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Original Article

Triple-Negative Breast Cancer: A Clinicopathological Study from Rural Tertiary Care Hospital of Central India

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Abstract

Introduction: Breast cancer is the most prevalent cancer among women in India, with higher incidence rates for aggressive molecular subtypes, like triple-negative breast cancer (TNBC).

Methods: In this single-center retrospective cohort study, we analyzed 140 breast carcinoma cases (70 cases each of TNBC and non-NBC) from June 2019 to June 2022. The clinical and morphological details taken were patient age, body mass, family history, tumor size, lymph node status, histological grade, molecular subtypes based on the immunohistochemical expression of ER, PR, and HER2/neu status, clinical stage, recurrence, metastasis, treatment Surgery/Adjuvant chemotherapy/radiotherapy received and survival.

Results: The incidence of triple-negative breast cancer is 20.21%. The median age for patients was 50.20 years with a clustering of 47.14% of cases between 40 and 59 years of age. Amongst TNBC 68.7% of cases were high-grade tumours as compared to 65.71% non- TNBC tumours. Most of the patients were in stage III, with 365 (48.08%) cases. The majority of patients with TNBC belong to TNM stage III (34; 48.57%), and IV (11; 15.71%). TNBC patients had higher chances of local recurrence, distant metastasis, and higher mortality as compared to non-TNBC patients (χ2- 4.51; P=0.03, S).

Conclusion: Triple-negative breast cancer tends to occur in young women and has higher chances of local recurrence, distant metastasis, and higher mortality as compared to non-TNBC patients. Amongst the molecular subtypes, TNBC is an aggressive tumor with poor survival outcomes. Further, studies with larger samples and careful analysis within the same geographical areas are needed.

Keywords: Breast cancer, Triple negative, Aggressive, Prognosis

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Introduction

Breast cancer is the second most common cancer worldwide, with age-standardized rates of 46.8/ 100,000 population. [1] In India, female breast cancer (BC) is the leading cause of cancer incidence and mortality, and accounted for 26.6% of new cancer cases and 13.7% of cancer-related deaths in 2022.[1,2]

Triple-negative breast cancer (TNBC) is a molecular subtype of BC in which tumor cell lacks estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2) on immunohistochemistry (IHC).[3] TNBC tends to occur in a young woman, and an aggressive tumor with a high fatality rate.[3-5] The incidence of TNBC is higher (27.9%) in India compared to the Western population.[6] According to recent epidemiological studies, physical inactivity, obesity and insulin resistance can increase the risk of TNBC. Physical inactivity increases the exposure to endogenous sex hormones and can also alter insulin-like growth factor-1 levels and immune responses.[5,6] TNBC has high metastatic potential, higher rates of local and disease recurrence and has a particular tendency for visceral metastases, most commonly to the lungs, bone and brain.[5,6]

Bajpai J et al (2022) at Tata Memorial Hospital, Mumbai, India analyzed clinicopathological features of 1297 operated cases of TNBC from 2013 to 2019. They concluded that the TNBC subtype is characterized a young age at presentation, a higher grade, and frequent relapse and recurrence.[7] Yavuz BB et al (2022) retrospectively compared the survival differences between TNBC and non-TNBC in patients undergoing adjuvant radiotherapy. They observed that the TNBC group had more grade II-III tumors, a higher ki-67 value, higher chances of local recurrence and more metastatic potential than the non-TNBC group. [8]

India is a heterogeneous country, and different state

has distinct demographic, economic, and cultural characteristics. Also, it has been observed that the incidence, and mortality rate of breast cancer are closely related to the geographical indicators of development [9] Most of the studies in the literature are from urban areas. In the present study, an attempt is made to study the clinicopathological features of triple- negative breast carcinoma particularly in rural populations, by assessing histomorphological features of TNBC; analyzing various parameters such as the age, site, tumor size, clinical features and treatment outcomes in triple- negative breast cancer; and by comparing these clinicopathological features with non – TNBC cases.

