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Original Article

Immunohistochemistry-Based Molecular Subtype Distribution of Invasive Breast Cancer and Its Clinicopathological Correlates in a Bangladeshi Cohort

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Abstract

Background/Context: Breast cancer is the second most common cancer worldwide, with approximately 2.3 million new cases annually. In Bangladesh, the disease predominantly affects premenopausal women and often presents at an advanced stage. The distribution of molecular subtypeswhich determine treatment strategies and prognosishas not been comprehensively characterised in largescale single institution studies from Bangladesh.

Objective: To determine the prevalence and distribution of molecular subtypes of invasive breast carcinoma using immunohistochemical (IHC) surrogate markers according to St. Gallen criteria, and to evaluate their associations with clinicopathological parameters in a Bangladeshi cohort.

Materials and Methods: This cross-sectional observational study enrolled 107 consecutive patients with histopathologically confirmed invasive breast carcinoma at the Department of Pathology, TMSS Medical College, Bogura, Bangladesh, from January 2024 to December 2024. IHC was performed for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 using standardised protocols with ASCO/CAP guidelines for HER2 and the Allred scoring system for hormone receptors. Tumours were classified into molecular subtypes (Luminal A, Luminal B, HER2-enriched, and triple-negative breast cancer (TNBC) per St. Gallen surrogate definitions. Clinicopathological associations were assessed using chi-square or Fisher's exact test ($p < 0.05$).

Results: The mean patient age was 45.4 ± 10.6 years, with 67.3% of patients in the 31–50 years age group. TNBC was the most prevalent subtype (33/107, 30.8%), followed by Luminal A (28/107, 26.2%), HER2-enriched (23/107, 21.5%), Luminal B (18/107, 16.8%), and Luminal B (HER2-positive) tumours (5/107, 4.7%). Statistically significant associations were identified between molecular subtype and histological grade ($p = 0.004$) and lymph node metastasis status ($p = 0.035$). Grade III tumours were predominantly TNBC (52.2%), while Luminal A predominated in Grade I (50%). HER2 enriched subtype was the most frequent subtype among cases with extensive nodal involvement (N3a, 60%).

Conclusions: This study reveals a distinctive molecular landscape of breast cancer in Bangladesh, characterised by a high prevalence of TNBC exceeding global rates. The significant associations between molecular subtypes and tumour grade and nodal status validate the St. Gallen IHC classification for clinical practice in resource-limited settings. These findings underscore the urgent need for robust chemotherapy infrastructure, access to HER2-targeted therapies, and enhanced early detection programmes.

Keywords: Immunohistochemistry, Molecular subtypes, Triple-negative breast cancer, Estrogen receptor, HER2 protein, Ki-67 antigen, Bangladesh

Introduction

Breast cancer is the most prevalent malignancy among women worldwide and represents a major global health burden. According to GLOBOCAN 2022 estimates, female breast cancer is the second leading cause of cancer incidence globally, with approximately 2.3 million new cases accounting for 11.6% of all cancer diagnoses. The disease ranks fourth in cancer-related mortality, responsible for

approximately 666,000 deaths (6.9% of global cancer deaths) annually.[1] Despite moderate improvements in survival attributable to advances in early detection and systemic therapies, breast cancer continues to claim an unacceptably high number of lives, owing largely to its heterogeneous biological behaviour, variable treatment responsiveness, and differential access to care worldwide.[2]

In Bangladesh, breast cancer constitutes a formidable public health challenge. Until 2015, it was the leading cancer among Bangladeshi women, contributing to 69% of female cancer deaths.[3] The age-standardised incidence rate is 22.5 per 100,000 women, with 12,764 new cases reported in 2018 according to the International Agency for Research on Cancer (IARC).[4] Uniquely, the disease disproportionately affects premenopausal women: epidemiological studies from Bangladesh demonstrate that 82.35% of patients present with advancedstage disease (Stage III–IV), and 77% of these patients are under 50 years of age.[5] This pattern contrasts markedly with Western populations, where breast cancer incidence peaks in the sixth decade.[6]

