Clinicopathological Characteristics of Triple-Negative Breast Cancer

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Abstract

Background: Triple Negative Breast Carcinomas (TNBCs) are a group of primary breast tumors with aggressive clinical behavior that have no targeted therapy at present. Comparative studies for subtypes of triple negative group of invasive breast carcinoma are needed to detect any specific morphologic features of this group.

Results: The study included 40 cases of triple negative breast cancer. All cases were subjected to pathological study regarding gross and microscopic features. Results showed that 25 cases (62.5%) were invasive ductal carcinoma (NOS), 10 cases (25%) were medullary and 5 cases (12.5%) were metaplastic. The majority of cases were found to be T2 (17 cases representing 42.5%). 11 cases (27.5%) showed negative axillary node deposits, 11 cases (27.5%), showed deposits in 1-3 lymph nodes; 14 cases (35.0%) showed 4-9 nodal deposits and only 4 cases (10.0%) showed more than 9 nodes (10%).

According to "Modified Bloom Richardson grading system", 24 cases (60%) were GIII.

Key Words: Basal phenotype breast cancer – EGFR – Targeted therapy – TNBC – Triple negative breast cancer.

Introduction

It is only in the last few years that professionals studying breast cancer have concluded that breast cancer is not one disease, but many different forms of cancer, all originating in the breast. Among the 1 million or more women worldwide who are diagnosed with breast cancer annually, an estimated 170,000 may be classified as having triple negative breast cancer [1].

Human breast cancers are heterogeneous in their morphology, clinical course and response to therapy. This heterogeneity may originate due to differences in the underlying target cell population and/or it may be the result of different combinations of mutations in a normal breast stem cell or committed progenitor cell [2].

Triple Negative Breast Carcinomas (TNBCs) are a group of primary breast tumors with aggressive clinical behavior [3]. TNBC has not only a common pattern of molecular and histologic characteristics but also distinct patterns of epidemiology and risk factors. Depending on its stage of diagnosis, triple negative breast cancer can be extremely aggressive and more likely to recur and metastasize than other subtypes of breast cancer [4].

Most TNBCs possess a Basal Phenotype (BP). The importance of recognizing these tumors came to light largely as the result of gene expression profiling studies that categorized breast cancer into 3 major groups. Two of these groups are defined by their respective expression of estrogen receptor and HER2-neu. TNBCs represent a third group and are defined by negativity for hormone receptors and HER2-neu [5].

Although adjuvant hormone therapy was shown to be effective for ER-positive breast cancers and adjuvant Herceptin therapy was also shown to improve the survival of HER-2-neu positive breast cancers TNBCs have no targeted therapy at present [6].

Material and Methods

Tissues:

The material of this study included 40 cases of invasive breast carcinoma.

The cases included formalin-fixed, paraffin-embedded tissue sections of previously diagnosed cases as well as fresh specimens obtained during the work of this study (January 2009 to December 2011). Serial sections of 5 microns thick were
prepared from each paraffin block; one of them was mounted on glass slide and stained by Hema-

toxylin and Eosin (H & E) for histological eval-

uation. Three were mounted on charged glass slide for immunohistochemical staining for Estrogen (ER), Progesterone (PR) receptors and Her-2neu.

Cases:

Clinical history:

Clinical history includes the age of patient; operative findings (whether modified radical mastectomy or breast conservative surgery). Cases of this study included 32 Modified Radical Mastectomy specimens (MRM), representing 80% of the study group, and 8 Conservative Breast Surgery specimens (CBS) representing 20% of the study group.

Pathological findings:

Pathological findings include gross as well as histopathological data. Data concerning gross description were obtained from previous reports as well as recording these features in fresh specimens received during the work of this study.

The detected pathologic data include: Size of the tumor, histologic subtype, regional lymph node status as regards the absence or presence of metastatic tumor deposits, presence of ductal carcinoma in situ and specification of its type and its percentage from the tumor bulk, marked lymphoplasmacytic reaction, tumoral necrosis and finally the tumor grade: According to Nottingham modification of the Bloom-richardson system [7].

According to nottingham modification of the bloom-richardson system:

Tubule formation: 1 point: Tubular formations in >75% of the tumor, 2 points: Tubular formations in 10-75% of the tumor and 3 points: Tubular formations in <10% of the tumor. Nuclear pleomorphism: 1 point: Nuclei with minimal variation in size and shape, 2 points: Nuclei with moderate variation in size and shape and 3 points: Nuclei with marked variation in size and shape. Mitotic count: 1 point: 0-5, 2 points: 6-10 and 3 points: >11.