Methods

In the present study we retrospectivelyanalyzed 140 breast carcinoma cases received at the Department of Pathology of a rural tertiary care hospital in central India over a period of three years (June 2019 to June 2022). The BC cases, in which IHC markers were assessed, such as Estrogen receptor (ER), progesterone receptor (PR) and HER2/neu markers, were included in the study. The tumors were classified as Luminal A (HR + ve, HER2/neu-ve), Luminal B (HR + ve, HER2/neu + ve), Her 2 Positive (HR -ve, HER2/ neu + ve) and TNBC (HR -ve, HER2/neu -ve). The Clinicopathological details histomorphological features and immunohistochemical findings of TNBC and non TNBC cases were reviewed and compared. Various clinical and morphological details of the patients including, age , body mass index (BMI) , family history, treatment Surgery/Adjuvant chemotherapy/radiotherapy received, morphological subtypes of laterality, tumor size ,lymph node status, histological grade molecular subtypes on the basis of immunohistochemical expression of ER, PR and HER2/neu status, clinical stage of the disease, recurrence and metastasis of the disease were retrieved from the hospital information system and also from patient's record kept in Medical Record Department. Various morphological features were analyzed for their frequency, mean and median. The study was approved by the Institutional Ethical Committee.

Immunohistochemistry (IHC) Scoring

Stained slides were re- examined by two pathologists (MA and PP) blinded to patients' clinical characteristics and outcomes. Semi-quantitative analysis of ER-stained tissue sections and PR-stained tissue sections was performed by Allred score.[9]

Tumors were categorized as follows: Allred scores of 0 and 2 are considered negative for ER (i.e., not actionable), while scores of 3 to 8 are considered positive (i.e., recommended for hormonal therapy).

Her-2/neu intensity of immunohistochemistry staining was scored according to the 2007 ASCO/CAP guidelines. Negative (score 0) with no staining observed; Score 1 -incomplete membranous staining or complete membranous staining in less than 10% of the tumor cells; moderate intensity (Score 2)- complete membranous staining in more than 10% of the tumor cells; or strong staining intensity Score 3)- complete membrane staining in more than 10% of the tumor cells. A score 3 was considered positive, a score 2 was equivocal positive, and scores of 1 and 0 were negative.[9]

Statistical Analysis

Statistical analysis was done by using descriptive and inferential statistics using Chi-square test and Student's unpaired t test. Univariate and multivariate logistic regression analyses will carry out to identify the prognostic factors associated with molecular subtypes of breast cancer i.e. TNBC with respect to non TNBC. A forward selection method is used to addvariables. Survival outcome were drawn by the Kaplan-Meier method and differences assessed by the stratified log-rank test. Software used for analysis was SPSS 27.0 version and GraphPad Prism 7.0 Version.

Results

In the present study, the incidence of triple negative breast cancer is 20.21%.

The peak incidence of overall breast carcinoma in the study was seen in the perimenopausal age group 40-59 years (47.14%) with the mean age of 50.20 years. Majority of the TNBC cases were in the premenopausal age (40-59 years), while Non -TNBC patients were in the postmenopausal age ($\geq$60 years) with mean age 51.65 $\pm$ 12.09. Amongst TNBC 68.7% cases were high-grade tumours as compared to 65.71% non- TNBC tumours. The majority (n = 46, 65.71%) of TNBC cases have a BMI $\geq$ 25 kg/m2 compared to controls and their difference is statistically significant ($\chi 2 = 18.34$, $P = 0.0004$).

The majority of cases (n = 48, 68.57%) and controls (n = 39, 55.71%) present with tumor size 2.1-5 cm. 12 cases and 8 controls were present with tumor size > 5 cm. Their difference is statistically significant ($\chi 2 = 6.85$, $P = 0.032$), suggest that cases have larger tumor size at the time of presentation. 15 TNBC cases and 12 non- TNBC cases present with locally advanced breast cancer, however their difference is statistically insignificant ($\chi 2 = 0.41$, $P = 0.52$). Table1: Clinicopathological features of TNBC and Non-TNBC cases in the study population.

