

CLINICOPATHOLOGICAL PROFILE AND HISTOMORPHOLOGY OF TRIPLE NEGATIVE BREAST CANCERS

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ABSTRACT

Background: Triple Negative Breast Cancer (TNBC) is an aggressive subtype of breast carcinoma characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression. TNBC exhibits poor prognosis and limited therapeutic options, emphasizing the need for clinicopathological correlation. The objective is (1) To evaluate the histopathological spectrum of TNBC, including tumor type, grade, and morphological patterns. (2) To assess tumor size, lymph node status, and other pathological parameters. (3) To correlate histopathological features with available clinicopathological data. **Materials and Methods:** This was a cross-sectional study of 54 TNBC cases diagnosed in the Department of Pathology, Tirunelveli Medical College, over a period of 2 years. Histopathological analysis was performed using hematoxylin and eosin staining, and immunohistochemistry (IHC) was conducted for ER, PR, and HER2. Tumors were classified and graded according to WHO classification and Nottingham's modification of the Bloom–Richardson system. Clinicopathological parameters were collected from patient records. **Result:** The majority of cases (87%) were invasive ductal carcinoma NOS. Most tumors (72.2%) were grade II, with 11.1% grade III. Tumor size ranged predominantly between 2–5 cm (64.8%). Lymphovascular invasion was present in 83.3%, lymph node metastasis in 63%, and tumor necrosis in 20.3%. Stromal reaction showed fibrosis in 53.7% and lymphocytic infiltration in 27.7%. Perineural invasion was rare (1.8%). **Conclusion:** TNBC predominantly presents as invasive ductal carcinoma NOS with intermediate to high grade, frequent lymphovascular invasion, and high nodal involvement. Histopathological features strongly correlate with clinical parameters, highlighting the need for early detection and tailored management strategies.

INTRODUCTION

Invasive breast carcinoma (IBC) is the most frequent cancer in women worldwide and the leading cause of female cancer related deaths.^[1] In Indian women, it accounts for about 30% of all cancers.^[2-4] Breast cancer is a complex multifactorial disease associated with age, diet, parity, hormonal status, lifestyle, environment, race and inherited genetic predisposition.^[2,3] This risk rises with age, peaking at 70-80 years.^[5] Prior biopsies revealing atypical hyperplasia or

proliferative changes increases risk. Alcohol consumption, environmental toxins, radiation exposure increases the risk of breast cancer. Lactation lowers the risk for developing breast cancers.^[5]

Breast cancer is a heterogenous disease classified into luminal A, luminal B, normal breast like, basal like 1 and Her 2 overexpressing type based on the gene expression profiling.^[6] Triple negative breast cancers (TNBCs) lacking Estrogen (ER), Progesterone (PR), and Human epidermal growth factor-2 (Her 2 receptors), accounts for about 12% to

14% of cases.^[7] TNBCs are usually aggressive with poor prognosis.^[8] Lehmann et al (2011) classified TNBCs into 7 Subtypes: basal-like1, basal-like2, immunomodulatory, mesenchymal, mesenchymal stem cell like, low androgen receptor and unclassifiable type.^[9]

Aim and Objective of the Study

1. To evaluate the histopathological spectrum of triple negative breast cancers, including tumor type, grade and morphological patterns.
2. To assess tumor size, lymph node status and other pathological parameters in triple negative breast cancers in our population.
3. To correlate histopathological features with available clinicopathological data.

Clinical features:

Invasive breast carcinomas manifest as palpable masses, skin changes, nipple inversion, discharge and breast shape alterations. Rarely, axillary lymphadenopathy is the only sign. These can mimic benign breast diseases, making imaging and histopathology essential for diagnosis.^[10]

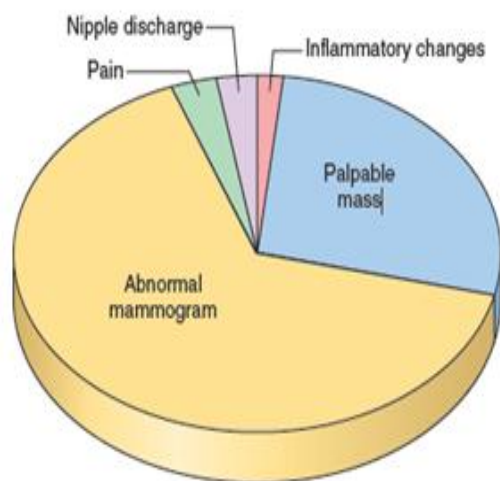


Chart:1 clinical presentations of breast cancer.^[17]

