoplasms, amplification of HER-2/neu protein has been identified in 10-34% of breast cancer patients. HER-2/neu protein over expression was first reported in situ breast cancer and

was associated with Comedo carcinoma variant.<sup>3-5</sup> Later in subsequent studies HER-2/neu protein expression associated with breast cancers has been confirmed., further evaluation of this receptor appear warranted to confirm whether this marker can be clinically useful in stratifying patients into low risk groups, which may be followed conservatively, and high risk groups that may require extensive post biopsy surgical procedures to prevent recurrence and to rule out invasive disease with an aggressive phenotype.<sup>6-7</sup> HER-2 /neu gene expression has generally not been specifically implicated in progression or prognosis assessment of lobular carcinoma of breast, but it is strongly associated with increased disease recurrence and a poor prognosis.<sup>8</sup> Over expression is also known to occur in ovarian, stomach, and aggressive forms of uterine cancer.<sup>9</sup> Multiple studies reported a significant co-relation of serum HER-2/neu protein levels with recurrence, metastasis or shortened survival. It also predicts the resistance of breast cancer to chemotherapy and absence of clinical response to hormonal therapy even when estrogen assays are positive.<sup>10</sup> Hence it is concluded that HER-2/neu amplification is an independent predictor of shorter disease with free survival in both node negative node and positive note patients.<sup>11</sup>

The objective of this study was to determine the prevalence of HER-2/ neu receptor amplification and its effect on the prognosis of the patients having breast cancer.

## METHODS

This retrospective cross-sectional study was carried out at the, Karachi Pakistan for a period of 03 years from 1<sup>st</sup> October 2014 to 30<sup>th</sup> September 2017. Sample size were calculated as 120 cases. After receiving ethical approval from the Institutional Review Board, all surgical patients operated for breast carcinoma on the basis of proven biopsy, sufficient record in hospital registry, having any stage of breast cancer and patients who received neo-adjuvant chemotherapy or radiotherapy were included in the study. Patients that were unfit for surgery due to severe co-morbidities and the patients, whose record present in hospital's registry was insufficient, were excluded from the study. Her-2/neu was assessed through immunohistochemistry. The paraffin-embedded blocks of breast cancer specimens were assessed for HER-2 status using a DAKO autoimmunostainer.<sup>12</sup>

Expression of Her-2/neu status was evaluated using the Hercep Test™. The score was determined by immunohistochemical analyses (Hercep Test™).

- Score 0 means that no staining or membrane staining that is incomplete and faint in <10 % of the tumour cells.
- Score 1<sup>+</sup> is also negative and indicates a faint/ barely perceptible membrane staining detected in >10% of

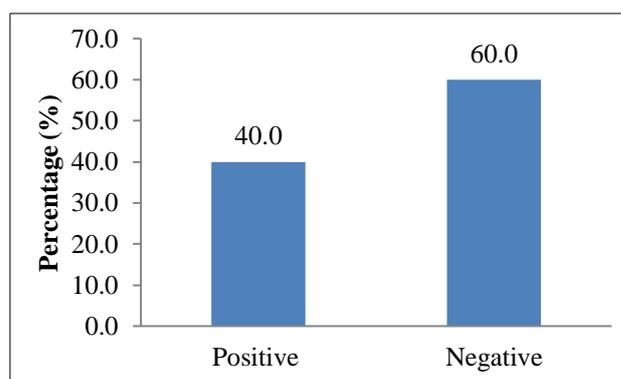
tumour cells. The cells are only stained in part of their membrane.

- A score 2<sup>+</sup> is equivocal or weakly positive that indicates a weak-to-moderate complete membrane staining in >10% of the tumour cells.
- A score of 3<sup>+</sup> by IHC was considered as strong positive and it shows circumferential homogenous dark staining in >10% of the tumour cells.<sup>13</sup>

Patients were followed for 20 to 36 months and at each follow up, prognosis of the breast cancer in terms of local recurrence, distant metastasis and disease free survival were recorded. SPSS version 20.0 was used for data analysis. For qualitative data, frequency and percentages were reported. Bar graph was used for to present the positive and negative cases of HER-2 neu.

## RESULTS

A total of 120 operated patients with biopsy proven breast carcinoma were included in this study. Information from the patients was collected after taking informed consent from patient, spouse or guardian. The data recorded, included clinical staging, patients taking neoadjuvant therapy or radiotherapy. Frequency of HER2/neu expression and each parameter has been depicted in the following tables and figures. Immunohistochemistry for the assessment of Her2/Neu was done with microscopic examination. The recorded results were then tested and associated with breast cancers prognosis. A total of 120 patients selected for the study, 48(40%) of patients had +ve expression of HER-2/ neu while majority of the patients 72(60%) were found to be negative for HER2/neu (Figure 1).



**Figure 1: Incidence of HER-2/ neu status.**

From the total of 120 patients, the range of age recorded was from 31 to 64 years having a mean age of 46.44±8.48. These were divided into different groups and their frequency and percentage was reported. The groups included age groups between 30-40 years, 41-50 years, 51-60 years and 60 years above. Maximum frequency recorded was in the age group of 41-50 present found as 61(50.8%) cases. Minimum frequency was recorded in second age groups in 30-40 and above 60 years 15 cases

each (12.5% in each group). Age group of 51-60 years had 29(24.1%) patients.

Among the 48 HER-2 positive patients, 17(35.4%) patients were reported to have local recurrence within 03 years while 21(43.7%) of patients were found to have distant recurrence. Among the 72 HER-2/neu negative patients, 20 (27.7%) of patients had local recurrence while 23 (31.94%) patients had distant recurrence within 03 years (Table 1).

