ORIGINAL ARTICLE

Clinicopathological comparison of triple negative breast cancers with non-triple negative breast cancers in a hospital in North India

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Abstract

Introduction: Breast cancer is the second most common cancer worldwide (1.3 million cases, 10.9%) and ranks 5th as cause of death from cancer overall (458,000 cases, 6.1%). Triple-negative breast cancer (TNBC) is a subtype of breast cancer with characteristic biological and pathological features. Among the subgroups of breast cancer, triple negative cancer is particularly feared because it is associated with poor outcome. However, clinical data on TNBC in Asian population are limited. The present study was aimed to find the prevalence of TNBCs and to compare various clinicopathological features of TNBC with non TNBC patients in our population.

Materials and Methods: Clinical and pathological data of 180 breast cancer patients who visited our department from January 2009 to December 2013 were analyzed. Statistical analysis was done using the Chi-square test and Mann–Whitney U-test.

Results: Of 180 cases, 62 (34.4%) had TNBC. Data analysis revealed significant difference in mean age, mean tumor size, tumor grade between TNBC and non-TNBC patients. Axillary lymph node metastasis and lymphovascular involvement were also more in TNBC patients however this was not statistically significant. Extranodal spread was recorded more in non-TNBC patients as compared to TNBC patients, but the results were statistically insignificant.

Conclusion: Triple negative breast cancer represented 34.4% which is higher than the range normally reported in the literature. TNBC are associated with younger age, large tumor size, high-grade tumors, and a higher rate of axillary lymph node metastasis.

Key words: Basal subtype, estrogen receptor, hormone receptors, human epidermal growth factor receptor-2/neu, progesterone receptor, triple negative

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Introduction

Breast cancer is by far the most frequent cancer among women worldwide with an estimated 1.38 million new cases of breast carcinoma diagnosed in 2008 (22.9%) and ranks 2nd among all cancers in both the sexes (10.9%).[1] It was estimated that 12.7 million new cancer cases and 7.6 million cancer deaths occurred in 2008 worldwide, with 56% of new cancer cases and 63% of the cancer deaths occurring in the less developed regions of the world.[1] Lung cancer remained the most common cancer in the world both in terms of cases (1.6 million cases, 12.7% of total) and deaths (1.38 million deaths and 18.2%).[1] Incidence rates of cancer breast varies from 19.3/100,000 women in eastern Africa to 89.9/100,000 women in Western Europe, and are high (>80/100,000) in developed regions of the world (except Japan) and low (<40/100,000) in most of

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the developing regions. The range of mortality rates is approximately 6–19/100,000. Breast cancer ranks as fifth cause of death from cancer overall (458,000 deaths), but it is still the most frequent cause of cancer death in women in both developing (269,000 deaths, 12.7% of total) and developed regions.[1]

Early detection of breast cancer and the use of aggressive multimodal treatment have successfully resulted in a decrease in mortality from the disease.[2] Prognostic and predictive factors have been widely used in treatment decisions. These factors include the Extent of axillary lymph node involvement, histopathologic grade, age of the patient, involvement of lymphatic or microvascular spaces, status of hormone receptors (HRs), and human epidermal growth factor receptor-2 (HER-2/neu).[2] Breast tumors are heterogeneous and consist of several pathological subtypes with different histological appearances of the malignant cells, different clinical presentations and outcomes, and the patients show a diverse range of responses to a given treatment. The cellular composition of tumors is a central determinant of both the biological and clinical features of an individual disease.[3]

Now a days, immunohistochemistry, microassay techniques, and cytogenetics are necessary for the exact diagnosis, better prognostication, and application of newer modalities of treatment.[4] Complementary DNA microassay profiling has identified five subtypes of breast cancer: Luminal A (estrogen receptor [ER]+ or progesterone receptor [PR]+ or both, HER-2 neu negative), luminal B (ER + or PR + or both, HER-2 neu+), basal-like (ER−, PR−, HER-2 neu±), HER-2 neu+ (ER−, PR−, HER-2 neu+) and normal breast-like. These are based on consideration that two distinct types of epithelial cells are found in human mammary glands: Basal (and/or myoepithelial) cells and luminal epithelial cells.[4,5] These two cell types can be distinguished immunohistochemically that luminal cells express ER, PR receptors, and they are positive for keratin 8/18, whereas basal cells are positive for keratin 5/6 and 17.[4,5] The term "basal-like cancer" and triple negative cancer have been used interchangeably by many authors, however they are not synonymous, approximately 75% of basal-like cancer are triple negative but 25% of them may express HER-2 neu or HRs.[4]

Triple negative cancers are characterized by lack of expression of ER, PR, and HER-2 neu.[6] These cancers occur in approximately 10–25% of all breast carcinomas.[4] Triple negative breast cancers (TNBCs) are clinically characterized as more aggressive and less responsive to standard treatment and are associated with poor overall patient prognosis.[7] African-American women have a higher incidence risk for TNBC and have a worse 5 years survival rate than Caucasian women with TNBC.[8]

The clinical data on TNBC in Asian population are limited. The present study was designed to investigate the prevalence of TNBC and non-TNBC (non-TNBC) in our study population and further we aimed to compare various clinicopathological characteristics of TNBC with non-TNBC in our study.