Diagnosis:

1. Clinical examination: Nowadays approximately 90% of breast cancers are identified by imaging, only 10% are found by palpation.^[18]

2. Imaging: Mammography: Detects stellate or circular lesions with or without calcifications.^[17]

Ultrasonography: Useful in women <40yrs and for axillary node assessment. USG guided core needle biopsy became the investigation of choice.^[17]

Magnetic resonance imaging: Highly sensitive, used in high risk population and for staging.^[5,11]

3. Cytology: Nipple discharge cytology has limited value. Fine needle aspiration from palpable mass is reliable [sensitivity 87%, specificity 100%].^[12,13] But may misinterpret benign lesions as malignant.^[13,14]

4. Core Needle Biopsy: Trucut biopsies are superior to FNAC as architecture features are maintained.^[15,16] ASCO/CAP recommends fixation for 6 to 72 before IHC testing.^[19,20]

Who classification of breast tumors^[21]:

Overview

- Epithelial tumors of breast
- Fibroepithelial tumors and hamartomas
- Tumors of nipple
- Mesenchymal tumors
- Hematolymphoid tumors
- Tumors of male breast
- Metastases
- Genetic tumor syndromes.

Invasive Carcinoma Subtypes

- Invasive breast carcinoma of no special type
- Microinvasive carcinoma
- Invasive lobular carcinoma
- Tubular carcinoma
- Cribriform carcinoma
- Mucinous carcinoma
- Mucinous cystadenocarcinoma
- Invasive micropapillary carcinoma
- Carcinoma with apocrine differentiation
- Metaplastic carcinoma

Prognostic and predictive factors:

1.Age: Postmenopausal women will have better prognosis than younger patients.^[22,23]

2.Early diagnosis: Earlier the screening and diagnosis, better the survival and prognosis.^[22]

3.Size of tumor: Larger tumors correlate with nodal involvement.^[24,25] Tumor staging is done based on greatest dimension of tumor,^[21,26] [Table1].

Table 1: staging based on tumor size.^[21]

Tumor stage	Tumor size in greatest dimension, in cm
T1	<2 cm
T2	2-5cm
T3	>5cm

4.Histologic type: Signet ring carcinoma, type of invasive lobular carcinoma of breast has poor prognosis and inflammatory carcinoma of breast has ominous prognosis.^[27]

5.Microscopic grade: The most preferred grading system is Nottingham's criteria of modified Bloom–Richardson system,^[21] [Table 2], based on nuclear grade, tubule formation and mitotic rate [Table 3 &

4]. Nottingham prognostic index is an effective prognostic tool for breast cancer.^[21]

Other factors: Skin invasion has decreased survival rate (27,28). Lymphatic and blood vessel tumour emboli has higher risk of poor prognosis (28,29). Lymph node metastasis has poor prognosis (29,30). Tumour necrosis is seen in high grade carcinomas.^[21,26] Perineural invasion is associated

with poor survival rate.^[21,26,31] Recurrence is seen in cases with positive surgical margins.^[31] Breast carcinomas infiltrated by lymphocytes has good response to chemotherapy.^[32,33]

Table 2: Microscopic Grading of Breast Carcinoma

Microscopic Grading Of Breast Carcinoma: Nottingham Modification Of The Bloom–Richardson System	
Tubule formation	
1 point: Tubule formation in >75% of the tumor	
2 points: Tubule formation in 10%–75% of the tumor	
3 points: Tubule formation in <10% of the tumor	
Nuclear pleomorphism	
1 point: Nuclei with minimal variation in size and shape	
2 points: Nuclei with moderate variation in size and shape	
3 points: Nuclei with marked variation in size and shape	
Mitotic count	
1, 2, or 3 points	

Table 3: scoring of mitotic count per 10 HPF

POINTS	Mitotic count in Field diameter (in mm)	
	0.59mm/0.274mm ²	0.44 mm/0.152 mm ²
1	0 to 9	0 to 5
2	10 to 19	6 to 10
3	>20	>11

Table 4: final grading score of breast carcinoma

Grade	Total Score	Degree of differentiation
I	3 - 5	Well differentiated
II	6 - 7	Moderately differentiated
III	8 - 9	Poorly differentiated

Hormone receptors: Estrogen receptor and Progesterone receptor plays important role in development of normal breast as well as breast carcinoma progression.^[34] Hormone receptors (ER and PR) positivity in the tumor tissue provides good response to chemotherapy and hormone therapy. ER-negative tumours are grade 3 tumors except few special tumour types like adenoid cystic carcinoma and secretory carcinoma.

