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*Original Research Article*

# Clinical presentation and histopathological spectrum of triple negative breast carcinoma at a specialized cancer care center: A retrospective study

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**Abstract**

**Introduction:** Triple negative breast cancers (TNBC) are defined as cancers lacking expression of estrogen receptor, progesterone receptor and Her-2-neu. The diagnosis is based on demonstration of negative receptor expression using immuno-histochemistry and/or Fluorescent in situ hybridization (FISH). The disease has an aggressive course with overall poor prognosis, so accurate and early diagnosis becomes all the more significant for management of these cancers.

**Aim and Objectives:** To assess the clinical and pathological characteristics of TNBC including clinical profile, histopathological type, tumor grade, Ki-67 proliferation index along with expression of estrogen receptor, progesterone receptor and Her-2-neu.

**Material and Methods:** A retrospective study was conducted over period of 3 years and 6 months and the above mentioned clinical and pathological findings were assessed for TNBCs. For estrogen and progesterone receptor evaluation, Allred scoring system was used and a total score of 0-2 was considered as negative. Her-2-neu was considered negative when the staining was negative/faint/barely perceptible in part of membranes (score of 0-1+). In equivocal cases of Her-2-neu expression (3 cases), FISH was done for definitive assessment.

**Observations:** 48 cases of Triple negative breast cancers were studied. Cytological evaluation was done in all the cases with 35 cases being reported as confirmed for a malignant lesion. All the patients were female with mean age of presentation being 46.2 years. The most common clinical presentation was breast lump. Clinically, T2 was the most common tumor stage with 22 cases. All the cases were diagnosed as 'Invasive Breast Carcinoma, No special type' with mean Nottingham score of 7.6. The histopathological grade was Grade 2 in 16 cases and Grade 3 in 32 cases. The mean Ki-67 proliferation index was 55%.

**Conclusion:** TNBCs occur in a younger population in India. Majority of patients have histological grade III tumors with high proliferation activity which is one of the reasons for adverse prognosis in these cancers. In cases where immuno-histochemistry is equivocal for Her-2-neu expression, FISH can be used to ascertain the exact status.

**Keywords:** Triple negative breast cancers (TNBC), Clinical and Pathological characteristics, Ki-67 Proliferation Index

## Introduction

Triple negative breast cancers are defined as cancers lacking expression of estrogen, progesterone and Her-2-neu, which can be determined by various methods but immunohistochemical evaluation on formalin fixed paraffin embedded (FFPE) tissue sections remains the most commonly used method [1]. The disease itself has aggressive nature including invasiveness, high metastatic potential and tendency to relapse, so overall prognosis is poor [2]. The diagnosis of triple negative breast cancer is based on immunohistochemical demonstration of negative receptor expression by Allred score in estrogen and progesterone receptor status [3-4] and Her-2-neu score of 0-1 [3-4]. In equivocal cases of Her-2-neu expression on immunohistochemistry (score 2), Fluorescent in-situ hybridization (FISH) may be used which is considered negative when there are less than 4 Her-2-neu copies per nucleus or FISH ratio of less than 1.8 [3].

The mean age of diagnosis of TNBC has been reported to range from 34 to 59 years in previous studies [5-8]. The most common presenting symptom of carcinoma breast is lump. Other symptoms include breast pain, skin or shape abnormalities and nipple abnormalities. Non-breast symptoms include fatigue, breathlessness, axillary lump, and back pain [9].

Histological grade and typing are important for TNBC which show marked molecular and morphological heterogeneity and although most are high grade tumors, few low grade entities have been described which have a comparatively better prognosis [10-11]. High histological grade irrespective of tumor size and stage has a poor prognosis and Abdollahi and Eteadi have reported grade II being the most common histological grade followed by grade III [12].

Similarly studies have documented TNBC to be highly proliferative tumors with high Ki-67 proliferation index. 30% cut off for the same can be used to classify tumors with high grade, propensity to metastasize and advanced stage with poor survival [13-14].

