Original Article

# Profile of molecular subtypes of breast cancer with special reference to triple negative: A study from Northeast India

**Gayatri Gogoi, Mondita Borgohain, Projnan Saikia, Syed Abul Fazal**

Department of Pathology, Assam Medical College, Dibrugarh, Assam, India

## ABSTRACT

**Background:** Different molecular classes of breast cancer (BRCA) correlate with prognosis and response to therapy. Triple-negative breast cancer (TNBC) is a newer concept and very limited studies were carried out in India. The aim of this study was to profile the molecular types with a particular emphasis on TNBCs. **Materials and Methods:** Prospectively evaluated descriptive study for 2 years from June 2014 to March 2016, was carried out in the Department of Pathology and Surgery in a tertiary care institute. Cases included were of invasive breast carcinoma in females, confirmed by histopathology. Ethical clearance was received. Data were analyzed using Statistical SAS software. **Results:** A total of 123 cases of invasive BRCA were studied and mean age was 44.64 years. The peak age group was 36–45 years (43.9%). Tumor sizes ≥2 cm was 30%, between 2 and 5 cm was 50.40%, over 5 cm was 19.51%. Invasive duct carcinoma was 82.11% and invasive lobular carcinoma 8.13%. Only 21% of subjects presented as early breast carcinoma. Cases of 1–3 nodes were 22.8%, 4–5 nodes 21.1%, more than five nodes were 34%. Histologic Grade 3 was 50.4%, Grade 2 was 41%, and Grade 1 was only 8.1. The American Joint Committee on Cancer, Stage 1 (17.9%) in Stage 2 (29.3%) Stage 3 was 46.3%, Stage 4 was 6.5%. Estrogen receptor was in 40.62%, progesterone receptor 35.77%, Her2/Neu 18.69% luminal A (19.51%), luminal B (21.13%), Her2/Neu type (17.88%), and triple negatives (38.21). **Conclusion:** The present study showed significantly higher TNBC with poor prognostic factors in younger women in a background of peculiar ethic spectrum in this geographical region.

**Key words:** Basal-like, breast cancer gene 1, premenopausal age, triple negative breast cancer

## INTRODUCTION

The incidence of breast cancer (BC) is increasing rapidly in India. Recent Indian Council of Medical Research data shows that BRCA incidence rates within India display a 3–4-fold variation across the country, with the highest rates observed in the Northeast and major metropolitan cities such as Mumbai, Chennai, Bengaluru, and New Delhi.[1] Reasons for this variations are not known.

BC is a heterogeneous disease with varied morphological appearances, molecular features, behavior, and response to therapy. Current routine clinical management of BC relies on the availability of robust clinical and pathological prognostic and predictive factors to support patient decision-making in which potentially suitable treatment options are increasingly available. The traditional staging on the basis of tumor size and lymph node status remains the cornerstone of outcome indicators; it has become clear that not all BC s presenting at the same stage have the same underlying biology or clinical behavior.[2]

Prognosis varies with tumor size, axillary lymph node status, histologic grade, histologic type and biologic markers such as estrogen receptor (ER), progesterone receptor (PR), Her2/Neu expression profile.[3] Recently

**Address for correspondence:** Dr. Gayatri Gogoi,
Assam Medical College, Dibrugarh - 786 002, Assam, India.
E-mail: gayatrigogoi303@gmail.com

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developed techniques that examine the DNA, RNA and proteins of carcinomas globally have provided a framework for new molecular classifications of this group of BC. Studies correlating the molecular subtypes of BC and pathologic features have found correlations between the two types.[4,5] Earlier a crucial development was the evaluation of BRCA has been the realization that the presence of ER and PR in the tumor tissue by immunohistochemistry (IHC) which was correlated well to the response by hormone therapy and chemotherapy.[6,7] IHC evaluation is one-way to derive molecular subtypes and further Basal-like from triple negative group. Luminal A (ER/PR+ and Her2/Neu−) cancers express hormone receptors (HRs) and are lower grade, include 40%–55% of all BCs.[8,9] The Her2/Neu subtypes overexpress Her2/Neu gene products and are higher grade which is within a range 15%–30% of BCs. Luminal B cancers are approximately 15%–20% (ER/PR+, higher grade, Her2/Neu+/−) have a worse prognosis than luminal A cancers; often have lower expression levels of HRs, higher Nottingham grade, and higher proliferative rates; and can be Her2/Neu+.[6,8,9] There is clinical interest in distinguishing the luminal B cancers from luminal A cancers because they may be a subset of ER+ cancers that derive benefit from more aggressive therapy.[7] The basal-like molecular subtype (13%–25%) appears to overlap that of "triple negative (ER, PR, and Her2/Neu−) type and high grade.[8,9] It is also associated with characteristic histologic features such as solid-pushing borders, geographic areas of necrosis, and dense lymphocytic infiltrates.[10-15] Triple-negative breast cancer (TNBC) is a newer concept and very little is known.[16] Various studies have been reported in Western literature on TNBCs representing basal-like cancers, all of which are highlighting the poor prognostic features of this molecular subtype in comparison to the other types of BCs.[4,12,14,17,18] However, reliable research data on TNBC from India is very scarce. The aim and objective of the study is to profile the molecular subtypes of BC with special reference to triple negative and correlate triple negatives with age and other prognostic parameters in a tertiary care institute from Northeast India.

### Study setting and design

A total of 123 invasive breast carcinoma of females confirmed by histopathology were studied. The clinical information and specimens were collected from the Department of Surgery and operation theaters. Then further analysis was performed in the Department of Pathology. The cases diagnosed by fine-needle aspiration or core biopsy or cases with recurrence were not included in the study. The invasive BC cases undergoing surgery with a curative intent such as lumpectomy or mastectomy were included in the study.

