Study of HER2/neu status in Qatari women with breast carcinoma

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ABSTRACT

Objectives: The study is aimed at determining the prevalence of HER2/neu overexpression in Qatari women with breast cancer and to assess the survival in patients with HER2/neu positive tumors.

Methods: This is a retrospective study of clinical data of 70 Qatari female patients diagnosed with breast cancer during the period 1991 through to 2001, at Hamad Medical Corporation, Doha, Qatar. We also performed a retrospective review of breast tissue sample for those patients using paraffin sections and applying immunohistochemistry staining-[Hercep test (DAKO Inc)] to determine the HER2/neu status.

Results: Eighteen patients (26%) were HER2/neu positive (2+ and 3+) with a mean age at diagnosis of 49.3 years, and 52 (74%) were negative (0 and 1+) with mean age at diagnosis of 46.6 years. Of the patients with positive HER2/neu, 5 (28%) had a relapse of the disease and 4 (22%) died of the disease during follow up. Of the patients with HER2/neu, negative test 9 (17%) had a relapse of the disease and 10 (19%) died of the disease. The median survival function at mean of covariates for HER2/neu positive patients was 26 months, and for HER2/NEU negative patients was 28 months.

Conclusion: The prevalence of HER2/neu over expression in Qatari female with breast cancer in this study is 26%, but due to a small sample size it may not reflect really the prevalence. Patient with HER2/neu positive were older at diagnosis than patients with HER2/neu negative, also they had higher relapse rate and mortality. Median survival function was better for HER2/neu negative patients.


Until recently the only biologic marker that has been utilized in decision making regarding specific treatment in breast cancer was the estrogen receptor (ER) or progesterone receptor (PR), or both. For the last 20 years advances in the molecular biology highlighted numerous tumor-associated markers, the most promising of these new markers was the HER2/neu.1 Human growth factor receptor HER2 gene is a proto-oncogene encoding the HER2 receptor. It is well known that in approximately 20-40% of patients with breast cancer tumor cells show an amplification or over expression of the tyrosine kinase receptor HER2/neu (c-erbB2), or both.2,3 This protein (p185) is a member of the epithelial HER family. This group also includes the epidermal growth factor receptor HER1, HER3 and HER4. In the epithelial cells more than 5 gene copies is an appropriate practical cut-off point for defining amplification and more than 10 gene copies is common in amplified state. HER2 gene amplification increases HER2 gene transcription, producing raised HER2 messanger ribonucleic acid (mRNA) levels and increased synthesis of HER2 protein. The HER2 protein is consequently over expressed on the cell surface. It is known that HER2 plays a central role in signal transduction by the HER family. HER2 amplification/over expression causes increased

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Methods. Study population. All Qatari women with invasive breast cancer (total 79 patients) who were diagnosed and treated at Hamad Medical Corporation, Doha, Qatar, during the period 1991 through to 2001 were included. Their files were reviewed, and clinical data collected. The paraffin sections of 70 out of 79 patients were available for study by IHC staining using HECEP TEST (Hoffman-La Roche) kits.

Hercep test immunohistochemistry assay procedure. For Hercep test study 3-4 microns thick sections were cut from the paraffin block, mounted on charged slides. Deparaffinizes in xylene and rehydrated in descending grades (95-100%) of ethanol. Sections then subjected to a heat-induced epitope retrieval by microwave for one minute in high power, then for 40 minutes in low power. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 5 minutes. Fluorescence in situ hybridization (FISH) is a direct method to detect amplification of the HER2 gene. This technique uses fluorescent deoxyribonucleic acid (DNA) probes to identify increased copies of the HER2 gene. The assay is rapid, nonradioactive, and requires little tumor material. Fluorescence in situ hybridization can be used to identify gene amplification in formalin-fixed, paraffin-embedded tissue samples. Determination of HER2/neu status appear to be of prognostic and predictive value and can be readily performed in most hospitals as part of the routine assessment for breast cancer patients. Amplification or over expression of c-erbB-2, or both may be associated with a poor prognosis, and has also been associated with relative sensitivity or resistance to several therapies, including endocrine therapy, chemotherapy, radiation therapy, and Herceptin (trastuzumab). Knowing HER2 status may help select the most appropriate therapy for individual patients. Standardization of the assay to assess HER2 gene amplification or receptor over expression is necessary so that it could be integrated into routine tumor marker testing. Furthermore, the HER2 receptor protein has now become a valid target for therapy as it provides an extracellular target for novel and specific anticancer treatment such as anti-HER2 monoclonal antibody "Herceptin".