In this scheme, the grade is obtained by adding up the scores of tubule formation, nuclear pleomorphism and mitotic count, each of which is given 1, 2 or 3 points. This results in a total score between 3 and 9 points, which is translated into the final grade by the following formula: 3-5 points: Grade I, 6-7 points: Grade II and 8-9 points: Grade III.

Immunohistochemistry:

Histologic sections were studied by immunohistochemistry with ER, PR and Her-2 using a standard avidin-biotin-peroxidase system.

Histologic sections approximately 4µm thick were deparaffinated in xylene and alcohol, rehydrated in distilled water for 5 minutes, and then washed in PBS for 5 minutes. To reveal the antigens, they were pre-treated by boiling them in a microwave 3 times for 10 minutes each with antigen retrieval solution, then washing them in PBS for 5 minutes.

The primary monoclonal antibodies to ER, PR and Her-2 (Dako, Denmark) were incubated for 60 minutes at 37°C. The sections were washed in PBS for 5 minutes.

A secondary antibody was applied for 60 minutes (Dako, Denmark). Two drops of the horseradish peroxidase conjugated streptavidin were added. The slides were incubated for 60 minutes, and then rinsed with PBS was performed. Finally, the reaction was visualized with DAB chromogen (Dako, Denmark). The slides were counterstained with dilute hematoxylin, and rinsed with ammonia and then with tap water. The sections were dehydrated with graded ethanol, cleared in xylene, and then cover slipped.

Histologic evaluation:

Tumour tissue sections were examined and scored under the microscope at high power magnification. As regards the evaluation of hormone receptors (ER and PR) in breast cancer tissue, the two parameters regarded in immunohistochemical preparations are the number of tumor cell nuclei stained and the intensity of the reaction. The American Society of Clinical Oncology (ASCO) in conjunction with the College of American Pathologists (CAP) produced guidelines for Estrogen Receptor (ER) and Progesterone Receptor (PR) testing in breast cancer [8].

The 2010 guidelines recommend that ER and PR should be recorded in a semi quantitative manner. In Harvey et al., study showing the clinical validity of ER and PR immunohistochemistry, the proportion and intensity of ER staining was converted into a total immunohistochemical score that is now known as the Allred score [9,10]. The Allred score combines an estimated proportion score on a scale of 0 to 5 with an average intensity score of 0 to 3 [11].

This reporting system is graded on a scale of 0 to 8, with 0 indicating a completely negative
result and 2 to 8 used as a means of semi quantifying the immunoreactivity. The Allred score has a limited dynamic range (0-8) because it is derived from a summation of proportion and intensity score [11,12].

Membranous staining for Her-2 was scored as score 0 and score 1 for negative, score 2 for weak, score 3 for strong membranous immunostaining [7]. (Table 1).

The positive control for ER, PR and Her-2neu staining was a section of known case of duct carcinoma with strong positive staining for ER, PR and Her-2neu respectively. Normal breast tissue as well, reacted as positive internal control for both ER and PR. The selected 40 cases of the study were the cases with triple negative immune profile.

Statistical analysis:

Data was analyzed using SPSSwin statistical package version 17 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. Results were considered to be statistically significant at \( p < 0.05 \).

Results

Histologic evaluation:

The present study included 40 cases of invasive breast carcinoma which are negative to Estrogen receptor, Progesterone receptor and Her-2-neu (triple negative).

The results of this study included clinical results (age of the patients and the type of surgical operation), pathological results (such as histological subtypes, tumor stage, grade lymph nodal status and others) and immunohistochemical results.

The ages of the patients of the whole triple negative study population ranged between 38 and 71 years with a mean age of 48.4 years. Twenty seven cases were less than or equal 50 years (representing 67.5% of the study group), and 13 cases were older (representing 32.5%).

The histological subtypes included 25 cases of invasive ductal carcinoma (NOS) constituting (62.5%) of the cases. Whereas 10 cases were of the medullary variant (25%) and 5 cases were metaplastic (12.5%). See (Table 2) and Figs. (1-3).

The majority of the cases (67.5%) showed no intra duct component (27 cases), whereas (32.5%), showed associated intraductal in situ changes (13 cases). As regards the histological types of the in situ component detected, varied between cribriform, comedo, and solid patterns. And the percentage of the intraductal carcinoma in situ, ranged between 5% and 40%. See (Table 3).

As regarding tumor size, the majority of cases were found to be T2 (17 cases representing 42.5%), 12 cases T3 (representing 30%), 7 cases were in T1 (representing 17.5%), and only 4 cases were end T4 (representing 10%). See (Table 4). As regarding axillary lymph nodes status, 11 cases (27.5%), showed negative axillary node deposits, 11 cases (27.5%), showed deposits in 1-3 lymph nodes; 14 cases (35.0%) showed 4-9 nodal deposits and only 4 cases (10.0%) showed more than 9 nodes (10%) See (Table 5).