Historically, breast cancer classification relied on clinicopathological features including tumour stage, histological grade, and morphological parameters. However, over 70% of breast cancers are classified as invasive ductal carcinoma of no special type (IDC-NST), indicating that morphology alone provides insufficient biological discrimination.[7] The recognition that cancers of identical histological type exhibit vastly different clinical behaviours prompted the development of molecular classification systems. Gene expression profiling studies pioneered by Perou and colleagues identified intrinsic molecular subtypes Luminal A, Luminal B, HER2- enriched, and Basallik ebased on hierarchical clustering of gene expression patterns.[8] These subtypes demonstrate distinct clinical behaviours, prognoses, and therapeutic responses. However, routine gene expression profiling is constrained by high cost, technical complexity, and limited availability, particularly in resource-limited settings such as Bangladesh.[9] Since 2011, the St. Gallen International Expert Consensus has adopted surrogate immunohistochemical (IHC) definitions utilising estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and the proliferation marker Ki-67. IHC used to approximate molecular subtypes in clinical practice.[10] This approach provides a cost-effective, reproducible, and clinically actionable classification system that is increasingly used worldwide.[11] Validation studies confirm that IHC-based surrogate classification effectively stratifies patients into distinct prognostic categories with significant differences in disease-free survival and overall survival.[12]

Epidemiological studies reveal significant geographic and ethnic variation in molecular subtype distribution. Western populations demonstrate higher prevalence of Luminal A subtype (40-50%). South Asian and African populations report elevated proportions of HER2 positive and triple-negative breast cancer (TNBC) subtypes.[13] Data from Bangladesh remain limited; previous small-scale studies have suggested higher rates of aggressive subtypes. But large, single-institution characterisations employing standardised methodology are lacking.[14] Comprehensive understanding of the molecular subtype landscape in Bangladesh is essential for informing healthcare resource allocation, guiding treatment policy, facilitating access to targeted therapies, and designing population-specific clinical trials.

Therefore, the aim of this study was to determine the distribution of molecular subtypes of invasive breast carcinoma using IHC surrogate markers according to St. Gallen criteria, evaluate the expression patterns of ER, PR, HER2, and Ki-67, and analyse the associations between molecular subtypes and clinicopathological parameters in a Bangladeshi cohort.

Materials and Methods

Study Design and Ethical Approval

This cross-sectional observational study was conducted at the Department of Pathology and Immunohistochemistry Laboratory, TMSS Medical College, Bogura, Bangladesh. The study was approved by the Institutional Ethical Review Committee of TMSS Medical College. All procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to tissue sampling and inclusion in the study.

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Study Setting

The study was conducted at a tertiary care academic medical centre in Northern Bangladesh. Data collection and laboratory analyses were performed between January 2024 and December 2024.

Study Population

Patients referred to the Department of Pathology with clinically suspected breast malignancy and requiring histopathological evaluation were screened for eligibility.

Inclusion criteria: (1) histopathologically confirmed invasive breast carcinoma; (2) adequate tissue available for IHC analysis; (3) complete clinicopathological data; and (4) documented patient consent for research use of tissue.

Exclusion criteria: (1) non-invasive breast lesions; (2) availability of adequate tissue for IHC analysis; (3) history of neoadjuvant chemotherapy or hormonal therapy; and (4) patients with incomplete clinical records.

A total of 107 consecutive patients meeting the inclusion criteria were enrolled. This sample size is consistent with published single-institution molecular subtype studies from South Asia and permits meaningful chi-square analysis for clinicopathological associations.

Specimen Collection and Processing

Tissue samples were obtained through core needle biopsy (CNB) or surgical excision (modified radical mastectomy, mastectomy, wide local excision, or lumpectomy). For CNB procedures, tissue was obtained using a 14-gauge needle with a sample notch length of 15–22 mm on a spring-loaded automated biopsy gun; five core biopsies were obtained per patient under local anaesthesia, with ultrasound guidance where required. Specimens were immediately fixed in 10% neutral buffered formalin for a minimum of 6 hours and a maximum of 30 hours prior to processing.

Fixed tissues underwent automated tissue processing with graded alcohol dehydration, xylene clearing, and paraffin infiltration. Sections of 3–4 μm thickness were cut using a rotary microtome and mounted on glass slides. Haematoxylin and eosin (H&E) staining was performed for histopathological diagnosis. Histological type was classified according to WHO criteria. Tumour grading was performed using the Nottingham modification of the Bloom–Richardson system, assigning grades I, II, or III based on tubule formation, nuclear pleomorphism, and mitotic count. Additional parameters recorded included tumour size, presence of lymphovascular invasion (LVI), and lymph node metastasis status (where surgical specimens were available).