**Table 1: Comparison of disease free survival among patients with Her-2 +ve and Her-2 -ve.**

Recurrence within 3 years	Her-2 +ve n= 48	Her-2 -ve n= 72
Local recurrence	17 (35.4 %)	20 (27.7 %)
Distant recurrence	21 (43.7%)	23 (31.94 %)

The disease free survival rate in 03 years was observed in 22(45.83%) out of 48 HER2 positive patients while 42 disease survival in three year (58.33%) out of 72 HER-2 -ve patients (Table 2).

**Table 2: Disease free survival within period of 3 years follow up.**

Receptor status	Disease free survival in 3 years
Her2 +ve	22 patients out of 48 (45.83 %)
Her2 -ve	42 patients out of 72 (58.33 %)

**DISCUSSION**

Around 1 in 5 types of breast carcinomas possess the gene mutation which causes excess of HER-2. Along with gene mutation, elevations in HER-2 levels can occur in other cancers in addition to breast cancers.<sup>14</sup> However, genetic mutation is limited only to cancers but the gene mutation is not transmitted generation to generation.<sup>15</sup>

It has been reported in a study that cancers having such gene mutations are mostly more aggressive than other type of breast carcinomas. Additionally, they are normally less responsive to hormonal treatment as compared to other cancer types. In another study it has been reported that multiple effective treatments are practiced which target the HER-2 positive breast cancers.<sup>16</sup> Furthermore, several other treatment regimens also persists which target HER-2 specific breast carcinomas which are currently under testing in clinical trials. At present, the standard chemotherapy drugs continue to be used for treating HER-2 positive cancers although they do not specifically target HER-2/ neu proteins.<sup>17</sup>

Results of this study reported that among 40% HER-2 positive female patients, local recurrence within 03 years was observed in 35% of patients while distant recurrence was seen in 44% of patients. Disease free survival within

03 years of follow up was reported for 46% of patients having HER-2 positive while 58% among HER-2 negative patients.

Studies have reported a significant co-relation of HER-2/ neu protein levels with recurrence, metastasis, shortened survival and predicted resistance of the breast cancer to chemotherapy with absent response to conventional hormonal therapy clinically even when estrogen assays are positive.<sup>18</sup> Therefore it can be concluded that HER-2/ neu amplification could be regarded as an independent predictor for shorter disease free survival amongst both node positive as well as node negative patients.<sup>19</sup> A study reported that 5 year disease free survival rate at 86.4 % and 97.2 % among patients having HER-2 +ve and HER-2 -ve cancers (p<0.001).<sup>20</sup> Another study found that HER-2 over-expression was present in around 20-30 % of all breast cancers.<sup>21</sup> A study observed that amplification or over-expression of HER-2 gene was seen in approximately 15-30 % of breast carcinomas.<sup>22</sup> In a study by Niwińska et al, HER-2 gene was reported to be significantly predicted to both overall survival (p<0.001) and time lapse (p<0.001).<sup>23</sup> In another study by Kamil et al, HER-2 expression was studied in node-negative breast carcinomas and was reported that among females with breast carcinoma having high over-expression of HER-2 were found to have 9.5 times greater risk for recurrence when compared to breast carcinomas with (-ve) HER-2 expression (p=0.001).<sup>24</sup> Yet another study observed that HER-2 amplification was linked to significant shorter disease-free survival (p=0.0027).<sup>25</sup>

Studies have reported that HER-2 amplification has also been significantly associated to pathological state of disease, the number of nodes involved in the tumour, type of histology as well as absent estrogen and progesterone receptor. It is seen that HER-2 over-expression is one of the early events in female breast carcinogenesis.<sup>26</sup> Over-expression of HER-2 is reported in almost half of all in situ ductal cancers regardless of evidence of invasiveness of the tumour and in which HER-2 status is maintained throughout cancer's progression to invasive disease, metastasis from lymph nodes as well as distant metastasis. Breast carcinomas with HER-2 over-expression have been found to be more sensitive to some specific cytotoxic chemotherapeutic agents and resist some certain hormonal treatments while showing increase in propensity for metastasis to brain.<sup>27</sup>

HER-2 expression in gastric cancers has been reported in around 10 to 30% of cancers and has correlated with poor outcomes due to a more aggressive disease.<sup>28</sup> Similarly, HER-2 expression in esophageal cancer has been reported with a wide range from 10 to 83% and in ovarian cancer between 20 to 30% and associated with poor disease free survival time period.<sup>28,29</sup> HER-2 expression in endometrial cancers has been found in around 14 to 80% of such cancers.<sup>30</sup> Over-expression of HER-2 in other cancers such as lung cancer has been reported in around 20% of such cancers and all have been

associated with poor outcomes.<sup>31</sup> Similar to the above reported ranges in our study as well, the reported disease free survival period was reported in only 46% of patients having HER-2 positivity.

Since HER-2 positivity significantly affects prognosis of disease, therefore it is important to detect and assess its status before undergoing any treatment therapies. Besides its (Her-2 receptor) resistance to multiple chemotherapy agents, it cause the increased recurrence rate of disease at same or distant site, hence influence the disease free survival as well and this is because again due to resistance of receptor towards many chemotherapy drugs.

Limitation and recommendations of the study was not free from limitations such as selection and observer bias and the fact that the study was conducted at a single centre with limited sample size. Therefore further multi-centered studies with greater sample size should be charred out would help in attaining better knowledge of research on the subject.

## CONCLUSION

According to the results of the study, 40% of patients in the study reported HER-2/ neu positivity among breast cancers and it was associated with poor outcomes and disease free survival time period in comparison to patient with HER-2/ neu receptor negative breast cancers.

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