ORIGINAL ARTICLE

Frequency of triple negative breast cancer: a local experience at JPMC

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Abstract

Objective: To find out the frequency of triple negative breast cancer.

Study Design: Prospective case - series study

Place and duration of study: The study was conducted in General surgery, ward 2, Jinnah Post Graduate Medical Center, Karachi, from January 2008 to December 2009.

Patients and methods: It was a descriptive case series of 170 patients. All patients clinically examined and then underwent for radiological investigations including ultrasound scan and mammography, as well as trucut biopsy and metastatic work up have been done. Triple receptors (i.e. estrogen, progesterone and herceptin receptors) status also assessed to differentiate triple receptor negativity or positivity in all patients. Among 170 patients, 13 found to have borderline between Her-2 receptor, therefore we performed fluorescent in situ hybridization (F.I.S.H) test. Statistically different variables analyzed, like age, tumor size, lymph node status, grade of tumor and lympho vascular invasion.

Results: In our series of patients, the percentage of frequency was 30.58% (p = 0.001, chi sq = 9.62) with 95% C.I. Among 170 patients, 13 were having borderline Her - 2 receptor, therefore fluorescent in situ hybridization (F.I.S.H) test performed, so 08 were negative while 05 were positive, hence 55 found to be triple receptor negative. The mean age was 47.3 years range (20 - 75), while common age group was premenopausal. Majority of the tumors were grade III, about 69.2%. Most of the tumors 44.2% were of T2 size(2 – 5 cm). 40% of the triple negative breast cancer were node negative while 60% were lymph node positive, and in 34.6% Lymphovascular invasion was seen.

Conclusions: Triple negative breast cancer is more common among young women Clinical behavior among triple negative breast cancer cases is aggressive. It is a different entity of breast cancers which have no targeted therapy, therefore requires research at the molecular level.

Keywords: Triple Negative Breast cancer, Flourescent in situ hybridization (FISH) test, Lymphovascular Invasion.

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Introduction:

Breast cancer is the most common cancer in women, with over 180,000 new diagnoses of invasive disease annually in the United States, based on recent estimates.[1] Despite advances in therapy, over 40,000 women still die of breast cancer each year in the US.[1] There are currently 8000 to 10,000 (15-20% of total) new cases of triple-negative breast cancer per year in the UK. The term of triple-negative breast cancer originated from hierarchical clustering analysis of gene expression microarray studies[2,3]. This subgroup accounts for 15% of all types of breast cancer. It represents a subtype of breast tumors with unique molecular and clinical characteristics, distinctive risk factors and patterns of recurrence, association with BRCA1 mutation status, poor prognosis, and expanding therapeutic op-

tions. There is strong relation between triple negative and BRCA–1 gene.[4]

It frequently involves African and African-American women who are premenopausal. There are different intrinsic breast cancer subtypes, which include several luminal subtypes characterized by expression of hormone receptor–related genes, and two hormone receptor–negative subtypes—the HER2-positive/ER-negative subtype and the "basal-like" subtype. Triple-negative breast cancer lacks the ER/PR and HER2 expression, which are known to fuel most of the breast cancers. Multiple studies have proved short survival among patients with triple-negative.

Material and methods:

Our study constitutes 170 patients, among them 52 were triple receptor negative. This study was conducted in General surgery Department, Unit II, of Jinnah Postgraduate Medical Center, Karachi from January 2008 to December 2009.

It was a prospective study. Different variables like; age, tumor size, lymph node status, grade of tumor, Her–2 status, Estrogen, Progesterone receptors status lympho vascular invasion were entered in to a Performa for analysis and pathology reports were also reviewed.

Our patients presented with different symptoms like lump in the breast, pain and discharge from nipple. We examined clinically all the patients and advised for radiological imaging including ultrasound scan and mammography, followed by trucut biopsy of mass and metastatic workup was assessed after histopathological confirmation.

Triple receptors (i.e. ER, PR, and HER2/neu receptors) status was obtained through standard clinical testing, using immunohistochemistry for ER and PR as well as the HercepTest for HER2/neu. There were 13 patients in whom we performed FISH test to further validate triple receptor negative status, as these patients were having the HER2 score of 2+. For ER and PR test, the scoring was done according to Allred guidelines. In HER2/neu (Dako) test, Score of 0 and 1 were considered negative, and a score of 3 was considered positive but there is uncertainty about the appropriate classification of HER2/neu 2+ scoring, therefore fluorescent in situ hybridization (FISH) was used to determine the more appropriate results.

All patients were treated with modified radical mastectomy or breast conservation surgery.

The statistical variables were considered significant if the p value is <0.05. The data was analyzed with the help of statistical program SPSS, version 12. Chi–square test applied for the assessment of different variables. P value considered significant if found < 0.05.