Materials and Methods

The present study included a total of 180 operated cases of breast cancer who reported to our department for adjuvant treatment from January 2009 to December 2013. The study was approved by hospital ethics committee. All necessary relevant details like presurgical investigations, details of metastatic workup, nature of surgery done with intraoperative findings, postoperative complete histopathological details like type of malignancy, modified Bloom–Richardson grade of tumor, lymph node metastasis, lymphovascular invasion, status of ER, PR, HER-2 neu overexpression, were noted in the proforma.

For this study, "triple negative" breast cancers were defined as those that were ER negative, PR negative, and HER-2 neu negative. "Other"/non-TNBC were defined as those that were positive for any of these markers.

Data of all patients were compiled, and a retrospective analysis was done to assess:

- Prevalence of TNBC and non-TNBC in the study population
- To compare various clinicopathological characteristics of TNBC such as age, histopathological type of cancer, modified Bloom–Richardson histological grade, size of the tumor, lymph node metastasis, and lymphovascular involvement with non-TNBC.

Patients were divided into two groups:ww

- Those with TNBC as (TNBC) group
- Those with "other" types of breast cancers as non-TNBC group.

Patients with histopathology showing benign tumors, incomplete surgical details and those patients in whom no/incomplete information about ER/PR and HER-2 neu status was available were excluded from the present study. Patients who had received neoadjuvant chemotherapy were also excluded from the present study.

Human epidermal growth factor receptor-2 neu score of 3+ was taken as positive by immunohistochemistry method. For equivocal results (2+), *in situ* hybridization tests were advised as per our institutional protocol.

The statistical analysis was done with the help of institutions statistician using Spss Inc, released 2007, version 16, Chicago, IL, USA. Chi-square test, Mann-Whitney *U*–test were employed for statistical analysis. Two-sided $P < 0.05$ was considered statistically significant.

Results

After proper scrutiny, a total of 180 operated breast cancer cases was included in the present study. Among these 180 patients, 62 (34.4%) were TNBC and 118 (65.5%) patients were non-TNBC patients.

In the TNBC group, the youngest patient was 23-year-old, and the oldest was 70-year-old. In the non-TNBC group, the youngest patient was 25-year-old, and the oldest was 80-year-old at the time of diagnosis. The mean age at diagnosis was significantly younger for the TNBC group compared to non-TNBC group patients (47.4 years vs. 52.0 years, respectively: $P < 0.014$).

In TNBC category, 8 (12.9%) patients were ≤35 years of age, 37 (59.6%) were 36–55 years of age and 17 (27.4%) were >55 years of age. In non-TNBC group, 10 (8.4%) cases were ≤35 years of age, 67 (56.7%) patients belonged to 36–55 years of age and 41 (34.7%) patients were >55 years of age. The characteristics of patients with TNBC and non-TNBC are compared in Table 1.

In TNBC group, 61 (98.3%) patients were females whereas 1 (1.6%) patient was male. In non-TNBC group, 112 (94.91%) patients were females and 6 (5.0%) were male patients.

In TNBC group, 60 (90.7%) cases were married whereas 2 (3.2%) cases were unmarried. In non-TNBC group,