### Ethical clearance

Ethical clearance was received before start of the study from the Institutional Ethics Committee for Human Research of the study institution.

## MATERIALS AND METHODS

The lumpectomy or mastectomy specimens were fixed on 10% neutral buffered formalin in a 10-fold volume immediately after surgery. Important information from patients was collected in performa which includes name, age, menopausal status, childbirth, lactation, present medical history, history, family history of breast and ovarian cancer in blood relatives. Then after explaining about the study, the consent was taken to carry out a study from the participants along with the witnesses. Grossing was done with recording of weight, length, breath, and depth, color of skin flap, any scar, recent surgical incision, edema, discoloration, peau de orange, puckering, Bulging, and ulceration. The tumor location was noted in terms of quadrant, distance from skin, nipple, muscle fascia, color consistency, borders, and margins such as circumscribed or infiltrating also features of, necrosis, hemorrhage, and calcification. Then, recording and careful search for axillary, apical or any other lymph nodes were done. At least four sections from tumor mass, one section each from skin and nipple and all palpable lymph nodes were submitted for processing besides one section from apparently normal tissue for IHC internal control. This was followed by standard histological processing according to laboratory standard operating procedure. Finally, routine hematoxylin and eosin (H and E) stain were done, and sections were studied in light microscope fitted with camera connected to desktop computer.

H and E sections were examined to confirm the presence of invasive cancer, ascertain histological types, histological Bloom–Richardson grade (BRG) modified by Ellis and Elston[19] and axillary lymph node involvement. The American Joint Committee on Cancer (AJCC) staging was assigned after histopathological confirmation of lymph node status and numbers. Staging was done by using radiological investigations such as X-ray chest, ultrasound abdomen for localized disease with the addition of bone scan and computed tomography, magnetic resonance imaging for locally advanced disease and metastatic disease. More pathological features such as necrosis, solid patterns, and lymphovascular invasions were noted. A thickness of 3–4 mm thin sections were taken in three aminopropyltriethoxysilane coated slides both from tests blocks and control blocks for IHC procedure. The steps followed were deparaffinization/rehydration in descending grades of alcohol followed by antigen retrieval with Tris buffer. A dedicated antigen retrieval system (EZ-Retriever

System V.3) was used for optimum retrieval of epitopes in a standardized laboratory condition. The nonspecific sites were blocked by peroxidase and power block inside a humidity chamber. Then, primary antibodies were incubated for 1 hour duration. Finally, secondary antibody components were used in multiple steps with super enhancer, polymer horseradish peroxidase (HRP), and 3, 3'-diaminobenzidine chromogen. The counterstaining was done by iron free hematoxylin. The tumor was immunostained with primary antibodies are of ready to use (RTU) (standardized for dilution with quality control) – anti-ER with, anti-PR, anti-Her2/Neu, anti-Ki-67, anti-cytokeratin 5/6 (CK 5/6), and anti-epidermal growth factor receptor (EGFR) (reference details - table: primary antibodies). The secondary detection system containing HRP with polymer detection method (Reference Super Sensitive Polymer-HRP IHC Detection System, Clone QD400-60KE) was from Food and Drug Administration approved clones and RTU (BioGenex: RTU).

Her2/Neu and ER, PR interpretation and scoring were based on American Society of Clinical Oncology/College of American Pathologist Recommendations 2010.[8,9]

### Estrogen receptor interpretation

Distinct nuclear staining in at least one or more tumor cells was interpreted as positive.

### Progesterone receptor interpretation

Distinct nuclear staining in at least one or more tumor cells was interpreted as positive.

However, we also followed Allred scoring system where the percentage of cells stained and intensity of staining were being counted but for this purpose of subtyping, only positive or negative data were extrapolated to categorize in different subtypes.

### Her2/Neu interpretation

- 3+: More than 30% invasive BRCA cells showing strong complete homogenous membrane positive by Her2/Neu was interpreted as positive
- 2+: More than 30% invasive BRCA showing moderate or incomplete membrane positive Her2/Neu was interpreted as equivocal
- 1+: Any proportion of invasive BRCA cells showing weak or incomplete membrane positive by Her2/Neu was interpreted as 1+, clinically taken as negative
- 0: No stain in any tumor cells, negative.

### Ki-67 interpretation

- Low: Nuclear staining up to 9 tumor cells per 10 high-power field examination
- Medium: Nuclear staining up to 19 tumor cells per 10 high-power field examination
- High: Nuclear staining in 20 or more tumor cells per 10 high-power field examination.

Clinically validated thresholds for CK 5/6 or EGFR staining are still lacking. The definition of basal-like BRCAs has been evolving and though there were no universally agreed on criteria to define it, the panel developed by Nielsen *et al*.[4] is generally accepted in practice. The basal-like cancers are negative for HRs and Her2/Neu, in addition to being positive for CK 5/6 or EGFR. We followed Neilson *et al*. and Cakir *et al*.[20]

CK 5/6: cytoplasmic pattern positivity in more than 10% of tumor cells was taken as positive in the presence of appropriate positive controls.

EGFR: Complete membrane positive in more than 10% of tumor cells was taken in the presence of appropriate positive controls.

IHC defined molecular subtypes were done as follows:

- Luminal A: ER/PR+, Her2/Neu−
- Luminal B: ER/PR+ and Her2/Neu±, high histologic grade, high Ki-67
- Her2/Neu type: ER−, Her2+
- Triple negative: ER−, PR−, Her2/Neu−
- Triple negative – further subdivided
  - Basal-like, CK 5/6+ or EGFR+
  - Nonbasal like, CK 5/6− or EGFR−.