The difference in histopathological grade between TNBC and non- TNBC tumours was insignificant. 42(60%) TNBC and 33(47.1%) non- TNBC cases had metastasis in axillary lymph nodes. Local complications like skin changes, positive lymph nodes were associated significantly large tumor size of $\geq$2cm in diameter. The mean value of lymph node ratio between cases and controls 0.18 and 0.23 respectively. The majority of patients with TNBC belong to TNM stage III (34; 48.57%), and IV (11; 15.71%) while in control non-TNBC group 33 (47.14%) and 20 (28.57%) patients belongs to stage II and Stage III and the difference is statistically significant ($\chi 2 = 11.198$, $P = 0.010$). Univariate analysis of the cases

Table 1. Clinicopathological features of TNBC and Non-TNBC cases in the study population

Clinical parameters	TNBC (cases)	Non-TNBC controls	χ2-value; *P* value
Age <60 years >60 years	56 (80%) 14 (20%)	32 (45.71%) 38 (54.29%)	17.86 *P*=0.0001, S
BMI (kg/m^2) <25 >25	24 (34.29%) 46 (65.71%)	49 (70%) 21 (30%)	8.34 *P*=0.0004, S
Family history of BC in first degree relative Present Absent	4(5.71%) 66(94.29%)	2(2.85%) 68(97.15%)	0.69 *P*=0.40, NS
Laterality Right Left	35(50%) 35(50%)	37(52.86%) 33(47.14%)	0.11 *P*=0.73, NS
Skin changes Present Absent	15(21.43%) 55(78.57%)	12(17.14%) 58(82.86%)	0.41 *P*=0.52, NS
Size of tumor ≤2 cm 2.1-5 cm >5 cm	10(14.29%) 48(68.57%) 12(17.14%)	23(32.85%) 39(55.71%) 8(11.42%)	6.85, *P*=0.032,S
Lymphovascular invasion Present Absent	42(60%) 28(40%)	43(61.43%) 27(38.57%)	0.02 p=0.86, NS
Perineural Invasion Present Absent	9(12.86%) 61(87.14%)	8(11.43%) 62(88.57%)	0.06 *P*=0.79, NS
In situ component present <25% >25%	13(18.57%) 57(81.43%)	16(22.86%) 54(77.14%)	0.39 *P*=0.53, NS
Lymph Node Ratio (LNR) Mean Median Range	0.25 0.015 0.1-0.33	0.22 0 0-1	0.99 *P*=0.32,NS
Tumor Grading Grade I Grade II Grade III	0(0%) 22(31.43%) 48(68.57%)	2(2.86%) 22(31.43%) 46(65.71%)	2.04; *P*=0.36,NS
Stage I Stage II Stage III Stage IV	9(12.85%) 16(22.85%) 34(48.57%) 11(15.71%)	11(15.71%) 33(47.14%) 20(28.57%) 6(8.57%)	11.198, *P*=0.010, S
Recurrence Present Absent	12(17.14%) 58(82.85%)	4(5.71%) 66(94.28%)	4.51 *P*=0.03,S
Metastasis Present absent	10(14.28%) 60(85.71%)	3(4.28%) 67(95.71%)	4.15 *P*=0.04,S
Death Alive	25(35.71%) 45(64.28%)	15(21.42%) 55(78.57%)	3.5 *P*=0.06,NS

shows a positive correlation of tumor size with BMI and stage indicate that larger tumor size is related to higher BMI and higher stage ($P=0.018$, and 0.038, respectively). The control shows positive correlation of tumor size with stage indicates that larger tumor size is related to higher stage ($P=0.003$). Table 2 Univariate analysis of various clinicopathological parameters

Non-compliance to the prescribed treatment was the most important factor behind adverse outcome later in the disease course. All 32/32 (100%) TNBC and 15/28 (39.47%) non-TNBC patients who could not complete the treatment presented with adverse outcomes (recurrence/ distant metastasis/ death) later while only 14/38 (36.8%) TNBC and 7/42(16.6%) non-TNBC patients who completed the treatment showed adverse outcomes. TNBC patients had higher chances of local recurrence, distant metastasis and higher mortality as compared to non-TNBC patients, and it was statistically significant. The number of positive lymph node ($P=0.002$) and treatment compliance (0.008) were significantly associated with survival outcome. Table 3: Correlation of treatment received (completed or incomplete) in cases (TNBC) and controls (non-TNBC) with respect to recurrence, metastasis and death rate.