Table 1: Characteristics of patients with TNBC and non-TNBC

Characteristics	Total (%)	TNBC (%)	Non-TNBC (%)	Chi-square	*P*
Mean age		47.45	52.04		0.014
Mean tumor size (cm)		4.22	3.25		0.001
Tumor size (cm)					
≤2	49 (27.2)	11 (17.7)	38 (32.20)	6.402	0.041
>2-5	101 (56.1)	36 (58)	65 (55)		
>5	30 (16.6)	15 (24.1)	15 (12.7)		
Tumor grade					
I	25 (13.8)	3 (4.8)	22 (18.64)	9.7	0.008
II	77 (42.7)	24 (38.7)	53 (44.9)		
III	78 (43.3)	35 (56.4)	43 (36.4)		
Clinical stage					
0	0	0	0		0.225
I	27 (15)	5 (8.0)	22 (18.6)		
II	80 (44.4)	28 (45.1)	52 (44.0)		
III	70 (38.8)	28 (45.1)	42 (35.5)		
IV	3 (1.6)	1 (1.6)	2 (1.6)		
Lymph node					
Positive	113 (62.7)	43 (69.3)	70 (59.3)	1.34	0.246
Negative	67 (37.2)	19 (30.6)	48 (40.6)		
Mean lymph node involved		4.35	3.5		0.5
Lymphovascular infiltration					
Present	69 (38.3)	27 (43.5)	42 (35.5)	0.778	0.3
Absent	111 (61.6)	37 (56.4)	76 (64.4)		
Pathology					
IDC	157 (87.2)	53 (85.4)	104 (88.13)	0.753	0.686
ILC	7 (3.8)	2 (3.2)	5 (4.2)		
Others	16 (8.8)	7 (11.2)	9 (7.6)		
ER status					
Positive	86 (47.7)	0	86 (72.8)	NA	
Negative	94 (52.2)	62 (100)	32 (27.11)		
PR status					
Positive	87 (48.3)	0	87 (73.72)	NA	
Negative	93 (51.6)	62 (100)	31 (26.2)		
HER 2/neu					
Positive (3+)	36 (20)	0	36 (30.5)	NA	
Negative	144 (80)	62 (100)	82 (69.4)		

HER 2=Human epidermal growth factor receptor 2; TNBC=Triple negative breast cancer; PR=Progesterone receptor; ER=Estrogen receptor; IDC=Infiltrating duct carcinoma; ILC=Invasive lobular carcinoma

117 (99.1%) patients were married, 1 (0.84%) patient was unmarried. Left breast was more commonly involved in both groups. 36 (58.06%) patients had left breast involvement and 26 (41.9%) patients had right breast involvement in TNBC group, 63 (53.3%) cases had left breast cancer and 55 (46.6%) cases had right breast involved in non-TNBC group. In both TNBC and non-TNBC group, upper outer quadrant was the most common quadrant involved [Table 2].

In TNBC group, modified radical mastectomy was the predominant surgery done in 50 (80.6%) cases. 10 (16.1%) cases had undergone conservative surgeries (lumpectomy with axillary clearance in 3 [4.8%], wide local excision with axillary clearance in 4 [6.4%], and quadrantectomy with axillary clearance in 3 [4.8%] cases). 2 (3.2%) cases in TNBC group had undergone modified radical mastectomy with reconstruction of breast as well. In non-TNBC group, 89 (75.4%) cases had undergone modified radical mastectomy whereas 19 (16.1%) cases had undergone conservative surgeries (lumpectomy with axillary clearance in 14 [11.8%], wide local excision with axillary clearance in 4 [3.3%], and quadrantectomy with axillary clearance in 1 [0.84%] cases). 10 (8.4%) cases in non-TNBC group had undergone modified radical mastectomy with reconstruction of the breast.

Table 2: Quadrant involvement in TNBC and non-TNBC patients

Breast involved	Quadrant involved	TNBC $n=62$ (%)	Non TNBC $n=118$ (%)
Left breast	Upper outer	21 (58.3)	30 (47.6)
	Upper inner	4 (11.1)	9 (14.2)
	Lower outer	3 (8.3)	9 (14.2)
	Lower inner	2 (5.5)	6 (9.5)
	Central	2 (5.5)	6 (9.5)
	More than one quadrant	4 (11.1)	3 (4.7)
Right breast	Upper outer	10 (38.4)	32 (58.1)
	Upper inner	3 (11.5)	6 (10.9)
	Lower outer	4 (15.3)	8 (14.5)
	Lower inner	1 (3.8)	1 (1.8)
	Central	3 (11.5)	4 (7.2)
	More than one quadrant	5 (19.2)	4 (7.2)

TNBC=Triple negative breast cancer

Infiltrating duct carcinoma (IDC) was the predominant morphological category with IDC not otherwise specified (NOS) in 53 (85.4%) cases of TNBC group. Invasive Lobular carcinoma was seen in 2 (3.2%) cases, whereas 3 (4.8%) cases had medullary carcinoma breast and 1 (1.6%) case each of Invasive duct carcinoma with mucinous features and Invasive duct carcinoma with lobular pattern and neuroendocrine features was seen in TNBC group. In non-TNBC group, IDC (NOS) was seen in 104 (88.1%) cases, Invasive lobular carcinoma was seen in 5 (4.2%) cases, invasive mucinous carcinoma breast in 2 (1.6%) cases, mixed tubular, cribriform and IDC in 2 (1.6%) cases, invasive duct carcinoma with lobular pattern in 2 (1.6%) patients, and 1 (0.8%) case each of invasive apocrine carcinoma, solid variant of papillary carcinoma breast, invasive duct carcinoma with neuroendocrine differentiation. No difference in the distribution of the pathological type was found between the two groups ($P = 0.686$).