With above considerations the present study was conducted to assess the clinical and pathological characteristics of TNBC diagnosed at our specialized cancer center.

## Material and Methods

The present study was conducted retrospectively for a period of 3 years and 6 months (01/05/2019 to 30/10/2022). Records of patients diagnosed with Triple negative breast carcinoma were assessed. All regulatory permissions were taken from the Institutional Ethics Committee.

**Inclusion Criteria:** All cases of TNBC diagnosed for the first time.

## Exclusion Criteria

- All other neoplastic breast lesions.
- Breast carcinoma patients who had received any type of radiotherapy or chemotherapy, before pathological diagnosis.

Clinical presentation and stage was documented for all cases.

Similarly histopathological tumor typing, grade, Ki-67 proliferation index along with scoring and expression of estrogen receptor, progesterone receptor and Her-2-neu were assessed.

Ki-67 proliferation index was graded into two categories as less than equal to 30% and greater than 30%.

## Observations

A total of 48 cases of Triple negative breast cancers were recorded in the given time period, with all the cases being diagnosed in females. All the cases had undergone cytological

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evaluation and were reported as confirmed (35 cases), suspicious (10 cases) and suggestive (05 cases) for malignant lesion. Out of these in 12 cases core biopsy samples were obtained prior to any therapeutic intervention and 36 were diagnosed post operatively but before any hormonal or neo-adjuvant chemotherapy.

On examination of Hematoxylin and Eosin stained tissue sections all the carcinomas were 'Invasive Breast Carcinoma, No special type' (Figure 1A) with mean Nottingham score of 7.6 with highest and lowest score of 9 and 6, respectively. The histopathological grade as per Modified Bloom-Richardson grading was Grade 2 in 16 cases and Grade 3 in 32 cases (Table 1).

**Table 1**

| Number of Cases | Nottingham Score | Modified Bloom-Richardson Grade |
|---|---|---|
| 0 | 3,4,5 | I |
| 16 | 6,7 | II |
| 32 | 8,9 | III |

The diagnosis of TNBC was given after immunohistochemical evaluation of formalin fixed paraffin embedded tissue sections using ER (Clone EP1), PR (Clone EP2) and Her-2-neu (Clone EP3) primary antibodies using standard immunohistochemistry staining protocol. For estrogen and progesterone receptor evaluation, Allred scoring system was used and a total score of 0-2 was considered as negative. (Figure 1B, 1C). Similarly Her-2-neu was considered negative when the staining was negative/faint/barely perceptible in part of membranes (score of 0-1+) (Figure 1D). An IHC staining score of 2+ (weak to moderate complete membrane staining) was considered equivocal and additionally Fluorescent in Situ Hybridization (FISH) was done in such cases, for a definite negative status of Her-2-neu expression.

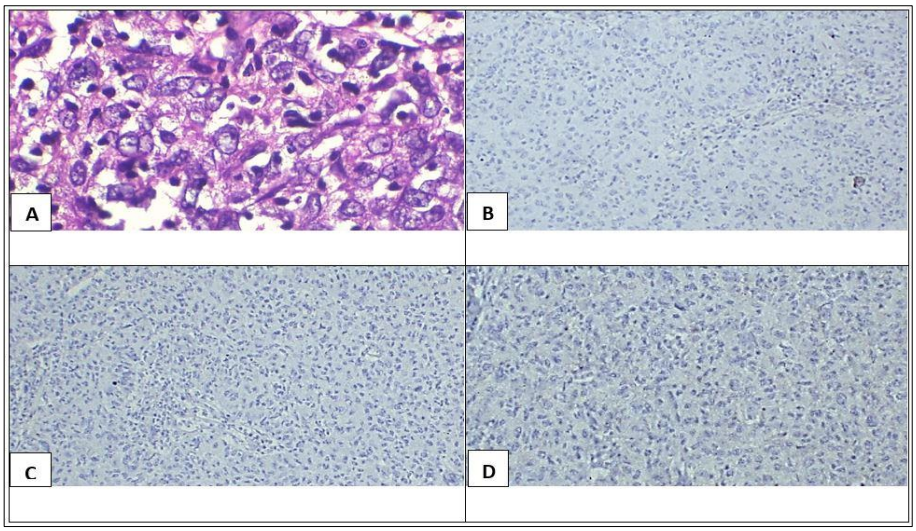


**Fig 1**: A- Invasive breast carcinoma (NOS) (H&E x400). B, C, D- Negative expression of Estrogen Receptor, Progesterone Receptor and Her-2-neu (Immuno-histochemistry x100).