Results:

During the two years, there were about 170 females, among them 52 (30.5%) found negative for all 3 receptors and 118 females (69,4%) were positive. The frequency of TNBC was 30.58% (p=0.001, chi - sq=9.62) with 95% C.I.

The mean age at diagnosis was significantly younger, that is 46.7 years (range 20 - 75) for the triple-negative group. Majority of our patients were in premenopausal age (Figure 1).

All patients presented with different presenting complaints and the average duration of symptoms was 2 - 6 months. The most common complain by the patients was lump in the breast and discharge from the nipple.

Patients of the triple-negative group commonly had grade III tumors whilst majority of the patients had tumor size of 2–5 cm (P < 0.01) (Table 1).

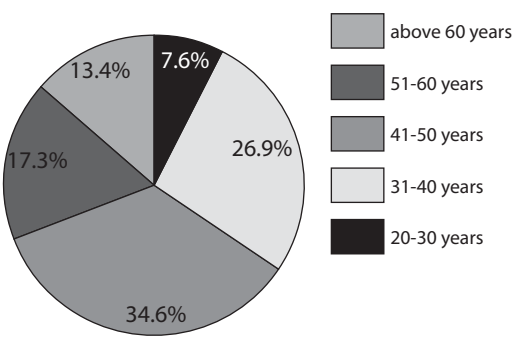

Figure 1: *Age distribution*

Table 1: *Grading and size of tumor in this study*

	No. of Patients(%)
Tumor grading	
Grade III tumors	36(69.2%)
Grade II tumors	15(28.8%)
Grade I tumors	1(1.9%)
Tumor size	
2–5 cm	23(44.2%)
More than 5 cm	20(38.5%)
Less than 2 cm	9(17.3%)

The rate of node positivity was slightly higher in the triple-negative group comprising 60%, in 19 (36.5%) 1–3 nodes were positive and in 12 (23.1%) > 3 lymph nodes were involved. 21(40%) patients were node negative. In our patients series one third (34.6%) of the patients had Lymphovascular invasion (Table 2).

Discussion:

Breast cancer is the most common cancer in women, with over 180,000 new diagnoses of invasive disease annually in the United States, based on recent estimates.[1] Despite advances in therapy, over 40,000 women still die of breast cancer each year in the US.[1]

Triple-negative tumors are negative for estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2), the so-called 'triple-negative' breast cancers. The term Triple-negative is based on clinical assays for ER, PR, and HER2, and its molecular phenotype initially defined by using cDNA microarrays.[5].

The Normal Breast Duct contains, central Luminal (glandular) cells, Basal/myoepithelial cells. All cells express numbers of factors like,: Luminal (glandular) cells expresses CK7, CK8, 18, CK19, MUC1 alpha-6 integrin, BCL1, ER, PR, and GATA3; while Basal/myoepithelial cells expresses: CK5/6, 14, 17, smooth muscle actin calponin, caldesmon, p63, beta-4 integrin, laminin, maspin, P-cadherin, caveolin-1, and nerve growth factor receptor (NGFR)).[6]

Table 2:

Variables	**Percentage**	**P - Value**	**Chi - square**
Frequency	30.58%	0.001 (Significant)	9.62
Grade III	69.2%	0.37 (Non Significant)	0.79
T2	44.2%	0.01 (Significant)	7.36
Nodal positivity	60%	0.17 (Non Significant)	1.82
Lymphovascular invasion	34.6%	0.001(Highly Significant)	574.79

TNBCs comprise approximately 15% of breast cancer cases [7]. In current study we found three fold 30.58% (52/170) frequency of TNBC which doesn't match with that of 15% international percentage, while Rebecca Dent et al[8] found 11.2% (180/1601) and Bruce et al[9] 24.2% frequency.

It has been usually observed that the majority of BRCA1-associated breast cancers are triple-negative and express a high proportion of basal-like cytokeratins (CK5, 14, 17), as well as P-cadherin and HER1/EGFR.[10]. BRCA1 is an oncogene that takes part in DNA repair, therefore mutation in this pathway holds therapeutic implications in the context of the triple-negative phenotype.

Population-based studies indicating that triple-negative breast cancers are more likely to occur among premenopausal women of African-American descent.[11]

The mean age at the diagnosis of our patients was 47.37 years; compared with that of 53 years[8], 43 years [12] and <50 years[9]. Our findings were also consistent with the literature that younger and premenopausal patients are more prone to develop TNBC, as we already compared with companion authors.

Most of the triple-negative breast carcinomas are ductal in origin; however, several other aggressive phenotypes can also be present, such as metaplastic, atypical or typical medullary, and adenoid cystic.[13,14] All of our patients were having Ductal carcinoma in situ, while Lesley A Stead et al[15] found different varieties of histological types like,34%medullary-like tumours, 32% ductal carcinoma and 9% were of other pattern.