At minimum follow up period of one year, 25 cases and 15 controls are dead shows that cases have highest death rate compare to controls. The results are statistically significant with treatment completed within the cases and controls ($P=0.005$ and 0.01, respectively) These findings suggested that among molecular subtypes, TNBC is an aggressive tumor and continued to have the poorest outcomes than non- TNBC group.

Discussion

In the present study, an attempt is made to study the clinicopathological features of triple- negative breast carcinoma particularly in rural populations, by assessing histomorphological features of TNBC; analyzing various parameters such as the age, site, tumor size, clinical

Table 2. Univariate analysis of various clinicopathological parameters

Clinicopathological Variables	CASES		CONTROL	
	P value	Correlation Coefficients	*P* value	Correlation Coefficients
AGE				
Family history	0.001	0.390	0.016	0.288
Grade	0.265	-0.135	0.895	0.016
Lymph node positivity	0.004	0.337	0.020	0.278
Recurrence	0.262	0.136	0.303	-0.125
Metastasis	0.778	-0.034	0.031	-0.259
Stage	0.041	0.245	0.465	0.089
BMI				
Family history	0.794	0.032	0.352	0.113
Grade	0.231	0.145	0.642	0.056
Lymph node positivity	0.049	0.229	0.217	0.149
Recurrence	0.002	-0.363	0.718	-0.044
Metastasis	0.003	-0.354	0.746	-0.039
Stage	0.019	0.279	0.723	0.043
TUMOR SIZE				
Age	0.111	-0.192	0.537	-0.075
Lymph node positivity	0.732	0.042	0.966	0.005
Grade	0.566	0.070	0.416	0.099
Stage	0.038	0.248	0.003	0.353
GRADE				
Lymph node positivity	0.468	-0.088	0.061	-0.225
Recurrence	0.922	0.012	0.763	-0.037
Metastasis	0.185	0.130	0.263	0.136
Stage	0.529	-0.075	0.386	-0.105
STAGE				
Lymph node positivity	0.0001	0.647	0.0001	0.670
Skin changes	0.175	0.164	0.015	0.288
Recurrence	0.340	-0.116	0.364	-0.110
Metastasis	0.144	-0.177	0.232	-0.145

features and treatment outcomes in triple- negative breast cancer; and by comparing these clinicopathological features with non – TNBC cases.

Breast cancer is a heterogeneous disease of different biologic subtypes and are known to possess various clinicopathological features with prognostic and predictive markers. [10] TNBC constitutes a large proportion of BC deaths despite its small percentage amongst all breast cancer cases. [10,11]

Several studies on TNBC were from urban areas with mostly small sample and been discordant results with regard to the clinicopathological and prognostic factors of TNBC patients.[6, 7,8,11] In the present study, an attempt is made to study the clinicopathological features of triple negative breast carcinoma particularly in rural population, by assessing clinical, histomorphological features, treatment outcomes in triple negative breast cancer. In the present study TNBC cases constitute 20.21% of all breast carcinomas. Our findings are similar to the

Table 3. Correlation of treatment received (completed or incomplete) in cases (TNBC) and controls (non-TNBC) with respect to recurrence, metastasis and death rate