Out of 48 cases, all cases were negative for estrogen and progesterone receptor on immunohistochemical evaluation, whereas in case of Her-2-neu expression, 3 cases on immuno-histochemistry were equivocal with a score of 2+ (Figure 2A), with high Ki-67 expression (Figure 2B). These equivocal cases of Her-2-neu expression were subsequently confirmed as negative by FISH. (Figure 2D)

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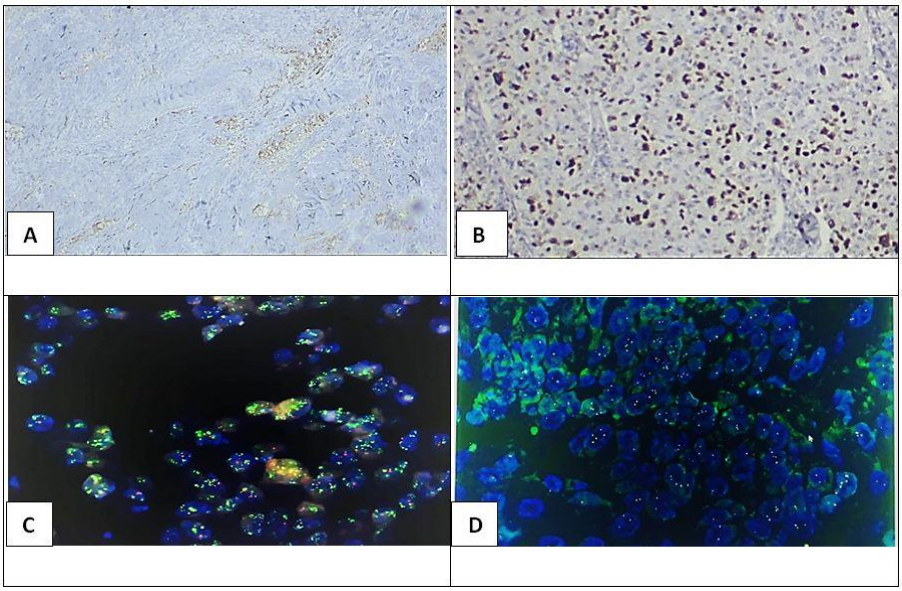


**Fig 2:** A- Her-2-neu equivocal, Score 2+ (x100). B- Ki-67 >50% (x400). C- Positive control FISH for Her-2-neu. D- Negative FISH in equivocal case of Her-2-neu expression on imuno-histochemistry.

The proliferation activity of these tumors was also assessed using Ki-67 proliferation index. Ki-67 index was determined using global method of evaluation on formalin fixed paraffin embedded tissue sections using MIB-1 clone. The mean Ki-67 proliferation index was 55% with minimum and maximum scores being 10% and 95%. 34 cases had Ki-67 proliferation index of greater 30% whereas in 14 cases, it was less than 30%.

## Clinical Profile

The mean age of presentation was 46.2 years with minimum and maximum age being 25 years and 80 years respectively. The maximum numbers of cases were seen in 31-40 years age group (13 cases) followed by 41-50 and 51-60 years age group which had 8 cases each.
The most common clinical presentation was breast lump seen in all the cases whereas additional clinical findings with lump were evident in 18 cases (Table 2). The mean size of lump on clinical evaluation was 7.2 cm in largest diameter with minimum size being 2 cm and largest being 14 cm, with fixation to underlying skin and involvement of whole of breast seen in 5 cases. Clinically, T2 was the most common tumor stage with 22 cases followed by T3 (14 cases), T4 (5 cases) and T1 (02 cases).