Results. The age of the patients at diagnosis ranged from 20 to 77 years, median (46 years). Tumor size, T1 was 14, T2 were 33, T3 were 12 and T4 were 6 patients. The median number of lymph nodes removed 11, range (0-25), N0 were 32, N1 were 27, and N2 were 3 patients. Metastasis (M), 66 patients were M0, 3 were M1 and 1 unknown M status. Histopathology included infiltrating ductal carcinoma in 63, lobular carcinoma in 2 mediulcar carcinoma in 3 and unknown in 2 patients. The grade G1 in 3, G2 in 22, G3 in 22, and unknown in 24 patients. Estrogen Receptor positive in 36 and negative in 34 patients. Progesterone Receptor was positive in 31, and negative in 39 patients. Fifty-two (74%) patients demonstrated no protein over expression (0 and 1+) (Figures 1 & 2) with a mean age at diagnosis of 45.5 years (20-77). HER2/neu protein over expression (2+ and 3+) was detected in 18 (26%) out of 70 cases (Figures 3 & 4), the mean age at diagnosis was 49.3 years (33-74). HER2/new positivity defined as 2+ or 3+ on a 0-3 scale. In the HER2/neu positive group 11 patients (61%) had lymph node involvement, while in the HER2/neu negative group 21 patients (40%) had lymph node involvement. Breast Conserving Surgery (BCS) carried out in 39 patients, modified radical mastectomy in 30 patients and type of surgery was unknown in one patient. Fifty-three patients received hormonal therapy and 17 did not receive it. The median progression free survival for the whole group was 68.9 ± 6 months (Kaplan-Meier). The progression free survival was not statistically significant for HER2/neu 2+, 3+ versus others (P=0.22). In univariate analysis, overall survival was not statistically significant for HER2/neu 2+, 3+ versus others (P=0.78). In multivariate analysis for age nodal status ER/PR, HER2/neu and grade Cox proportional hazard model did not predict any independent variable for survival (P=0.27).
Figure 1 - Hercep 0 (Negative). No membrane staining.

Figure 2 - Hercep 1+ (Negative). The tumoral cells show incomplete membrane staining.

Figure 3 - Hercep 2+ (Positive). Weak to moderate complete membrane staining in >10% of the tumoral cells.

Figure 4 - Hercep 3+ (Positive). More than 10% of the tumoral cells with strong and complete membrane staining.

Figure 5 - Survival function curve.

Figure 6 - Hazard function curve. Cum - cumulative
and diminished survival. High-risk patients with the propensity for early relapse suggests that overexpression of HER2/neu protein is an important molecular marker to identify a subset of patients. In the adjuvant setting, it would be of value, with some studies having used this receptor overexpression as an indicator of poor prognosis.

Expression of HER2/neu appears to be associated with a worse prognosis in patients with breast cancer. Therefore, assessment of HER2/neu status is crucial. Overexpression of HER2/neu has been shown to be associated with worse prognosis in patients with HER2/neu positive breast cancer and possibly with a worse prognosis in patients with HER2/neu negative breast cancer. Determination of HER2/neu status is a simple and accurate test for predicting prognosis.

A number of techniques have been used to assess HER2/neu expression in breast cancer. These include immunohistochemistry (IHC), in situ hybridization (ISH), fluorescent in situ hybridization (FISH), and others. IHC is a widely used technique that involves staining tissue sections with antibodies against HER2/neu protein. FISH is a technique that involves the use of fluorescent probes to detect gene amplification. Both techniques have advantages and disadvantages, and the choice of technique depends on the availability of resources and the specific research question.

Table 1 - Lymph node status and the stage of the disease in relation to HER2/neu status.

<table>
<thead>
<tr>
<th>HER2/neu status</th>
<th>HER2/neu positive n (%) of patients</th>
<th>HER2/neu negative n (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Node positive</td>
<td>22 (61)</td>
<td>21 (40)</td>
</tr>
<tr>
<td>Node negative</td>
<td>5 (28)</td>
<td>26 (50)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (11)</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Disease stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>0 (0)</td>
<td>8 (15)</td>
</tr>
<tr>
<td>Stage II</td>
<td>15 (83)</td>
<td>37 (71)</td>
</tr>
<tr>
<td>Stage III</td>
<td>1 (5.5)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>1 (5.5)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Unstaged</td>
<td>1 (5.5)</td>
<td>2 (4)</td>
</tr>
</tbody>
</table>

In the HER2/neu positive 5 (28%) patients had disease relapse, 4 (22%) died of the disease, while in the negative group only 9 patients (17%) had disease relapse and 10 (19%) died of the disease. Overall survival was better for the HER2/neu negative patients than the HER2/neu positive patients.