Twenty-four cases were negative for tumoral necrosis (60%), while the other 16 cases showed necrosis (40%). By re-examining the selected cases, 6 cases (15%) showed vascular invasion, and 34 cases (85%) were negative for invasion. See Fig. (4).

Ten cases (25%) of the study group showed marked lymphoplasmacytic reaction; and 30 cases (75%) were negative. The majority of cases were found to be T2.

As regarding tumor grade, 24 cases were GIII, constituting (60%) of the cases, and 16 were GII (40%); the high grade tumors ranged between invasive ductal carcinoma (NOS), medullary and metaplastic histological subtypes.

Table (1): Grading of the immunohistochemical staining of HER-2-neu overexpression regarding both the staining intensity as well as the percentage of stained tumour cells [7].

<table>
<thead>
<tr>
<th>Staining pattern</th>
<th>Score HER-2-neu result</th>
</tr>
</thead>
<tbody>
<tr>
<td>No staining is observed or membrane staining in less than 10% of the tumor cells</td>
<td>Negative</td>
</tr>
<tr>
<td>A faint membrane staining in more than 10% of the tumor cells. The cells are only stained in part of their membrane.</td>
<td>1+ Negative</td>
</tr>
<tr>
<td>A weak to moderate staining is observed in more than 10% of the tumor cells.</td>
<td>2+ Weakly positive</td>
</tr>
<tr>
<td>A strong complete membrane staining is observed in more than 10% of the tumor cells.</td>
<td>3+ Strongly positive</td>
</tr>
</tbody>
</table>
Table (2): Histological subtype in the selected cases.

<table>
<thead>
<tr>
<th>Histological subtype</th>
<th>Number of cases</th>
<th>Percentage</th>
<th>DCIS</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDC (NOS)</td>
<td>25</td>
<td>62.5</td>
<td>Absent</td>
<td>27</td>
<td>67.5</td>
</tr>
<tr>
<td>Medullary</td>
<td>10</td>
<td>25.0</td>
<td>Present</td>
<td>13</td>
<td>32.5</td>
</tr>
<tr>
<td>Metaplastic</td>
<td>5</td>
<td>12.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100.0</td>
<td>Total</td>
<td>40</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (3): Intra duct component in the selected cases.

<table>
<thead>
<tr>
<th></th>
<th>Number of cases</th>
<th>Percentage</th>
<th></th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>27</td>
<td>67.5</td>
<td>Present</td>
<td>13</td>
<td>32.5</td>
</tr>
<tr>
<td>Present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100.0</td>
<td>Total</td>
<td>40</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (4): Tumor size in the selected cases.

<table>
<thead>
<tr>
<th>Tumor size</th>
<th>Number of cases</th>
<th>Percentage</th>
<th>Nodal status</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>7</td>
<td>17.5</td>
<td>T0</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>T2</td>
<td>17</td>
<td>42.5</td>
<td>T1</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>T3</td>
<td>12</td>
<td>30.0</td>
<td>T2</td>
<td>14</td>
<td>35.0</td>
</tr>
<tr>
<td>T4</td>
<td>4</td>
<td>10.0</td>
<td>T3</td>
<td>4</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100.0</td>
<td>Total</td>
<td>40</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (5): Nodal status in the selected cases.

Fig. (1): Medullary carcinoma GIII (X 200).

Fig. (2): Metaplastic carcinoma GIII with squamoid features (X 200).

Fig. (3): Invasive duct carcinoma, with spindle cell features, GIII (X 200).

Fig. (4): Metaplastic carcinoma GIII with prominent angio-invasion, (arrow), (X 200).
Discussion

The present study included 40 cases of triple negative breast cancer. The ages of the patients ranged between 38 and 71 years with a mean age of 48.4 years. These results were close to the results recorded by Anderson et al., in 2006 [18], with a mean age of 50 years. Salomon et al., 2007 [14], reported an older age of patients with a mean age of 60 years. This discrepancy may be attributed to small sample size or to racial and geographic differences. 67.5% of our study group were less than 50 years; this finding agreed with the results of Dolle et al., in 2009 [15].

Regarding the histological subtypes, invasive duct carcinoma (NOS) represented the majority of cases. This subtype accounted for 62.5% of all cases included in this study, close results were reported by Kumar et al., 2005 [16], where invasive ductal carcinoma (NOS) constituting 61.9% of the cases. Chen et al., 2007 [17], reported that invasive ductal carcinoma (NOS) constituted 97% of their study cases. Medullary variant represented 25% and the rest of cases were metaplastic (12.5%). The percentage of metaplastic carcinoma was higher than that stated by Livasy et al., 2006 [18], where it constitutes 9% of the cases (Table 2).