**Table 2**

| Presenting Complaint | Number of cases |
|---|---|
| Lump only | 30 |
| Lump with skin ulceration | 02 |
| Lump with nipple discharge | 04 |
| Lump with nipple retraction | 04 |
| Lump involving whole breast with skin fixation | 05 |
| Lump with ipsilateral lymph node enlargement | 03 |

## Discussion

TNBC are aggressive tumors with median age of presentation being 49 years in Northern India as documented by Suresh P *et al.*, [6] which was comparable to the mean age of presentation in our study which was 46.2 years with minimum and maximum age being 25 years and 80 years respectively. The maximum number of cases was seen in fourth decade and this was in conjunction with findings of Rajendran and Prasad who reported a median age of 34 years in their study [5]. So, it is postulated that TNBC occurs in younger population in India when compared to population in America where median age was 59 years [8].

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The clinical tumor stage in our study was similar to the study of Suresh P *et al*., who reported stage II in 62% of the cases [6], followed by stage III (15%) whereas in our study T2 was present in 45.8% cases followed by T3 in 29.1% of the cases. A significant difference was found in percentage of T4 and T1 patients where in their study they found only 11% of patients in T4 whereas in our study it was 20.8%, which can be attributed to inherently aggressive nature and advanced stage with poor survival associated with TNBC [13-14]. Conversely in case of T1 disease their study had 12% patients whereas we found it to be much lower, 4.1%, the possible reason for this is lack of awareness towards breast cancer and significance of self examination in our study population. The ipsilateral node positivity was evident in 6.25% cases only, with all cases associated with T2 stage tumors with lump only clinical presentation, so there was no association with T stage and node positivity and this highlighted the fact that TNBC have inherently aggressive nature with tendency to spread, irrespective of tumor size as documented by Dent *et al.* [15].

The tumor grade on examination of hematoxylin and eosin stained section was found to be grade III in 66.7% of cases and 33.3% as grade II with no grade I tumors and this again may be attributed to aggressive nature of TNBC where the majority have been classified as high-grade invasive carcinomas of no special type, with pushing invasive borders, necrosis, brisk lymphocytic infiltrates, marked nuclear pleomorphism, and numerous mitoses [16], a finding similar to our study where all cancers were of 'No Special Type'.

Allred score and Her-2-neu staining pattern were taken as scoring criteria for immunohistochemical evaluation of estrogen receptor, FFPE progesterone receptor and Her-2-neu expression on formalin fixed paraffin embedded tissue sections. Allred Score was 0-2 in all cancers and was taken as negative whereas in case of Her-2-neu a score of 2 was given and the staining was considered equivocal in three cases. Although under ideal conditions both immunohistochemistry and Fluorescent in situ hybridization (FISH) may be used independently to determine Her-2-neu expression but in cases of equivocal expression on FFPE, FISH may be used for exact status of Her-2-neu with a FISH score of less than 4 Her-2-neu gene copies per nucleus or a FISH ratio of less than 1.8 taken as negative [3-4]. All three cases in our study returned negative on FISH analysis.

Ki-67 index with values greater than 30% have been associated independently with worse prognosis along with reduced overall survival and disease free survival in TNBCs [14]. Ki-67 index of greater than 30% was seen in 70% of cases thereby potentiating the fact that TNBC are cancers with high rate of proliferation contributing to their aggressive nature.

## Conclusion

TNBCs occur in a younger population in India as compared to the West. Majority patients have histological grade III tumors with Invasive Breast Carcinoma, No special type as most common subtype. Ki-67 index evaluation should be done for all breast carcinoma cases as it is directly shown to effect prognosis.

## Limitations

1. The sample size in our study was small and further studies with more cases can add further information to the clinical and pathological characteristics of these aggressive cancers.
2. The study did not include the radiological assessment of these tumors.

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