Hormone receptors status can be evaluated in formalin fixed paraffin embedded tissue by immunohistochemistry, fluorescent in situ hybridization and Polymerase chain reaction.^[35] It is now considered as a standard care to evaluate new breast cancers for the presence of these markers.^[36]

Assessment of hormone receptors:

Allred scoring system for ER & PR:

There are many scoring systems for assessment of the hormonal receptors- ER and PR receptors status but most widely used system is ALLRED scoring system.^[21] [Table 5,6].

Table 5: scoring of proportion of stained nuclei (for ER & PR)

SCORE FOR POSITIVE NUCLEI	PERCENTAGE OF STAINED NUCLEI
0	No staining
1	<1 %
2	1 – 10 %
3	11 – 33 %
4	34 – 66 %
5	67 – 100 %

Table 6: Scoring of intensity of staining (for ER & PR)

SCORE FOR INTENSITY	INTENSITY OF STAINING
0	No staining
1	Weak staining
2	Moderate staining
3	Strong staining

Human Epidermal Growth Factor – 2 Receptor

(Her2): Human epidermal growth factor-2 receptor is encoded by ERBB2 gene. Her-2 amplification and overexpression is seen in 15-20% of breast cancers.^[37,38] Her 2 positive tumors are usually aggressive cancers with high histological grade, high mortality rate and high rate of recurrence. Her 2 positive tumours [Figure 1] have poorer prognosis than Her-2 negative [Figure 2] tumors.^[38] So Her2 receptor status is essential which can be assessed by IHC and fluorescent in situ hybridisation (FISH).

The recent guidelines for Her-2 scoring is given by American Society of Clinical Oncology and College of American Pathologist (ASCO/CAP) protocol [Table 7].

The use of anti Her-2 antibody, Traztusumab as a targeted therapy is an important mile stone in therapy of breast cancers. This management is found to reduce recurrence of breast carcinomas, increase in response rate to therapy and increase in disease free survival of patient and also duration of progression of disease.^[39]

Table 7: Scoring of the immunohistochemical staining for Her2 overexpression

Staining pattern	Score	Her2 overexpression Assessment
No staining is observed or faint, incomplete membrane staining is observed in ≤10% of tumor cells	0	Negative
Incomplete membrane staining that is faint/barely perceptible and within >10% of tumor cells	1+	Negative
Weak and moderate complete membrane staining in >10% of tumor cells	2+	Equivocal
Circumferential membrane staining that is complete, intense, and within >10% of tumor cells	3+	Positive

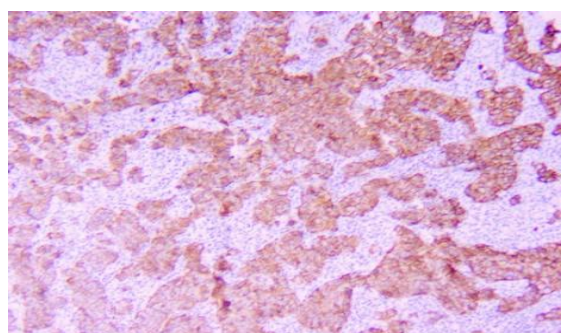


Figure 1: Her2 -control [positive]

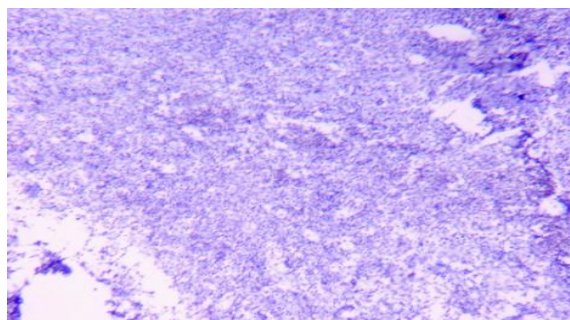


Figure 2: HER 2 - Negative

Molecular classification of breast cancers: The breast cancers are earlier classified based on morphological features. In year 2000, Perou and colleagues classified breast cancers into Molecular subtypes depending on gene expression profiles using microarray.^[36,40,41]

The molecular subtypes of breast carcinomas are based on their gene expression profiling.^[6] [Table 8,9].