Data were analyzed in Statistical SAS 9.4 software (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA). Chi-square test and ANOVA were done to find statistical significance and correlation between parameters.

## RESULTS

A total of 123 cases of invasive BC were studied during 2 years. The age of the study subjects were ranged from 24 to 75 years with the mean age of presentation was 44.64 years. The premenopausal women were diagnosed more frequently with 58.5% than postmenopausal women of 41.5%. The occurrence of cancer in the left breast was commoner 55% than on the right side 45%. The peak age group was 36–45 years which was accounted for 43.9% of BC. Moreover 63.4% women under 45 years were diagnosed BC, whereas women over 46 years was 37.6% only. The average age of TNBC type diagnosis was 35.77% years.

Tumor sizes of 2 cm or less was 30% at the time of diagnosis. The size between more than 2 cm to 5 cm was 50.40%. The tumor size over 5 cm was 19.51%. The histology type of invasive duct carcinoma (IDC), 82.11%, was the most common followed by invasive lobular carcinoma (ILC) 8.13% and others invasive papillary 3.25%, micropapillary

2.43%, mucious carcinoma 1.62%, metaplastic type, adenoid cystic type and cribriform carcinoma type were one each [Figures 1-4]. Only 21% of subjects presented as early breast carcinoma without regional lymph node involvement. Cases of 1–3 nodes were 22.8%, 4–5 nodes 21.1%, more than 5 nodes were 34% in the study. Histologic Grade 3 were 50.4%, Grade 2 were 41%, and Grade 1 was only 8.1%. The tumor size and lymph node status in subjects were found to have a statistical association with $P \leq 0.001$ staging according to AJCC staging showed in Stage 1 (17.9%) in Stage 2 (29.3%) Stage 3 were highest with 46.3%, whereas Stage 4 accounted for 6.5% [Table 1].

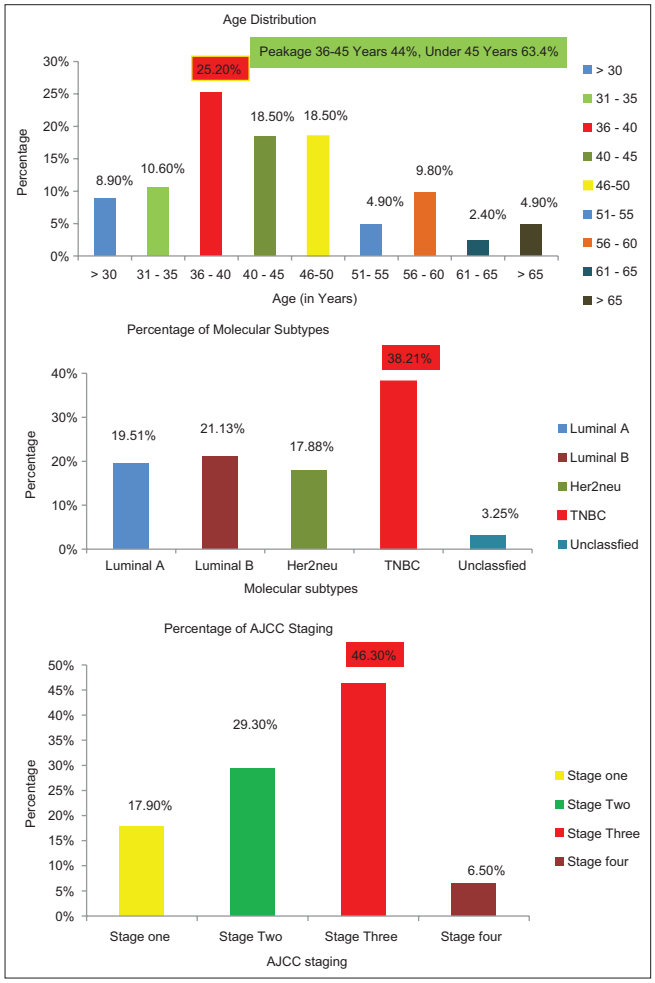


**Figure 1:** Bar diagram of age distribution pattern, molecular subtypes and AJCC stages

IHC evaluation of tumors showed ER+ in 40.62%, PR+ 35.77%, Her2/Neu+ 18.69%. The derivation of molecular types were luminal A (19.51%), luminal B (21.13%), Her2/Neu overexpressed 22 (17.88%), and TNBC (38.21%) [Figures 5-10]. Then, TNBC were tested to further classify by CK 5/6, EGFR into basal type 53.19% and nonbasal type 46.80% [Table 2]. Cross-tabulations between age and molecular subtypes revealed that age diverges significantly among molecular types ($P = 0.007$). Luminal B was expressed in young women (41.1 years), whereas Her2/Neu over-expressed was found in middle age women, that is, 50.4 years. Younger age and TNBC types were showing strong statistical significance.