Immunohistochemistry Protocol

IHC was performed manually at the Immunohistochemistry Laboratory, Department of Pathology, TMSS Medical College. Fresh 3–4 μm sections were cut from selected paraffin blocks and mounted on positively charged slides. Sections were deparaffinised in xylene and rehydrated through graded alcohols. Heat-induced epitope retrieval (HIER) was performed by microwave antigen retrieval for 20 minutes at 98°C in Tris-EDTA buffer (pH 9.0). Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 10 minutes.

Primary antibodies were applied as follows:

- Anti-ER: Clone 1D5 (Dako, Code N1575), ready-to-use, prediluted
- Anti-PR: Clone PgR 636 (Dako, Code N1630), ready-to-use, prediluted
- Anti-HER2/neu: Clone 4B5 (Ventana), ready-to-use, prediluted
- Anti-Ki-67**:** Clone MIB-1 (Dako), ready-to-use, prediluted

Primary antibody incubation was performed for 60 minutes at room temperature. Detection was achieved using the Labelled Streptavidin–Biotin (LSAB) method (Dako) with diaminobenzidine (DAB) as chromogen. Sections were counterstained with Mayer's haematoxylin, dehydrated, cleared, and coverslipped.

Controls

Three tiers of controls were employed for each IHC run: (1) strong external positive control (known strongly positive breast cancer tissue); (2) weak external positive control (known weakly positive breast cancer tissue); and (3) internal positive control

(normal breast epithelium within the tumour section where available). Negative controls were prepared by substituting the primary antibody with antibody diluent. All IHC-stained slides were independently reviewed by two experienced histopathologists; discordant cases were reviewed jointly to achieve consensus.

Scoring and Interpretation

ER and PR: Expression was evaluated using the Allred scoring system combining proportion score (0–5) and intensity score (0–3) for a total score of 0–8. Scores of 0–2 were classified as negative; scores of 3–8 as positive. Nuclear staining of at least 1% of tumour cells was the threshold for positivity.

HER2: Scored according to ASCO/CAP guidelines:

0 = no staining or membrane staining in < 10% of cells;

1+ = faint/incomplete membrane staining in > 10% of cells;

2+ = weak to moderate complete membrane staining in > 10% of cells (equivocal);

3+ = strong complete membrane staining in > 10% of cells (positive).

Scores 0 and 1+ were classified negative; 3+ positive. Score 2+ cases were classified as negative in the absence of fluorescence in situ hybridisation (FISH) confirmation, a limitation acknowledged in this study.

Ki-67: The labelling index was determined as the percentage of Ki-67 positive nuclei among at least 500 tumour cells in areas of highest proliferative activity (hot spots). Ki-67 < 14% was classified as low; ≥ 14% as high, per St. Gallen recommendations.

Molecular Subtype Classification

Tumours were classified according to St. Gallen surrogate definitions [10,11]:

- Luminal A: ER-positive and/or PR-positive, HER2-negative, Ki-67 < 14%
- Luminal B (HER2-negative): ER-positive and/or PR-positive, HER2-negative, Ki-67 ≥ 14%
- Luminal B (HER2-positive): ER-positive and/or PR-positive, HER2-positive, any Ki-67
- HER2-enriched: HER2-positive, ER-negative, PR-negative
- Triple-negative (TNBC/Basal-like): ER-negative, PR-negative, HER2-negative
- Luminal B (HER2-positive): ER-positive and/or PR-positive, HER2-positive

For analytical purposes, Luminal B (HER2 negative) and Luminal B (HER2 positive) were combined into a single Luminal B category.

Statistical Analysis

Data were entered and analysed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR) as appropriate. Associations between molecular subtypes and clinicopathological variables were assessed using the chi-square test or Fisher's exact test for categorical variables. Statistical significance was defined as $p < 0.05$ with 95% confidence interval (CI).