Parameter	Cases		Controls	
	Treatment Completed	Treatment Incomplete	Treatment Completed	Treatment Incomplete
Recurrence				
Present	4(5.71%)	8(11.42%)	1(1.42%)	3(4.28%)
Absent	33(48.57%)	25(34.28%)	41(58.57%)	25(35.71%)
χ2, p value	2.56, P=0.109, NS		2.16, P=0.141, NS	
Metastasis				
Present	2(2.85%)	8(11.42%)	1(1.42%)	2(2.85%)
Absent	35(51.42%)	25(34.28%)	41(58.57%)	26(37.14%)
χ2, p value	5.52, P=0.018, S		0.92, P=0.33, NS	
Death rate				
Dead	8(11.42%)	17(24.28%)	5(7.14%)	10(14.28%)
Alive	29(42.85%)	16(21.42%)	37(52.85%)	18(25.71%)
χ2, p value	7.78, P=0.005, S		5.65, P=0.01, S	

findings of various Indian and Asian studies of Sarkar S et al[12], and Krishnamurthy J et al[13], where overall prevalence of TNBC was 25.04%, 18.5%, and 18% respectively which was higher in comparison to the western studies in the literature. Yavuz BB et al[8] observed lower incidence of TNBC < 15% of all invasive breast cancer whereas Roy et al[14] noticed the pooled prevalence of breast cancer as high as 31% (95% confidence interval [CI]: 27–35%). The differences in hormone receptor positivity between Indian patients may be a true ethnic variance or it could be the effect of younger average age at presentation and diagnosis.

We observed TNBC patients present at young age than Non TNBC cases (χ2 = 17.86, P = 0.0001). These findings were well correlated with other studies like, Akhtar M et al[15] with mean age of 48.1 years, Chintapalani SR et al[16] with mean age of 50 years and Anand AS et al[17] with mean age of 53 years. We found BMI [overweight (BMI = 25-29.9), and obesity (BMI ≥ 30)] to be associated with TNBC compared with non-TNBC ((χ2- 18.34, P = 0.0004). Some studies Chen H et al and Nagrani R et al have suggested that a higher BMI is the risk of triple negative breast cancer.[18,19]

The important mechanism of central obesity promoting TNBC progression is the disruption of the 'insulin-leptin-adiponectin' axis. Central obesity is an independent predictor of insulin resistance and higher levels of free insulin-like growth factor-1 (IGF-1) with peripheral obesity. Increased level of insulin and IGF-1 in central obesity were mitogenic agents and promoted breast cancer cell proliferation directly, also promote tumor cell growth and migration by activating tumor angiogenesis.[18,19] As obesity is a modifiable risk factor that has been shown to be associated with increased risk of developing breast cancer, it is important to evaluate the impact of obesity on the clinical outcome of TNBC.

Family history of breast carcinoma in one or more first-

or second-degree relatives is an important risk factor for early development of breast cancer. Total six BC cases had positive a family history, of which four cases had TNBC (5.71%) and two had non -TNBC (2.85%). Our finding correlates well with the findings of Anderson K et al[20] who found the prevalence of a family history of IBC in a first-degree relative was almost twice in TNBC (24.3%) patients than non-TNBC (15.5%) patients.

The age of the patients at the time of diagnosis was significantly correlated with positive family history in both TNBC ($P=0.001$) and non-TNBC patients ($P=0.016$). Also, age of the patient was significantly associated with lymph node positivity ($P=0.004$), advanced stage of the disease ($P=0.041$) in TNBC while with lymph node positivity ($P=0.020$) and metastasis ($P=0.031$) in non-TNBC group. Sharma P et al[21] and Anderson K et al[20] also noticed a direct correlation of age of the TNBC patient at diagnosis and positive family history in first relative of breast cancer patients. Our findings exactly matched with Yin L et al[22] who compared clinicopathological features as prognostic marker in 216 very young (<35 years) and older (>35 years) TNBC cancer patients. They found very young patients with TNBC had more positive lymph nodes ($P=0.006$) and advanced clinical stage ($P=0.049$).

Among TNBC cases, we noticed significant correlation of BMI with tumor size ($P=0.018$), age of the patients ($P=0.022$), lymph node positivity ($P=0.049$) and stage of the disease ($P=0.019$) (Table). Our observations are consistent with Kodali D et al[23] and Gerhsuni V et al[24] studies. Kodali D et al[23] found that 50.3% of Asian, African TNBC patients and 32% of Caucasians TNBC were obese of which 43% of obese patients had node-positive disease at diagnosis. They concluded that obesity in TNBC patients, was associated with a higher incidence of lymph node involvement at diagnosis, and shorter interval of time to development of distant metastatic disease. Therefore, they recommend healthy food and exercise will help to prevent obesity, and thus may reduce the risk of developing TNBC and improve breast cancer outcomes.