Table 8: Major molecular subtypes of breast cancer by gene expression profiling.^[6]

Molecular Subtype				
	Luminal A-Like	Luminal B-Like	Her2- Enriched	Basal-Like
Gene Expression pattern	Positive for LMW cytokeratins, strong positive for ER, PR & associated genes.	Positive for LMW cytokeratins, moderate to weak positive for PR and associated genes.	Strong positive for HER2 and other genes Low positive for ER and associated genes	Strong positive for basal CKs. Low positive for ER & HER2 and associated genes
Clinical features	60% of invasive breast cancers. ER/PR – positive. HER2 – negative. Ki-67-Low.	10% of invasive breast cancers ER positive. PR low positive. HER2-variable. Ki-67-high	15% of invasive breast cancers ER/PR – negative, HER2 – positive High Ki67. TP53 mutation –common, Usually high grade and node positive	15% of invasive breast Cancers, Triple negative, ER/ PR and Her2 – negative, High Ki67, TP53 mutation, BRCA1 dysfunction .
Histologic Type	Tubular & Cribriform carcinoma. Low grade IDC NST & Classic lobular carcinoma	Micropapillary carcinoma. Invasive ductal carcinoma NST	High-grade invasive ductal carcinoma NST.	High-grade IDC NST. Carcinoma with medullary features. Metaplastic carcinoma.
Treatment and outcome	Responds to hormone (tamoxifen & aromatase inhibitors). Chemotherapy is not indicated. Good prognosis	Responds to endocrine therapy but not as for luminal A. Variable response to chemotherapy. Prognosis not as good as luminal A	Responds to trastuzumab Responds to anthracycline Chemotherapy. Generally poor prognosis, But has better with HER2- targeted therapy	No response to endocrine therapy or Trastuzumab. Responds to platinum-based chemotherapy & PARP inhibitors. Poor prognosis

Table 9: IHC as surrogate marker for the molecular subtypes of breast cancers(6).

IHC	Basal like	Her-2 enriched	Luminal A	Luminal B
ER/PR	negative	negative	positive	positive
PR	negative	negative	High positive	low or Moderate positive
Her-2	negative	positive	negative	Variable
Others	CK5/6 + EGFR+	-	Ki 67 <14%	Ki 67 >14%

Triple-Negative Breast Cancer (TNBC)

TNBCs lack expression of estrogen (ER), progesterone (PR), and human epidermal growth factor 2 (Her-2) receptors, accounts for about 15-20% of breast cancers.^[42] These tumors have very aggressive behavior with later stage at diagnosis, high mitotic activity and BRCA1 mutations. Cytotoxic chemotherapy is the main medical treatment for patients with TNBCs with no identified specific targeted therapies and has poor clinical outcome. The median survival of patient is 13 months. Hence TNBCs are studied in elaborated fashion to widen our concept regarding these cancers.^[42,43]

MATERIALS AND METHODS

The study was conducted in Tirunelveli Medical College in department of pathology after getting approval from Institutional Ethical committee of Tirunelveli Medical College in Tirunelveli. The

study was conducted for 2 years. Out of 246 breast carcinomas, 54 cases were reported as TNBCs.

Resources: 54 breast carcinoma patients with triple negative primary breast carcinoma (ER, PR & Her-2 negative) were selected. The specimen was received from Surgical oncology and general surgery department and processed and archived tissue blocks were included for the study. Detailed histories and clinical findings collected from medical records department. 4- 5 micron thick tissue sections were stained with routine hematoxylin and eosin staining and the tumors were classified based on WHO Classification of breast tumors and graded according to Nottingham's criteria. The immunohistochemical examinations for ER, PR & Her-2 were done and ALLRED scoring system was used.^[21]

Sample size: 54 cases of triple negative breast cancers included in the study.

Inclusion Criteria

Cases reported as Triple negative breast cancer by histopathological examination and immunohistochemical staining

Exclusion Criteria

Post chemotherapy and post radiotherapy breast specimens.

Materials Required

1. Formalin fixed tissue blocks of primary TNBCs.
2. Hematoxylin and Eosin stained tissue sections .

Methodology: The clinical history and examination findings of the triple negative breast cancer cases was obtained from medical records department. The following analysis was performed.

RESULTS

Cells appear with dark blue nucleus due to hematoxylin & pink cytoplasm due to eosin. Sections was assessed for prognostic parameters such as tumor type, tumor grade, stromal reaction of

tumour, tumour necrosis, tumour associated insitu components, perineural invasion & angiolymphatic tumor emboli, status of skin, nipple, areola & all resected margins for tumor involvement and lymph node metastases. The tumor type and grade was given based on recent WHO Classification of breast carcinoma and modified Bloom – Richardson grading system.