Results

Demographic and Clinical Characteristics

A total of 107 patients with histopathologically confirmed invasive breast carcinoma were enrolled. The age of patients ranged from under 30 years to over 50 years, with a mean age of 45.4 ± 10.6 years (Table I). The majority of patients (72 cases, 67.3%) belonged to the 31–50 years age group, followed by the > 50 years group (28 cases, 26.2%), and the < 30 years group (7 cases, 6.5%). This age distribution reflects the characteristic earlier age at breast cancer presentation in South Asian populations.

Regarding specimen type (Table II), modified radical mastectomy specimens accounted for the largest proportion (46 cases, 43.0%), followed by core needle biopsy specimens (45 cases, 42.1%), mastectomy specimens (13 cases, 12.1%), lumpectomy specimens (2 cases, 1.9%), and wide local excision specimens (1 case, 0.9%). Overall, the specimen distribution was predominantly represented by modified radical mastectomy and core needle biopsy specimens.

Tumour Characteristics

Tumour size was evaluable in 86 cases where surgical specimens were obtained (Table III). The majority of

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measurable tumours (72/86, 83.7%) were in the 2.1–5 cm range (pT2 stage). Only 4 tumours (3.7%) measured ≤ 2 cm (pT1), and 10 tumours (9.3%) were > 5 cm (pT3). Tumour size could not be assessed in 21 cases (19.6%) with core biopsies only.

Histological grading was performed on 96 cases (Table IV). Grade II tumours were most common (49 cases, 45.8%), followed by Grade I (24 cases, 22.4%) and Grade III (23 cases, 21.5%). Grading was not determinable in 11 cases (10.3%) due to limited tissue or processing artefacts.

Lymphovascular invasion (LVI) was assessable in 35 cases with adequate tissue (Table V). LVI was present in 31/35 assessable cases (88.6%; 29.0% of total cohort) and absent in 4/35 (11.4%; 3.7% of total). This parameter was unevaluable in 72 cases (67.3%), predominantly core biopsies.

Lymph node status was determined in 48 surgical specimens with axillary lymph node dissection (Table VI). Of these, 21 cases (43.8% of assessed; 19.6% of total) were node-negative (N0). Nodal metastasis was present in 27/48 assessed cases: N1a (1–3 positive nodes) in 8 cases (7.5%), N2a (4–9 positive nodes) in 10 cases (9.3%), N3a (≥ 10 positive nodes) in 5 cases (4.7%), and N1 (not further classified) in 4 cases (3.7%). Nodal status was unavailable in 59 cases (55.1%) where core biopsies or mastectomy without axillary dissection were performed.

Immunohistochemical Marker Expression

The distribution of individual IHC markers is presented in (Table VII). Estrogen receptor (ER) positivity was detected in 51 cases (47.7%), with 56 cases (52.3%) ER-negative. Progesterone receptor (PR) positivity was observed in 40 cases (37.4%), with 67 cases (62.6%) PR-negative. HER2 overexpression (score 3+) was identified in 29 cases (27.1%), with 78 cases (72.9%) HER2-negative.

Molecular Subtype Distribution

Classification according to St. Gallen criteria (Table VIII) revealed the following distribution:

- Triple-negative breast cancer (TNBC): 33 cases (30.8%)- most prevalent subtype
- Luminal A: 28 cases (26.2%)- second most common
- HER2-enriched: 23 cases (21.5%)- third most common
- Luminal B: 18 cases (16.8%)- fourth most common
- Luminal B (HER2-positive): 5 cases (4.7%) - least common

Combined hormone receptor-positive subtypes (Luminal A + Luminal B + Luminal B (HER2-positive) accounted for 47.7% of all cases. Total HER2-positive disease (HER2-enriched + Luminal B HER2-positive)constitute 26.2% of the cohort.

Association Between Molecular Subtypes and Clinicopathological Parameters

Results of subtype–clinicopathological associations are summarised in Table 9 and described below.

Age distribution: No statistically significant association was found between molecular subtype and patient age group (p = 0.525). Among patients ≤ 30 years (n = 7), Luminal A accounted for 42.9%, HER2 enriched 28.6%, and TNBC 28.6%. In the 31–50 years group (n = 72), TNBC was most prevalent (29.2%), followed by HER2-enriched (23.6%), Luminal A (20.8%), and Luminal B (19.4%). Among patients > 50 years (n = 28), TNBC and Luminal A were equally prevalent (35.7% each). As shown in Figure 2, aggressive subtypes were represented across all age groups.