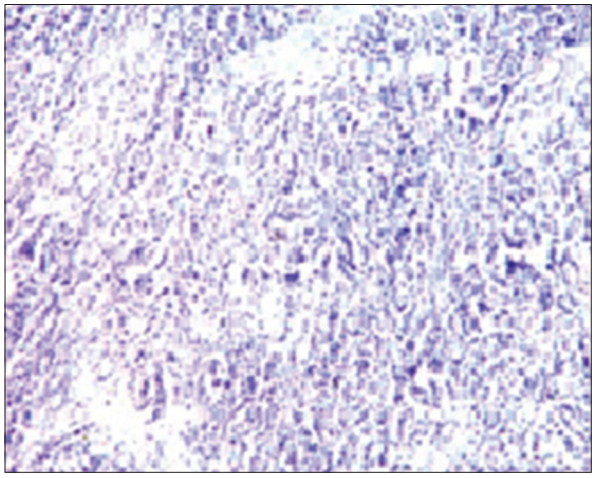

**Figure 2:** Classic invasive lobular histology

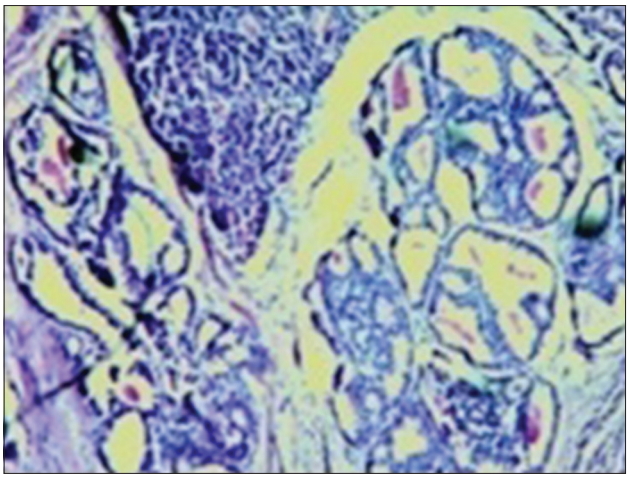

**Figure 3:** Adenoid cystic carcinoma histology

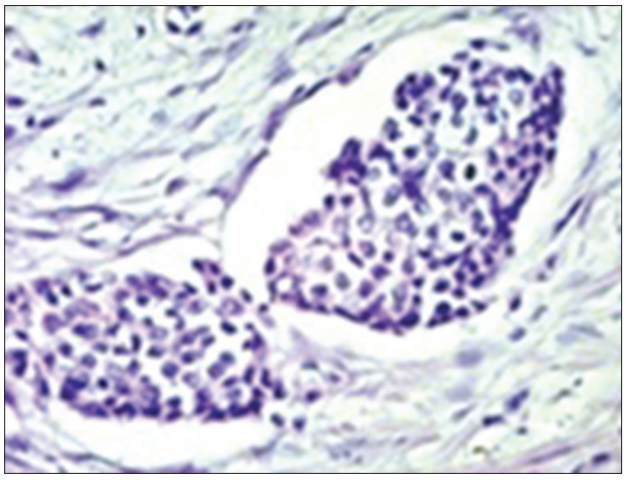

**Figure 4:** Lymphovascular invasion

**Table 1: Characteristic features and their profile data**

| Characteristic parameters | Percentage |
|---|---|
| Age (years) | |
| >30 | 8.9 |
| 31-35 | 10.6 |
| 36-40 | 25.2 |
| 40-45 | 18.5 |
| 46-50 | 18.5 |
| 51-55 | 4.9 |
| 56-60 | 9.8 |
| 61-65 | 2.4 |
| >65 | 4.9 |
| Menopausal status | |
| Premenopausal | 56.5 |
| Postmenopausal | 41.5 |
| Family history of breast cancer | |
| Present | 3.25 |
| Absent | 96.75 |
| Tumour size ($cm^2$) | |
| <2 | 30 |
| 2-5 | 50.4 |
| >5 | 19.6 |
| Histologic types | |
| Invasive duct carcinoma NOS | 82.11 |
| Invasive lobular carcinoma | 8.13 |
| Invasive papillary carcinoma | 3.25 |
| Mucinous carcinoma | 1.62 |
| Micropapillary carcinoma | 2.43 |
| Others | 2.46 |
| Modified Bloom-Richardson histologic grades | |
| 1 (low) | 8.1 |
| 2 (moderate) | 41.4 |
| 3 (high) | 50.5 |
| Axillary node status | |
| Negative (0 node) | 20.32 |
| Positive (1-3) | 21.95 |
| Positive (4-5) | 21.95 |
| Positive >6 | 33.78 |
| Estrogen receptor | |
| Positive | 40.62 |
| Negative | 59.38 |
| Progesterone receptor | |
| Positive | 35.77 |
| Negative | 64.23 |
| Molecular subtypes | |
| Luminal A | 19.51 |
| Luminal B | 21.13 |
| Her2/Neu | 17.88 |
| TNBC | 38.21 |
| Unclassified | 3.25 |
| Her2/Neu | |
| 0 (zero) | 55.28 |
| 1+ | 17.88 |
| 2+ | 9.75 |
| 3+ (positive) | 18.69 |
| K-i67 | |
| Low | 6.50 |
| Moderate | 32.52 |
| High | 60.16 |
| TNBC subtypes | |
| Basal | 53.19 |
| Nonbasal | 46.80 |
| Total cases | |
| AJCC staging | |
| Stage 1 | 17.9 |
| Stage 2 | 29.3 |
| Stage 3 | 46.3 |
| Stage 4 | 6.5 |

TNBC: Triple negative breast cancer, AJCC: American Joint Committee on Cancer, NOS: Not otherwise specified