IHC - Immunohistochemistry is the diagnostic and research tool, used to localise antigens or proteins in tissue sections with labelled monoclonal antibodies by antigen- antibody interactions. We had used an antigen retrieval buffer and a wash buffer from provider path-in-situ.

i. Antigen retrieval buffer- Tris EDTA buffer with pH 9 for all markers.

ii. Wash buffer - Tris buffer with pH 7.6.

Observation and Results

The study results were as follows:

AGE: [Table 13, Chart 2].

Table 13: Age distribution of patients (n=54)

Age Group	No of cases	Percentage
30-39	4	7.41%
40-49	17	31.48%
50-59	19	35.19%
60-70	11	20.37%
>70	3	5.56%
Grand Total	54	100.00%

Table 14: menopause status of patients

Menopause Status	No of cases	Percentage
attained	42	77.78%
not attained	12	22.22%
Grand Total	54	100.00%

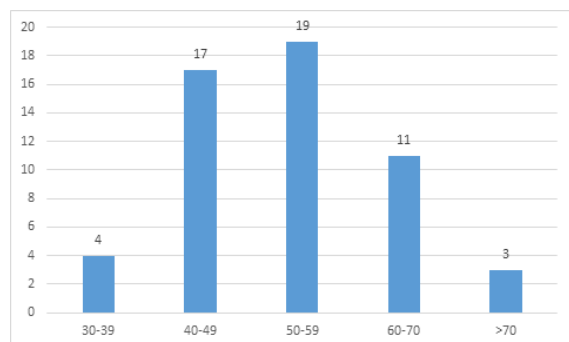


Chart 2: Age Distribution Of Patients

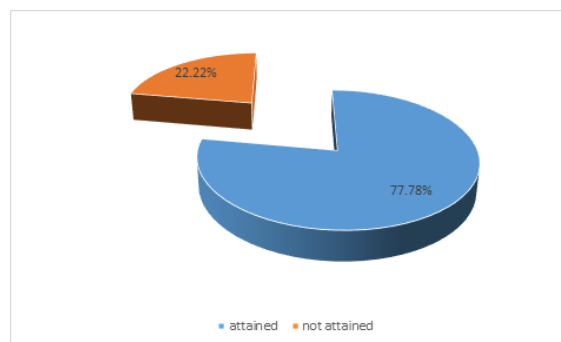


Chart 3: menopause status of patients

Menopause status of TNBC cases: [Table 14, Chart 3]

General parameters of tumor:
Tumor Size: [Table 15, Chart 4]

Table 15: tumor size of patients (n=54)

Tumor size (cm)	No of cases	Percentage
<2	4	7.41%
2-5	35	64.81%
>5	15	27.78%
Grand Total	54	100.00%

Table 16: distribution of cases based on histological type

Histological type	No of cases	Percentage
IDC NST	47	87.04%
IDC WITH MEDULLARY FEATURES	2	3.70%
IDC WITH NEUROENDOCRINE FEATURES	1	1.85%
INVASIVE LOBULAR CARCINOMA	1	1.85%
INVASIVE PAPILLARY CARCINOMA	1	1.85%
METAPLASTIC CARCINOMA	1	1.85%
TUBULAR CARCINOMA	1	1.85%
Grand Total	54	100.00%