In TNBC group, 11 (17.7%) cases had tumors of ≤2 cm, 36 (58%) patients had tumors with size between >2 cm and 5 cm, and 15 (24.1%) patients had tumors larger than 5 cm. In non-TNBC group, 38 (32.2%) patients had tumors of ≤2 cm, 65 (55%) cases had tumors with size between >2 cm and 5 cm and 15 (12.7%) patients has tumors larger than 5 cm. The Chi-square showed a statistical difference in the tumor size between TNBC and non-TNBC patients ($P = 0.041$). The mean tumor size was bigger in TNBC cases 4.22 ± 2.2 cm as compared to non-TNBC 3.25 ± 1.7 cm ($P = 0.001$). Overall TNBC patients had bigger tumors than non-TNBC patients.

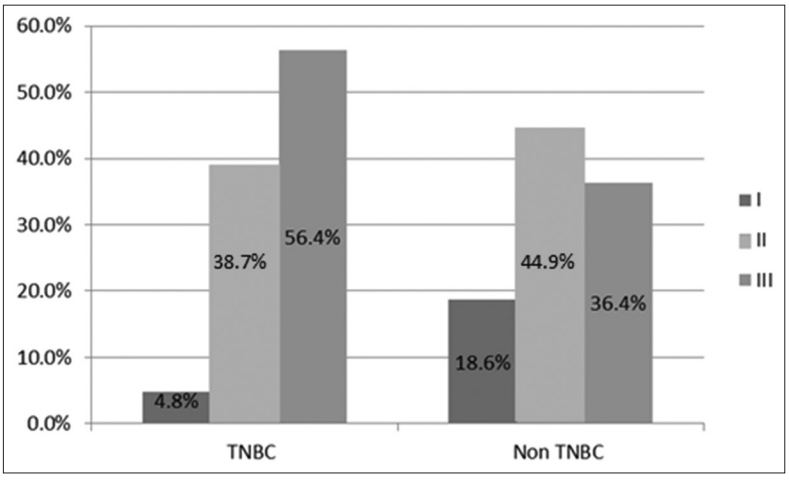

Figure 1: Modified Bloom–Richardson grade of tumor in triple negative breast cancers (TNBCs) and non-TNBC

In TNBC group, majority 35 (56.4%) patients had modified Scarff Bloom–Richardson grade III tumors, whereas 24 (38.7%) had grade II and 3 (4.8%) had grade I tumors. In non-TNBC group, 22 (18.6%) patients had grade I tumors, 53 (44.9%) had grade II and 43 (36.4%) had grade III tumors. The Chi-square test showed a significant difference in the distribution of histologic grade of the diagnosed TNBC and non-TNBC patients ($P = 0.008$) [Table 1 and Figure 1]. American joint committee on cancers TMN staging system for breast cancer was followed to determine the clinical stage. In TNBC group, 5 (8.0%) cases were diagnosed with disease at stage I, 28 (45.1%) cases at stage II and III, 1 (1.6%) case at stage IV. In non-TNBC group, 22 (18.6%) cases were diagnosed with disease at stage I, 52 (44.0%) cases at stage II, 42 (35.5%) cases at stage III and 2 (1.6%) cases at stage IV.

In the TNBC group, 43 (69.3%) cases had lymph node metastasis diagnosed on histopathological examination, Of them, 27 (62.7%) cases had metastasis in 1–3 lymph nodes, 9 (20.9%) had metastasis in 4–9 lymph nodes, and 7 (16.2%) cases had metastasis in >9 lymph nodes. In TNBC group, 19 (30.6%) cases had negative lymph node metastasis on histopathological examination. In non-TNBC group, 70 (59.3%) cases had lymph node metastasis, of them, 32 (45.7%) cases had metastasis in 1–3 lymph nodes, 24 (34.2%) had metastasis in 4–9 lymph nodes, and 14 (20%) cases had metastasis in >9 lymph nodes. Forty-eight (40.6%) cases had negative lymph node metastasis in non-TNBC group. Although lymph node metastasis was more in TNBC group, however, the Chi-square test did not reveal a significant difference in lymph node positivity between TNBC and non-TNBC patients ($P = 0.246$). Mean number of lymph nodes dissected out in TNBC group was 12.1 whereas in non-TNBC, it was 10.4 nodes. The mean number of lymph nodes involved in TNBC group was higher (4.3 nodes) than in non-TNBC group (3.5 nodes) ($P = 0.5$). Extra nodal spread was seen in 6 (9.6%) cases in TNBC group and 15 (12.7%) cases in non-TNBC group ($P = 0.72$).