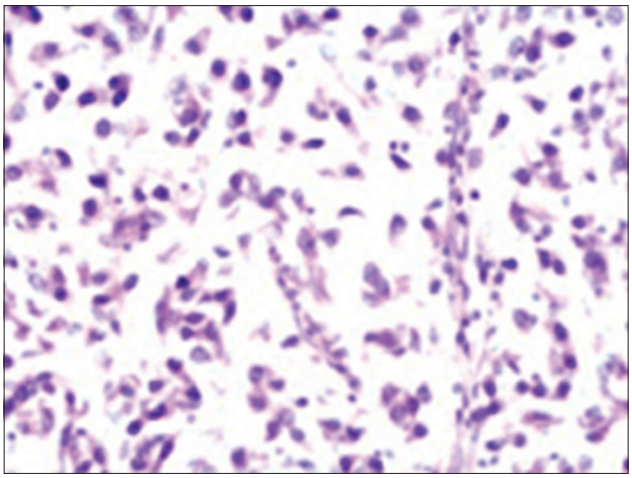

**Figure 5:** Micro papillary carcinoma

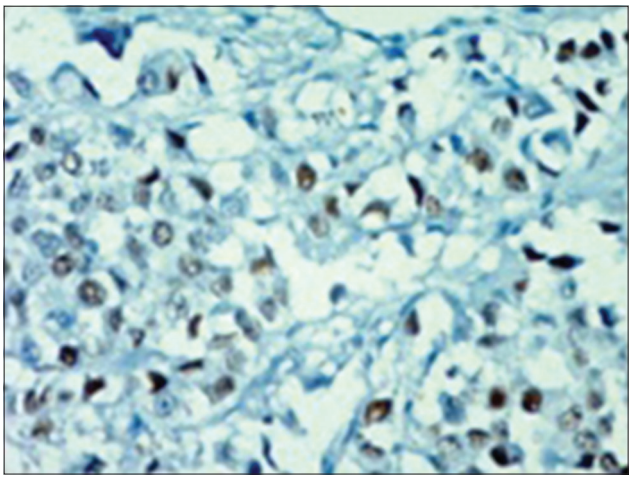

**Figure 6:** Estrogen receptor positive in micro papillary carcinoma

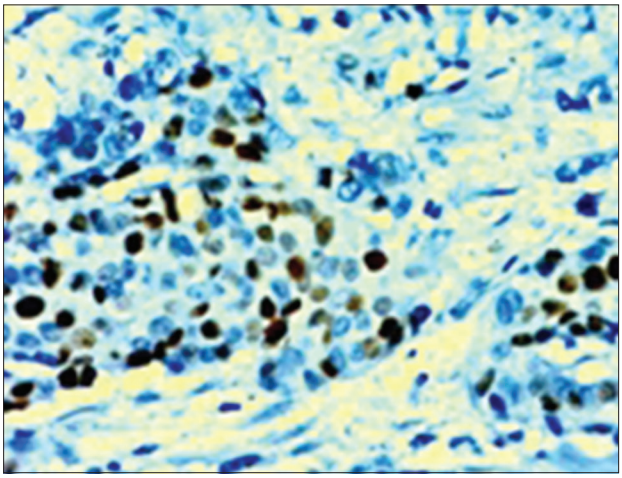

**Figure 7:** Strong estrogen receptor expression in low grade invasive ductal carcinoma

## DISCUSSION

The age of the study subjects was ranged from 24 to 75 years with mean age of presentation was 44.64 years which was

same with the previous study done in this tertiary care setup on total 112 subjects of invasive BRCA of females.[21] Mean age of Indian BRCA were found to be lower than the Western countries with an average difference of one decade, Sandhu *et al*. 2010,[22] Saxena *et al*. in 2005[23] found mean age of presentation as 47.8 years. Over 58% of premenopausal women were diagnosed compared to 41.5% of postmenopausal women affected in this study. Our study was showing slight higher incidence of premenopausal women affected than study done earlier in same institute showing 52% at the time of diagnosis.[21] The peak age group of BC occurrence was 36–45 years which was accounted for 43.9%. The women under 45 years age group was amounted to 63.4% of cases, whereas women over 46 years was 37.6% only. The median age of TNBC occurrence was 39 years only in this study.

The present study found only four cases of family history of blood BC in blood relatives which was comparable to the study[21] done by Gogoi *et al*., 2012 where only three cases had family history of BC in first-degree blood relatives. The histological types of tumor most common was IDC not otherwise specified was 82.11% which was followed by ILC 8.13% and a very small fractions belonged to papillary, micropapillary, mucinous, metaplastic and adenoid cystic type of breast carcinoma [Figure 11]. No histological types showed statistical significance with grades, stages, lymph nodal status or ER, PR Her2/Neu expression pattern. However, it was observed that lobular, tubular, mucious types of carcinoma almost invariably showed hormonal receptor positive which was in concordance with existing literature. IDC type ranged from low histological grades to high grades or represented to all molecular subtypes in the present study. Our study demonstrated a higher number of lymph node positive (80%) cases at the time of diagnosis. Indian and Asian studies have documented a greater percentage of breast carcinomas with lymph nodal metastasis compared to the Western figures.[18,22]