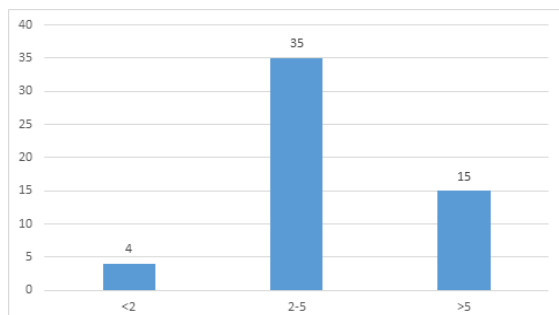
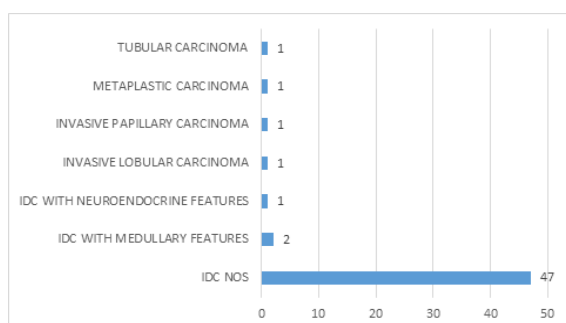
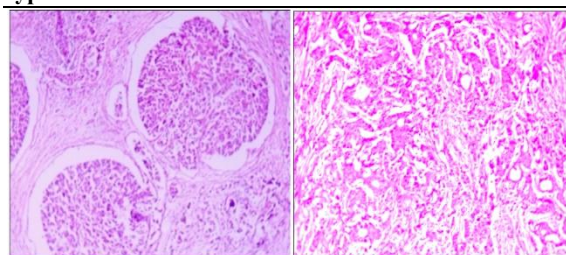
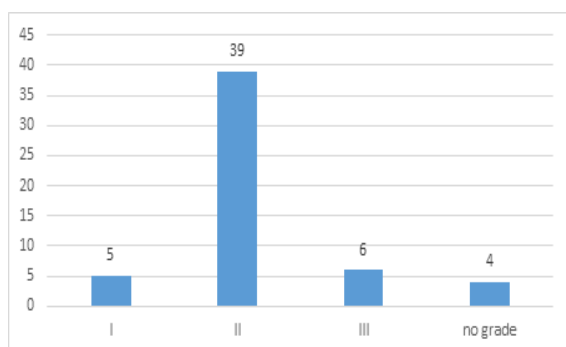
**Chart 4: tumor size****Histopathological parameters:****Type of tumor-** [Table 16, Chart 5 & Figure 3]**Chart 5: distribution of cases based on histological type****Figure 3: H and E OF IDC NST****Tumor Grade:** [Table 17, Chart 6].**Chart 6: distribution of cases based on tumor grade**

Table 17: distribution of cases based on tumor grade

Grade	No of cases	Percentage
I	5	9.26%
II	39	72.22%
III	6	11.11%
no grade	4	7.41%
Grand Total	54	100.00%

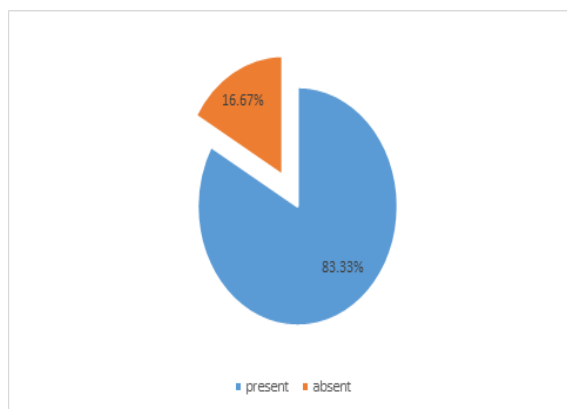
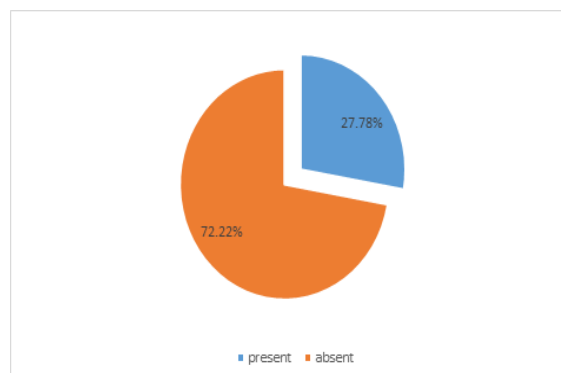
Lymphovascular Invasion: [Table 18, Chart 7]

Table 18: lymphovascular invasion status of patients

LVI Status	No of cases	Percentage
Present	45	83.33%
Absent	9	16.67%
Grand Total	54	100.00%

Table 19: distribution of cases based on presence of in situ component

In situ component	No of cases	Percentage
Present	15	27.78%
Absent	39	72.22%
Grand Total	54	100.00%

**Chart 7: lymphovascular invasion status of patients****Chart 8: distribution of cases based on presence of in situ component**

In SITU Component: [Table 19, Chart 8].