In TNBC group, 27 (43.5%) cases had lymphovascular invasion whereas only 42 (35.5%) cases in non-TNBC group had lymphovascular invasion [Table 1]. This difference was however not statistically significant ($P = 0.3$).

Discussion

In world cancer report 2008,[9] South East Asia region SEAO in which India comprises of 67% of total population of the region, it was estimated that there were 1,589,000 incident cases of cancer in year 2008 (758,000 in men and 831,000 in women) and 1,072,000 deaths from cancer (approximately 557,000 in men and 515,000 in women). The overall rate of TNBC in our study (34.4%) is comparable to results obtained by Saha *et al.*[6] (30.4%) and Keam *et al.*[10] (32.4%), however our results are higher as compared to studies by Ambroise *et al.* (25%),[11] Krishnamurthy *et al.* (18.5%),[4] Tan *et al.* (17.6%),[12] Li *et al.* (12.1%),[8] Bauer *et al.* (12.5%),[13] Dent *et al.* (11.2%).[14]

In our study, the mean age at diagnosis was significantly younger in TNBC patients (47.4 years) as compared to non-TNBC group (52 years) ($P = 0.014$). Similar results were seen by Saha *et al.*[6] in a study involving 1026 patients of which 312 patients represented TNBC. Mean age at diagnosis in TNBC was 48.8 years as compared to 53.6 years in non-TNBC ($P < 0.002$). Dent *et al.*[14] at women's college hospital Toronto studied 1061 breast cancer patients of which 180 patients were TNBC. Mean age at diagnosis was 53 years compared to 57.7 years ($P < 0.0001$) in non-TNBC patients. Similarly, Krishnamurthy *et al.*[4] and Rao *et al.*[15] reported mean age at diagnosis in TNBC as 46.6 years and 46.8 years, respectively.

In the present study, 82.2% patients diagnosed with TNBC had a tumor size of >2 cm compared to 67.7% patients in non-TNBC group. Patients in triple negative category had relatively large tumors (mean tumor size 4.2 cm as compared to 3.2 cm in non-TNBC, $P = 0.001$). Our results are comparable to Dent *et al.*[14] who reported mean tumor size to be larger in triple negative patients than in nontriple negative group (3 vs. 2.1 cm, respectively, $P < 0.001$). More patients in TNBC group presented with grade III tumors as compared to nontriple negative patients. Similar findings were reported by Dent *et al.*[14] and Patil *et al.*[2] Krishnamurthy *et al.*[4] retrospectively analyzed 50 TNBC patients and reported grade III tumors in 48 (96%) cases.

Lymph node metastasis in breast cancer is a prognostic factor of greatest importance and data about its status have a great impact on decision making regarding postoperative adjuvant therapy. Our results showed lymph node involvement more in TNBC group (69.3%) as compared to non-TNBC group (59.3%). Mean number of lymph nodes involved was more in TNBC patients as compared to mean number of lymph nodes in non-TNBC group, although the difference was also not statistically significant. Studies by Dent *et al.*,[14] Saha *et al.*[6] and Li *et al.*[8] also showed a higher propensity for Lymph node involvement in TNBC in 54.4%, 56.4%, and 71.3% patients respectively. Similarly, lymphovascular involvement was found more in patients with TNBC as compared to non-TNBC however this was not statistically significant. Our results are comparable to those of Dent *et al.*[14] who reported 39.6% lymphovascular involvement in TNBC patients compared to 32.3% patients in non-TNBC category ($P = 0.06$). In the present study, only 8.0% patients with TNBC were diagnosed at stage I, whereas in non-TNBC group 18.6% patients were diagnosed at stage I however overall no difference in the distribution of the clinical stage of tumor between TNBC and non-TNBC patients was found statistically ($P = 0.22$). Li *et al.*[8] also showed no statistical significance in the

distribution of the clinical stage of the tumor between TNBC and non-TNBC cases.

Conclusion

Triple negative breast cancers represented 34.4% patients in our study which is higher than the range normally reported in the literature and is associated with more aggressive clinicopathological features. TNBC occurred at younger age, presented with high histopathological grade and larger tumor size as compared to non-TNBC tumors. TNBC patients also had a high rate of axillary lymphnode metastasis and lymphovascular involvement as compared to non-TNBC patients, but this finding was not statistically significant. Although extra nodal spread was seen more in non-TNBC patients as compared to TNBC, but the results were statistically insignificant.

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