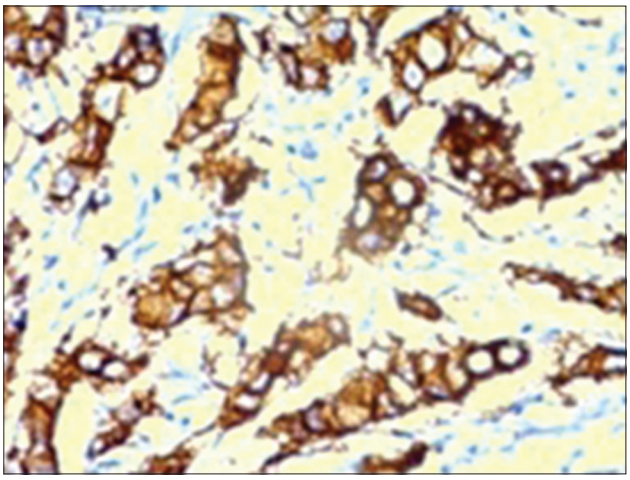

**Figure 8:** Her2 neu positive in invasive lobular carcinoma

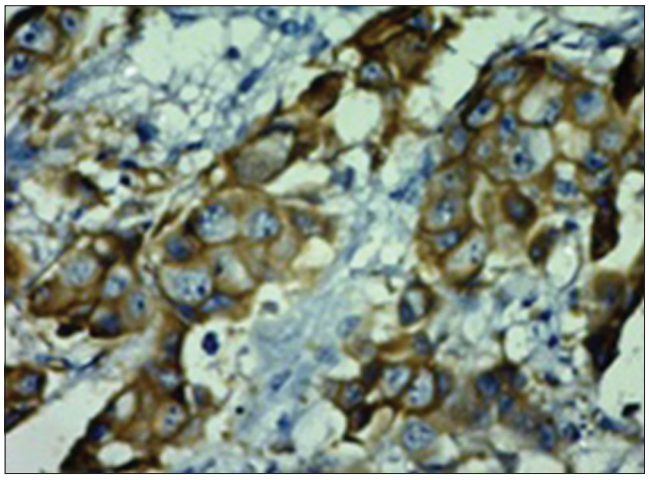

**Figure 10:** Cyokeratin 5/6 positive in a triple negative type of breast carcinoma

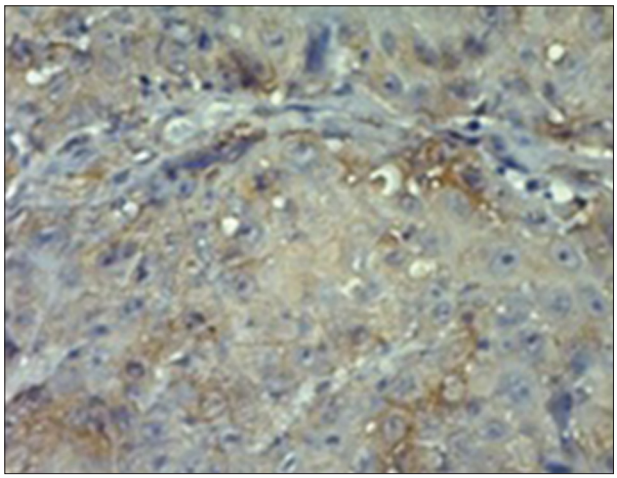

**Figure 9:** Epidermal growth factor receptor positive in a triple negative type of breast carcinoma

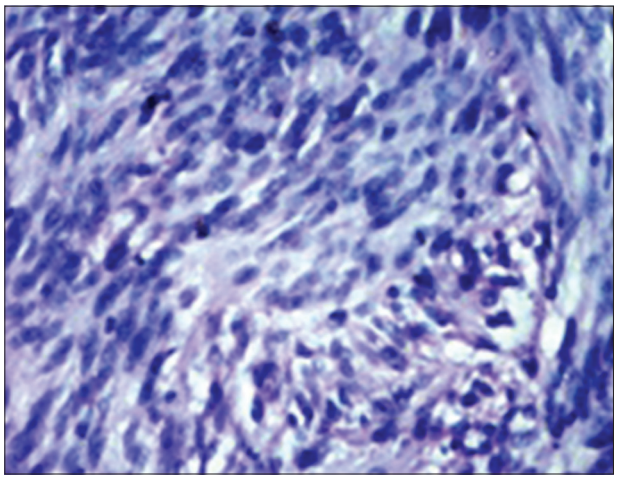

**Figure 11:** Metaplastic type, negative for estrogen receptor, progesterone receptor, Her2, epidermal growth factor receptor, cytokeratin 5/6, a nonbasal like carcinoma

**Table 2: Triple negative breast cancer type profile**

| Characteristic feature | Percentage |
|---|---|
| TNBC type | 38.19 |
| Mean age of TNBC | |
| Non-TNBC | 44.64 |
| TNBC | 35.77 |
| Lymph node | |
| 0 node | 14.89 |
| 1-3 nodes | 14.89 |
| 4-5 nodes | 25.53 |
| >6 nodes | 44.64 |
| Histologic grade | |
| Grade 1 | 10.63 |
| Grade 2 | 38.29 |
| Grade 3 | 51.06 |
| AJCC stage | |
| Stage 1 | 25.53 |
| Stage 2 | 19.14 |
| Stage 3 | 48.93 |
| Stage 4 | 6.38 |
| TNBC subtypes | |
| Basal-like | 53 |
| Nonbasal like | 46 |
| AJCC advanced stage (3 and 4) | |
| Basal-like | 60 |
| Nonbasal like | 50 |

TNBC: Triple negative breast cancer, AJCC: American Joint Committee on Cancer

The incidence of ER and PR in the present study was lower as compared to Western studies, but they were comparable with various studies done in India and other Asian countries. Desai *et al*.[24] obtained low incidence of ER (23.6%) and PR (42.1%) in a study done at Tata Memorial Hospital, India in 2000. Col V Dutta *et al*. in 2006[25] in a study done in New Delhi also found less numbers of BCs were ER (30.66%) and PR (42.66%) positive compared to Western values. Azizun-Nisa *et al*.[26] in a study done in Pakistan in 2008 also found similar results with only 32.7% ER and 25.3% PR positivity. In another study in Pakistan by Nadeem *et al*. in 2008[27] reported 45.83% ER positivity and 50% PR positivity, the findings of which were concordant to the present study. Kaul *et al*. (2011)[28] also reported lower level of ER (34.5%) and PR (36.5%) expression in Indian women with BC. The QuickScore of ER for 151 breast carcinomas by Mudduwa *et al*.[29] PR for 145 breast carcinomas showed 46.7%, and PR was 49.3%, indicating <50% HR expression in the majority of Asian and Indian by studies. Another recent study Sharma *et al*. 2014 published from Regional Cancer of Northeast India showing ER+ 58% and PR+ 49% after excluding TNBC category so actual statistical calculation would be lower than above figures.[30]