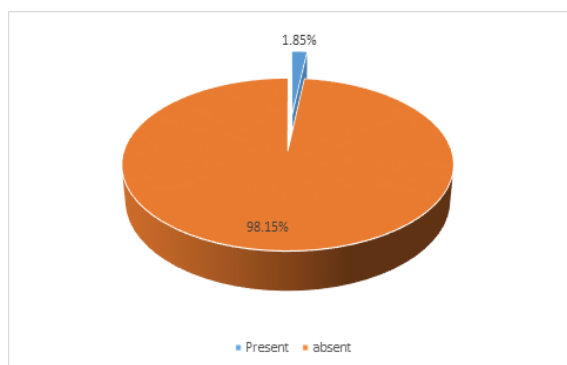
Perineural Invasion: [Table 20, Chart 9]

Table 20: perineural invasion status of cases

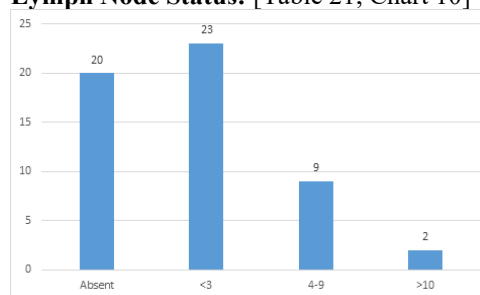
PNI Status	No of cases	Percentage
Present	1	1.85%
absent	53	98.15%
Grand Total	54	100.00%

Table 21: distribution of cases based on lymph node involvement

Node status	No of cases	Percentage
Absent	20	37.04%
<3	23	42.59%
4-9	9	16.67%
>10	2	3.70%
Grand Total	54	100.00%

**Chart 9: perineural invasion status of cases**

Lymph Node Status: [Table 21, Chart 10]

**Chart 10: distribution of cases based on lymph node involvement**

Tumor Necrosis: [Table 22, Chart 11].

Table 22: distribution of cases based on tumor necrosis

Necrosis	No of cases	Percentage
Present	11	20.37%
Absent	43	79.63%
Grand Total	54	100.00%

Table 23: distribution of cases with stromal reaction

Stromal reactions	No of cases	Percentage
Fibrosis	29	53.70%
Fibrosis & lymphocytic infiltration	10	18.52%
Lymphocytic infiltration	15	27.78%
Grand Total	54	100.00%

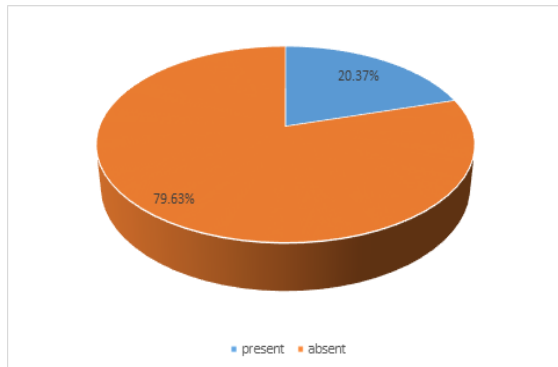


Chart 11: distribution of cases based on tumor necrosis

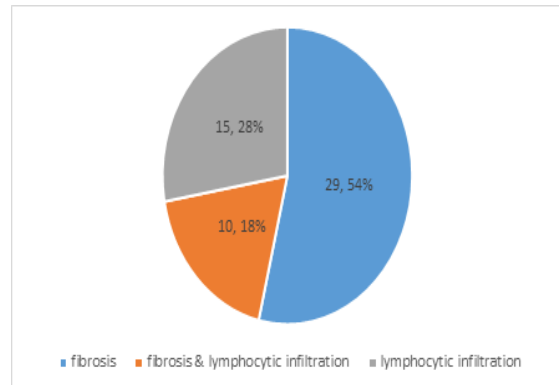


Chart 12: distribution of cases with stromal reaction

Stromal Reaction: [Table 23, Chart 12].

Nipple & Areola: [Table 24, Chart 13].

Table 24: distribution of cases with involvement of nipple and areola by tumor

Nipple & areola	No of cases	Percentage
Free	51	94.44%
Involved by tumor	3	5.56%
Grand Total	54	100.00%

Table 25: distribution of cases with skin involvement by tumor

Skin Involvement	No of cases	Percentage
free	51	94.44%
Involved by tumor	3	5.56%
Grand Total	54	100.00%