More than 90% of TNBCs show an invasive ductal histology and high histological grade, with high mitotic index , central necrotic zones

and pushing borders as well as a conspicuous lymphocytic infiltrate[16,17].

There are multiple risk factors for the development of triple negative receptor breast cancer like multiparity, decreased breast-feeding, oral contraceptive pills use before the age of 40, young age <50 yrs, low socio economic Status, obesity and metabolic syndromes. [18]

ER expression is determined by using monoclonal antibodies staining against ER. while PR by LSAB system and scoring performed according to Allred Guidelines while score 2 categorized as negative. HER2 expression is determined by immunohistochemistry. Tumors that showed 2+ Her2/neu immunohistochemistry staining based on HercepTest criteria were definitively assessed by fluorescence in situ hybridisation (FISH). Tumors were designated as being HER2+ if they showed 3+ Her2/neu immunohistochemistry staining (based on HercepTest criteria) or if they were FISH positive [19,20]

We also carried out the radiological investigations like ultrasound scan and mammography in all patients. Rare scientific data indicate that a reduced incidence of microcalcifications and peritumoral ductal carcinoma in situ represent typical mammographic characteristics comprise [17,21]. Consistent with its more aggressive biology, this BC subtype very often manifests itself as an interval cancer [i.e. diagnosed between (screening) mammograms][4,21]. Magnetic resonance imaging (MRI) carries a particular potential to predict response to neoadjuvant chemotherapy in TNBC[22]. Furthermore, TN breast tumors show enhanced 2-[fluorine-18]fluoro-2-deoxy-D-glucose (FDG) uptake allowing for detection of TNBC with a high sensitivity by using FDG–positron emission tomography (FDG–PET)[23]. Furthermore, unifocality, mass lesion type, smooth mass margin, rim enhancement, persistent enhancement pattern, and very high intratumoral signal intensity on T2-weighted magnetic resonance images are typical features associated with TNBC [24].

Mostly TNBC presented as a high grade tumor. In our patients grade III tumor was frequent, comprising 69.2% of the total. In one of the research paper, 52.9% were grade III tumors [25] and Rebecca Dent et al [8]described grade III tumor in 66%. Lesley et al[15] also found the high grade tumor in non black population.

In the present study, higher percentage seen in T2 (size 2–5 cm)stage, 44.5%. Bruce G et[9] al found 26%, another author 55.6%[8]. Rebecca[8] compared the two groups of patients and found that nodal positivity was frequent in TNBC 54.6% versus 45.6%, respectively. He also mentioned that among the triple-negative group, there was no correlation between tumor size and node status among women with tumors <5 cm. Even the small tumors in the triple-negative group had a high rate of node positivity; 55% of women with tumors of 1 cm had at least one positive lymph node. Traditionally, as tumor size increases, the rate of node positivity increases, and this relationship was not seen among the triple-negative group. Foulkes et al[26] reported that this phenomenon is also present in BRCA-associated cancers and suggested that the mode of spread of these cancers is hematogenous. Bruce G et al[9] However did not find any significant difference in the nodal status of patients included in his study and the overall population (25% node positive v 26% node positive, respectively). In our patients, 60% were node positive, in whom 36.5% were having 1–3 nodes and 23.1% revealed >3 nodes positivity. In our patients we found that lypmhovascular invasion was present in 34.5% of cases.

Despite of different therapeutic options, survival after diagnosis of brain metastasis remains only 6 months and 1-year survival approximating 20%.[27]

Although adjuvant chemotherapy is highly effective in the treatment of triple-negative breast cancers, the prognosis of triple-negative cancers remains poor. Population-based studies have also demonstrated reduced breast cancer–specific survival among patients with triple-negative disease.[4] More recently, scientific efforts aimed at dissecting the biology of triple-negative breast

cancer have revealed several promising targeted strategies including EGFR-targeted agents, anti-angiogenic agents, and PARP inhibitors.

Studies have shown that more aggressive visceral and soft-tissue relapse are more common than bone relapse[28]. TNBC is one of the aggressive subtype of breast cancer because; the peak risk of recurrence occurs within the first 3 years after initial treatment of the disease with the majority of deaths occurring in the first 5 years [4] and after diagnosis of metastatic disease, a significantly shorter survival was observed in TNBC[4]. Conversely, the risk for late recurrences (i.e. beyond 5 years of diagnosis) is decreased by 50% compared with HR-positive disease[29]. However, differences between TNBC and non-TNBC regarding overall survival (OS) wear off at 10 years of follow-up.

Conclusion:

We have shown by using the simple commonly available immunohistochemical markers ER, PR and HER2/neu that triple negative breast cancers are frequent in our setup. Triple negative breast cancer is more common among young women Clinical behavior among triple negative breast cancer cases is aggressive. It has relatively poor prognosis, with significantly shorter survival and a poorer response to treatment. It is a different entity of breast cancers which have no targeted therapy therefore requires research at the molecular level.

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