The over expression of Her2/Neu is associated with poorer prognosis, high grades features and resistant to usual chemotherapy and suitable for trastuzumab. Her2/Neu profile was 18.75% in this study and was found to be comparable to most of the Indian and Western studies. We found Her2/Neu, were negative in largest percentage (66.07%) of invasive breast carcinoma. This finding was consistent with universally accepted Her2/Neu overexpressed in 15%–30% of cases. This finding is in concordant with Asian study of 19.1% done in Sri Lanka 2009 by Mudduwa.[29] In a study from Bengaluru, South India Vaidyanathan *et al*., found a figure of 43.2% positivity by IHC in contrast to our findings.

The report, Vaidyanathan *et al*. 2010 also showed significant correlation with Her2/Neu and lymph node status, tumor size, and ductal carcinoma type histology.[18] Similarly, Her2/Neu expression was correlated in high-grade tumors, whereas low-grade tumors were expressing higher ER and PR in our study, comparable to study,[26] Azizun-Nisa *et al*. 2008. We found Her2/Neu+ tumors were belonged to mostly IDC histology and sometimes, ILC (high grade), proliferative marker Ki-67 which had shown a direct relationship. Her2/Neu score 3+ associated with a higher Ki-67 expression in invasive component, which was comparable to other studies.[21,31] This suggests the tumor has potential to behave aggressively.[32]

This study classified the tumors into molecular types using protein expression pattern in IHC. Proportion of types found were luminal A (19.51%), luminal B (21.13%), Her2/Neu overexpressed (17.88%) and triple negatives (TNBC) (38.21%). However, four cases remained uncategorized in due to equivocal expression of Her2/Neu protein without ER PR expression due to lack of facility for fluorescent *in situ* hybridization. ER, PR expression with equivocal Her2/Neu category were categorized in luminal B BC. Then TNBC category was tested with CK 5/6 and EGFR to distinguish and found basal type 53.19% and nonbasal type 46.80%.

Although the terms basal-like BC and TNBC are often used interchangeably, they are not synonymous. TNBC refers to the immunophenotype of the BC that is immunologically negative to ER, PR, and Her2/Neu. These immunological studies were done on formalin-fixed and paraffin-embedded tumor sections. Basal-like BRCA refers to the molecular phenotype of the tumor that has been defined by complementary DNA microarrays. Of these TNBCs, about 75% of them are of the basal-like type were the first to describe the various molecular subtypes or molecular profiles of BCs.[33] Perou *et al.* since then, by multiple studies of gene expression profiling, had advanced the understanding of the molecular diagnosis of BC, thus providing the background for oncologists to use the triple negative phenotype to describe the basal-like molecular subtype.[34-36]

The luminal subtypes of BCs express high amounts of luminal CKs and express genetic markers of luminal epithelial cells and normal breast cells. In contrast,

basal-like BCs tend to express CKs associated with basal types of cancers, as they arise from the outer basal layer.[15,37] Basal-like BCs are typically high-grade and poorly differentiated when examined morphologically. While the TNBC phenotype is defined by immune-histochemistry, no established diagnostic criteria have been identified for basal-like BC on a morphological basis. Some have the histomorphology of medullary carcinoma or metaplastic carcinoma. It has also been reported that almost 82% of basal-like BRCAs express p53 compared with 13% in the luminal A subgroup.[38]

TNBC type constitutes 12-24% of BCs[11,15] based on mostly Western literatures such as Dent *et al*. 2007[11] and Rakha *et al*. 2009.[15] Dent *et al*. study found 11.2% and Rakha *et al*. found 16.3% of tumors were TNBC type. Our TNBC types constituted 38.19% from tertiary care center covering a population mostly Assam and adjoining areas of Northeastern states comparable to few studies[20,39] shown in the list in contrast to available Western literature. The median age of TNBC type of BCs were 39 years with mean age 35 years. Out of 47 cases of TNBC and only 10 cases were postmenopausal, whereas rest 37 were premenopausal which was similar to study of findings of more premenopausal women suffer from basal type of BC.[20] Another recent study Sharma *et al*. 2014 published from Northeastern region found 31.9% TNBC type with a median age of 40 years which also was indicative of an association of TNBC type with younger age at diagnosis.[30] The list comparing the TNBC data in Indian setting and Western setting significantly differ. All Indian studies showing a higher proportion of TNBC except one study.[33]

### Comparison of triple negative breast cancer types of breast cancer in Indian and Western studies

1. Dunwald *et al*. 2007, USA 25%
2. Bauer *et al*. 2007, California 12.50%
3. Rakha *et al*. 2007, UK 16.30%
4. Adedayo *et al*. 2009, USA 13.40%
5. Ghosh *et al*. 2011, India 29.80%
6. KK Ma *et al*. 2012, Hong Kong 12%
7. Chun-Yan Li *et al*. 2013, China 12.18%
8. Isil Somali *et al*. 2013, Turkey 15%
9. Suresh *et al*. 2013, India 12.5%
10. SyedaJubeda *et al*. 2013, India 46%
11. Mousumi Sharma *et al*. 2014, India 31.9%
12. Lakshmaiah *et al*. 2016, India 26%
13. Present study 2016 38.21%.