Lymph node ratio (LNR) between cases and controls was found to be insignificant in the present study. However, in contrast to our findings, He M et al recommended that LNR can be used as an additional prognostic factor for TNBC patients with positive lymph node involvement, and can be considered while making treatment decision.[25]

High grade tumors (grade III) were prevalent in the study area irrespective of the stage of clinical presentation. Though the difference in grade between cases and controls was found to be insignificant ($\chi2$- 2.04, $P=0.36$). The stage of BC at the time of presentation in Indian patients is very different from the Western world. Most of the patients in the Western countries report in early stages (stage I and II), while cases with locally advanced breast cancer (LABC) and metastatic cancers are very low. These findings are consistent with other Indian studies like Chandra D et al[26] and Bajpai J et al[7] who observed, TNBC cases present with advanced stage of breast cancer stage III [34(48.57%)] and Stage IV [11(15.71%)]. The probable reason for this advanced presentation is multifactorial, such as, lack of awareness, low socioeconomic status, lack of effective screening program, lack of sufficient health care resources etc.[27] The lump of breast cancer is usually asymptomatic, and majority of rural patients ignore the lump or symptoms for a very long time. As a result, most of the cases report to the health centre only when there is a large lump or secondary changes such as skin ulcerations, bloody discharge from the nipple or oedema are obvious. Non-availability of tertiary care centres nearby is also an important factor in not being able to seek early treatment.

Surgery along neoadjuvant chemotherapy was the prescribed treatment in the majority of patients because of their clinical presentation in advanced-stage disease. However, most of the patients did not comply with the prescribed treatment plan, possibly due to lack of accessibility or personal and socio-economic reasons. The adverse outcome in the form of recurrence of the disease and metastasis was noted in 12 (17.41%) and 10(14.28%) TNBC patients respectively, which suggests that TNBC patients had higher chances of local recurrence, distant metastasis and it was statistically significant [($\chi2$- 4.51, $P=0.03$) and ($\chi2$- 4.15, $P=0.04$)]. Also, in TNBC patients had higher mortality as compared to non-TNBC patients, this difference was statistically insignificant ($\chi2$- 3.5, $P=0.06$).

The only Indian study on treatment compliance we could find, by Anand AS et al in Kerala.[16] The incidence of recurrence in this study was 17.5% which was almost equal to our results 17.41%. Though Anand AS et al[17] did not take loco regional and socio-economic factors into consideration, we suggest that socio economic factors and illiteracy amongst study population may be the reason behind this high non- compliance rate. More studies on socio-economic aspects of breast carcinoma patients are warranted, as these are manageable parameters which could significantly improve the prognosis of breast cancer patients. The limitation of study was small sample size and it includes the patients from singe tertiary care hospital.

A future study proposal on clinicopathological TNBC should focus on identifying how age and racial background might influence TNBC outcomes, and understanding the molecular heterogeneity of TNBC.

Conclusion

The overall prevalence of triple negative breast cancer in the present study was 20.21%. The majority of TNBC patients were in perimenopausal age group. High body mass index and positive family history of breast cancer in first degree relatives emerged as significant risk factors associated with TNBC. A significant correlation was seen with tumour size, positive lymph node status, stage of triple negative breast cancer. The majority of TNBC patients had TNM stage III (34; 48.57%), and IV (11; 15.71%) disease. These TNBC patients had higher

chances of local recurrence, distant metastasis and higher mortality as compared to non-TNBC patients. Amongst the molecular subtypes, TNBC are aggressive tumor with poorer outcome.

Further, studies with larger samples and careful analysis within the same geographical areas are needed.

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Authors' Contribution

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Competing Interests

The authors have no conflicts of interest to declare.

Ethical Approval

This project was approved by ethical committee of Mahatma Gandhi Institute of Medical Sciences.

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