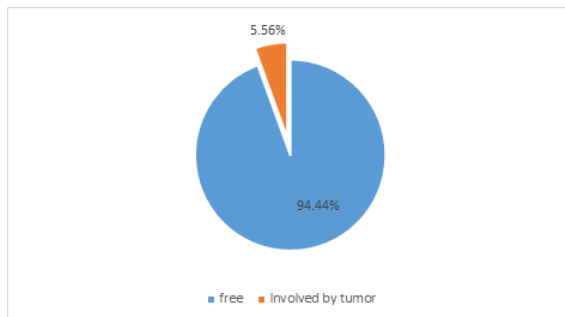


Chart 13: distribution of cases with involvement of nipple and areola by tumor

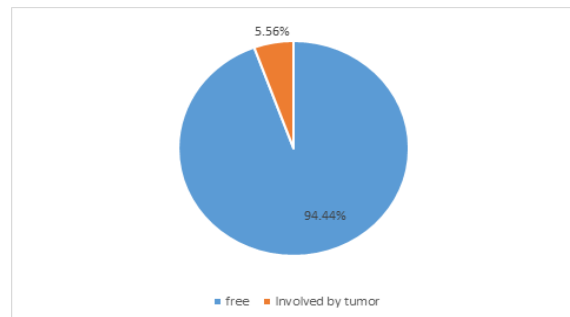


Chart 14: distribution of cases with skin involvement by tumor

Skin Involvement: [Table 25, Chart 14].

Resected Margin Status: [Table 26, Chart 15]

Table 26: distribution of cases with resected margin involvement

Margins Involvement	No of cases	Percentage
free	51	94.44%
involved by tumor	3	5.56%
Grand Total	54	100.00%

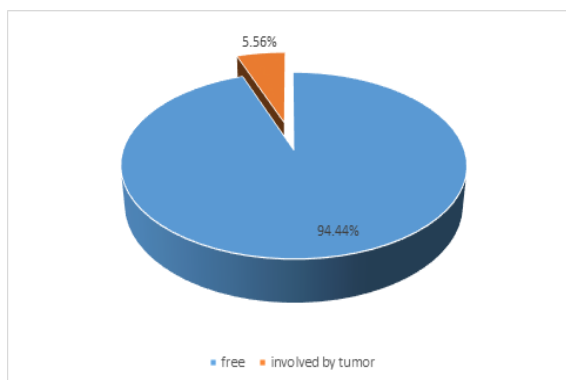


Chart 15: distribution of cases with resected margin involved by tumour

DISCUSSION

The study highlights the clinicopathological spectrum of TNBCs in a South Indian Cohort. Above 50 years aged (61%) cases, followed by patients with the age group less than 50 years (39%) at the time of diagnosis is studied. There was increased percentage of invasive ductal carcinoma NST type constituting about 88%, whereas other special types constitute about 12% only. Similar to the study conducted by Moo-Hyun lee et al, our study also included triple negative breast cancers which are predominantly of grade II and grade III tumours constituting about 72% and 11% respectively. In our study, majority of cases falls within the tumour size >2 cm to <5cm which constitute about 64.8%.

Further, we noted that lymphnode metastasis of N1 stage was present in 42.5% of cases, N2 in 16.6% cases and 3.7% were N3 stage. 20 cases (37%) were negative for lymph node involvement.

Summary

This study was conducted in the Department of pathology, Tirunelveli Medical College, in 54 cases of TNBCs. The clinical and histopathologic parameters such as age, tumor size, histologic type, histologic grade, tumor necrosis, lymph node metastasis, stromal reaction, lymphovascular invasion and DCIS component were assessed in hematoxylin and eosin stained sections of the tumor tissue, followed by immunohistochemical analysis of ER, PR and HER2 in formalin fixed paraffin embedded tissue.

In the 54 cases studied, 47 cases were invasive ductal carcinoma NOS type, 2 cases were IDC with medullary features, 1 case was IDC with neuroendocrine features, 1 case each was lobular carcinoma, invasive papillary carcinoma, tubular carcinoma and metaplastic carcinoma. 4 cases are carcinomas with special types and they could not be categorised in any grade, 5 cases of grade I histology, 39 cases of grade II histology and 6 cases were of grade III histology. 4 cases were <2 cm of size along the largest dimension, 35 cases were 2 - 5 cm and 15 cases were >5 cm of size. 45 cases showed lymphovascular invasion, 1 case showed

perineural invasion, 15 cases had associated DCIS component in the tumor tissue, 11 cases had areas of tumor necrosis, 29 cases had stromal fibrosis, 15 cases had lymphocytic infiltration and 34 cases had lymph node metastasis at the time of diagnosis.

Limitations

1. Sample size is small.
2. A larger multicentric trial can be preferred.

CONCLUSION

TNBC is a heterogeneous and aggressive subtype of breast cancer. Histomorphology combined with clinicopathological parameters provides valuable insights into prognosis. Future research should focus on molecular profiling to identify potential therapeutic targets.

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