One study published from North India by Suresh *et al*. 2013 a median age was 49 years. One hundred and three patients (60%) were <50 years and only 2 (1.2%) were >70 years.[40] The present study showed the youngest median age (39 years) of TNBC type than other studies[20,39,40] of median age 49 years and the ones described in Western data,[11] of median age 53 years. This finding of younger median age was likely reflective of the general trend of BCs occurring a decade earlier in India[40] and also probably characteristic tumor biology which leads to receptor negative status. Hence, this required further study whether sporadic BRCA mutation had contributed to the development of this higher proportion of TNBC or basal – nonbasal types tumor biology as described in study Dent *et al*.[11] The present study showed statistically significant relation of TNBC type with age ($P$ = 0.0161). Our data showed that age group 35–45 years was at high risk of TNBC.

TNBC subjects data demonstrated lymph node zero in 14.89% of cases, whereas 14.89% in 1–3 nodes, 25.53% in 4–5 nodes and 44.64% were in 6 or more nodes. TNBC types and axillary lymph node involvement had shown a statistically significant relationship. This finding showed a strong discordance with study by Suresh *et al*.[40] having node-negative patients were the largest group (65%) followed by N1 (28%), N2 (4%), and N3 (3%) from India. In general, basal-like breast carcinoma, are morphologically consistent with a high nuclear grade, high mitotic count, and necrosis (such as a Grade 3 IDC, not otherwise specified).[33] Basal-like carcinoma biology reflects TNBC types of BRCA, so our study findings were in concordance with findings of few other studies.[15,37]

TNBC types of cancers were belonged to higher histologic grades (BRG) with, 51.06% Grade 3, 38.29% Grade 2 and only 10.63% in Grade 1 category in our study. It showed statistically Significant association ($P$ = 0.0393) with histologic grades. These findings were comparable to study TNBC with 61% high histologic grade.[40] There were no statistically significant correlation between tumor sizes and lymph node positive status which was a concordance finding in the study from India.[40] This was nicely highlighted in the study by Dent *et al*.[11] where they had shown that in TNBCs even small tumors have a high chance of lymph node positivity.

When TNBC types data were studied from clinical and pathological stages, according to AJCC criteria, Stage 1 in 25.53% of cases, Stage 2 in 19.14% cases, Stages 3 in 48.93% of cases and Stage 4 in 6.38% of cases. Cross-tabulation of these data did not show any statistical significance. Our study reflected a larger proportion of cases belonged to Stage 3 in contrast to another study findings of Stage 2 was the most common stage (62%) followed by Stage 3 (15%).[33]

Basal-like BCs have been found to be more common in younger women of African-American descent, are more aggressive cancers with shorter relapse-free survival, a

tendency to visceral rather than bone metastases and a significant likelihood of BC susceptibility Type 1 mutation.[11] To date, studies on patients with basal-like BRCAs have been limited by small sample sizes and short follow-up times and have been restricted to Western literature. There is not much literature from India available on subclassification of TNBC. Hence, this was the novel step to stratify TNBC to basal-like phenotype is based on IHC staining of tumor tissue on slides using anti-keratin and anti-EGFR antibodies which were still to be in general clinical use. In the clinical setting, it was found that this "basal-like" category of tumors is composed almost entirely of TNBCs.[11] Hence, it has been an area of the attention of pathologists and oncologists as an easily recognizable poor prognostic group of BC that commonly lack targeted therapy. Reliable data on TNBC with its subgroups in Indian setting is very scarce[41] and hence, we undertook the study the clinicopathological background of these cancers in our setting.

We found 53% are basal-like and 46% are nonbasal like of TNBC in our study. Out of basal-like group 56% belonged to high histologic grade in contrast to 40% in nonbasal like group. Again Basal-like were diagnosed in advanced clinical and pathological stage in 60% cases against nonbasal in 50% cases. Both groups had a trend of positive lymph node status and more commonly occurred in premenopausal woman.

Basal-like tumors occur frequently in premenopausal young patients.[42] They were associated with larger tumor size and distinctive histological features, including high histological grade with high mitotic rate and nuclear/cytoplasmic ratio, the presence of spindle cell or squamous metaplasia, pushing growth pattern, central acellular areas of hyalinization or necrosis and lymphocytic infiltrate.[14,42] In our study too high histologic grade, high mitotic count, squamous metaplasia or sarcomatoid change, pushing borders by tumor cells, high degree of necrosis were observed in TNBC/basal type. IDC with medullary features was more likely to be TNBC/basal-like similar to study done by Cakir *et al*. findings of medullary and atypical medullary carcinomas, myoepithelial carcinomas and metaplastic carcinomas may also show the phenotype of basal-like carcinomas.[20] However, we also observed few specific histologic types such as adenoid cystic and apocrine type; even though, TNBC but had low histologic grades similar to reviewed study.[33] A subset of TNBC and basal-like BRCA that is of low histological grade includes secretary, adenoid cystic, acinic cell, and apocrine breast carcinoma.[33]

## CONCLUSION

The finding of higher proportion of TNBCs which were known for biologically aggressive behavior with poor prognostic factors could be one of the contributory factors of higher mortality rate in India. The younger women more frequently diagnosed as triple negative was significant in a background of peculiar ethic spectrum in this geographical region. This data and data supporting by recent Indian studies could be used to improve health care seeking behavior at much younger age such as screening by mammography and by clinical breast examination for early diagnosis. Molecular research should be undertaken to look for relation of sporadic BRCA1 gene mutation with TNBC/basal-like BC among different ethnic groups in this geographical population of Northeast India and associated risk factors in particular in the near future.

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### Conflicts of interest

There are no conflicts of interest.

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