Discussion. Until recently, the only biologic marker that has been utilized in decision making regarding specific treatment in breast cancer was the ER or PR, or both. For the last 20 years advances in the molecular biology enabled researchers to discover a number of tumor-associated markers, the most promising of these new markers was the HER2/neu.

Overexpression of HER2/neu has been shown to be associated with a worse prognosis in patients with HER2/neu positive breast cancer and possibly with a worse prognosis in patients with HER2/neu negative breast cancer. Determination of HER2/neu status appears to be of prognostic and predictive value for patient and physician, and can be readily performed in most hospitals as part of routine clinical assessment for breast cancer patients (Table 1). The development of HER2 assay that is simple to apply is crucial. Standardization of the assay to assess HER2 gene amplification or receptor overexpression is necessary so that they can be integrated into routine tumor marker testing. Further more, the HER2 receptor protein has now become a valid target for therapy because it provides an extracellular target for novel and specific antitumor agents.

The HER2 receptor protein has now become a valid target for therapy because it provides an extracellular target for novel and specific antitumor agents. The HER2 receptor protein is an important molecular marker to identify a subset of high-risk patients with the propensity for early relapse and diminished survival. The assessment of the HER2 status has gained increasing importance in the clinical management of patients with breast cancer. HER2 overexpression in node positive cases is linked to poorer prognosis that is longer disease free interval and shorter survival time, and a similar link age might also exist in node negative cases. A number of techniques have been used to assess protein or mRNA expression and clearly IHC appears to be the most suitable for this purpose. Recently, a standardized IHC kit for the evaluation of HER2/neu protein overexpression (Hercep test) has been approved by the Food and Drug Administration (FDA) represent an IHC test kit using rabbit polyclonal antibody with standardized procedures and evaluation criteria. The assay is rapid, nonradioactive, and requires little tumor material. It is used to identify HER2 overexpression in fresh, frozen, or paraffin-embedded tissue samples. Fluorescence in situ hybridization is a direct method to detect amplification of the HER2 gene. This technique uses fluorescent DNA probes to identify increased copies of the HER2 gene. The assay is rapid, nonradioactive, and requires little tumor material. Fluorescence in situ hybridization can be used to identify gene amplification in formalin-fixed, paraffin-embedded tissue samples. Both the IHC and the FISH produce useful and accurate information if performed correctly. It is believed that HER2/neu overexpression is likely to be associated with poor prognosis, in our study patients with HER2/neu positive receptors 11 patients (61%) had lymph node involvement while in the patients with HER2/neu negative receptors 21 patients (40%) had lymph node involvement. Due to the variety of tests of different accuracy used to determine HER2 status over the past 20 years this association remains controversial. In the meta-analysis by Ross and Fletcher only 6 of the 47 studies failed to reveal any association between HER2 status and the prognosis; 4 of these used the IHC to detect HER2 overexpression and 2 used the Southern blotting and slot-blot analysis of HER2 gene amplification. Thus 41 studies revealed at least univariate correlation between HER2 status and breast cancer prognosis whatever technique is used. Ross and Fletcher stress that IHC on fresh or frozen sections would be an ideal method of detection.

There is evidence that women in whom tumor overexpression of HER2/neu are likely to get greater benefit from therapy with anthracycline-containing regimens than from alkylating agents. It is therefore, reasonable to assess HER2/neu on all primary breast tumors at the time of diagnosis.

In contrast, to most studies, our patients with HER2/neu positive receptor were older than those with HER2/neu negative receptors. Mean age at diagnosis was significantly higher in patients with HER2/neu positive. In our study patients with HER2/neu positive receptors were 49.3 years while for those with HER2/neu negative it was 46.6 years. The median progression free survival for the whole group was 68.9 ± 6 months (Kaplan-Meier). The progression free survival was not statistically significant for HER2/neu 2+, 3+ versus others (P=0.22). In univariate analysis, overall survival was not statistically significant for HER2/neu.
HER2/neu positive versus HER2/neu negative (P=0.78). In multivariate analysis for age nodal status ER/PR, HER2/neu and grade Cox proportional Hazard model did not predict any independent variable for survival (P=0.27) (Survival curve Figure 5). The hazard function at mean of covariates was higher for HER2/neu positive patients (hazard function curve Figure 6).

In conclusion, the prevalence of HER2/neu receptor in Qatari women with breast cancer was 26%. Unlike other studies, the mean age of patients with HER2/neu positive was higher than HER2/neu negative patient and this may in part is due to small sample size. Lymph node involvement was higher in HER2/neu positive patients. The relapse rate and mortality was higher in the HER2/neu positive group. Overall patients with HER 2 neu over expression had shorter median survival function at mean of covariates, and higher hazard function at mean of covariates but all these were statistically not significant.

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References