As regards axillary lymph nodes status, 11 cases (27.5%), showed negative axillary node deposits, 11 cases (27.5%), showed deposits in 1-3 lymph nodes; 14 cases (35.0%) showed 4-9 nodal deposits and only 4 cases (10.0%) showed more than 9 nodes. Thus cases with axillary lymph nodes deposits represented 72.5% of the study cases; this disagreed with Nielsen et al., 2004 [19], who detected regional nodal metastasis in only 39% of the cases.

Moreover, Bhargava et al., in 2009 [20], stated that triple negative tumors show a low tendency for lymph nodes metastasis. Also, Nassar et al., 2010 [21], in their study, mentioned that triple negative carcinomas have a low incidence of axillary lymph node involvement (accounted for 15% of their study group). Thus; data concerning lymph node metastasis are conflicting. Although some have reported higher prevalence of lymph node metastasis, others found no difference or even lower rates of lymph node involvement Dent et al., 2007 [22], found that in the triple-negative group of breast cancers, there was no correlation between tumor size and node status among women with tumors smaller than 5cm. It is interesting to note that Foulkes et al., 2003, found the same discrepancy between tumor size and lymph node status in women with BRCA1-related breast carcinomas [23].

As regards ductal carcinoma in situ, the majority of the cases (67.5%) showed no intra duct component. As regards the histological types of the in situ component detected, it was of high grade, varied between cribriform, comedo, and solid patterns. Close results were reported by Lerma et al., 2007, where they found DCIS component in 45% of the cases [24]. Livasy et al., 2006 pointed out that the prevalence of high grade DCIS including comedo type suggest a probable precursor lesion for the associated invasive component [18].

Concerning tumor necrosis, 60% of the cases were negative for tumor necrosis, while the other 40% showed necrosis. These results disagreed with the results reported by Livasy et al., 2006, who found tumoral necrosis in 74% of cases; again, this discrepancy may be attributed to small sample size [18]. Triple negative tumors have a poor prognosis and extensive necrosis as a common feature of these aggressive tumors [17].

In the present study, only 25% of the study group showed marked lympho-plasmacytic reaction; this disagreed with the results reported by Livasy et al., (2006) [18], who recorded such reaction in 56% of their triple negative breast cancers; and also disagreed with the results recorded by Lerma et al., 2007, (49%). This variability observed could be explained by the absence of an agreed consensus for assessment of such finding [24].

In the current study, only 15% of the cases showed vascular invasion, and 34 cases (85%) were negative for invasion. Close results were reported by Billar et al., 2010, where they found vascular invasion in 18% of the cases [25]. However Gauchotte et al., 2011, reported vascular emboli in 30% of metaplastic carcinomas [26].

Regarding tumor size, the majority of cases were found to be T2 (42.5%). This agreed with the study made by Rakha et al., 2007, which stated that the majority of cases presented at stage 2; and attributed the large tumor size to the rapid rate of growth of these tumors [27].

Regarding tumor grade, 60% were poorly differentiated, and 40% were moderately differentiated; the poorly differentiated cases ranged between invasive ductal carcinoma (NOS), medullary and metaplastic histological subtypes. Thus all cases included in this study were high grade (none of the cases was Grade I). This agreed with Nassar et al., 2010, who found that the majority (77%) of cases were high grade [21].
Conclusions and recommendations:

Breast carcinoma with a triple negative immunophenotype (ER, PR and Her-2-neu negative) is a heterogeneous group of breast cancer; this mandates thorough search for a variety of biological markers that might serve as possible predictors for the biological behavior of the tumors and/or targets for possible therapeutic agents.

Certain histopathological features are frequently observed among triple negative breast cancer, including marked lymphoplasmacytic reaction, tumoral necrosis and atypical medullary features. Triple negative breast cancers are high grade tumors with modified bloom richardson Grade II and III.

The majority of basal breast cancer, medullary carcinoma and metaplastic carcinoma are triple negative cancers; none of the morphologic criteria is diagnostic, therefore immunohistochemical confirmation is mandatory.

Epidemiology based studies for triple negative breast cancer as well as its subsets are recommended to identify their incidence and risk factors. Clinicopathological as well as follow up studies on the triple negative breast cancer group are required, including a more expanded panel of immunohistochemical markers and prognostic markers.

Sensitive detection of triple negative breast cancer requires application of "FISH" technique for Her-2-neu stained cases having a score of +2 in order to be able to add cases with negative "FISH" to this group.

Ethical approval:

All procedures and the study were approved by the Ethical Group of Kasr El-Aini, Cairo University, Egypt.

References