Tumour size: No significant association was observed between tumour size and molecular subtype (p = 0.428). Among tumours in the 2.1–5 cm category (n = 72), TNBC was most prevalent (33.3%), followed by Luminal A (31.9%), Luminal B (16.7%), and HER2 enriched (15.3%).

Histological grade: A highly statistically significant association was identified between tumour grade and molecular subtype (p = 0.004; Table 9). Grade I tumours (n = 24) were predominantly Luminal A (50%), with HER2-enriched at 29.2% and TNBC at only 4.2%. Grade II tumours (n = 49) showed a more heterogeneous distribution: TNBC 32.7%, Luminal A 26.5%, HER2-enriched 16.3%, Luminal B 14.3%, and Luminal B (HER2-positive) 10.2%. Grade III tumours (n=23) demonstrated striking enrichment for aggressive subtypes: TNBC 52.2%, HER2-enriched 21.7%, Luminal B 17.4%, and Luminal A only 8.7%. Figure 3 demonstrates the grade–subtype relationship.

Lymph node metastasis: A statistically significant association was identified between lymph node status and molecular subtype (p = 0.035; Table 9). Among node-negative cases (N0, n = 21), Luminal A predominated (47.6%), followed by TNBC (33.3%) and Luminal B (14.3%). In cases with N3a disease (≥ 10 positive nodes, n = 5), HER2-enriched accounted for 60% (3 cases) and Luminal A for 40% (2 cases). N1 cases showed TNBC predominance (75%).

Lymphovascular invasion: No statistically significant association was found between LVI status and molecular subtype (p = 0.582). Among LVI-positive cases (n = 31), Luminal A represented 38.7%, TNBC 32.3%, HER2-enriched 12.9%, and Luminal B 9.7%.

Age and tumour grade: The association between patient age and tumour grade was non-significant (p = 0.402; Table 10). Grade II was the most common grade across all age groups.

Table I. Patient Age Distribution (n = 107)

Age Group	n	Percentage (%)
< 30 years	7	6.5
31–50 years	72	67.3
> 50 years	28	26.2
Total	107	100.0

Mean age: 45.4 ± 10.6 years. The demographic breakdown presented in highlights that the majority of the patients fell within the 31–50 years age spectrum.

Table II. Specimen Type Distribution (n = 107)

Specimen Type	n	Percentage (%)
Modified Radical Mastectomy	46	43.0
Core Needle Biopsy	45	42.1
Mastectomy	13	12.1
Lumpectomy	2	1.9
Wide Local Excision	1	0.9
Total	107	100.0

As outlined in, modified radical mastectomy and core needle biopsy constituted the primary specimen modalities evaluated in this study.

Table III. Tumour Size Distribution (n = 86 assessable cases)

Tumour Size	n (of 107)	Percentage (%)
≤ 2 cm (pT1)	4	3.7
2.1–5 cm (pT2)	72	67.3
> 5 cm (pT3)	10	9.3
Not assessable (core biopsy only)	21	19.6
Total	107	100.0

The classification of tumor dimensions in illustrates a distinct predominance of pT2 stage lesions (2.1–5 cm) within the assessable cohort.

Table IV. Histological Grade Distribution (n = 96 assessable cases)

Nottingham Grade	n (of 107)	Percentage (%)
Grade I (Well-differentiated)	24	22.4
Grade II (Moderately differentiated)	49	45.8
Grade III (Poorly differentiated)	23	21.5
Not determinable	11	10.3
Total	107	100.0

Documents the Nottingham histological grade distribution, demonstrating that moderately differentiated (Grade II) malignancy was the most frequent finding.

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Table V. Lymphovascular Invasion Status (n = 35 assessable cases)

LVI Status	n (of 107)	Percentage (%)
Present	31	29.0
Absent	4	3.7
Not evaluable	72	67.3
Total	107	100.0

The proportion of cases demonstrating lymphovascular invasion among the pathologically evaluable specimens is specified below the matrix.

Table VI. Lymph Node Metastasis Status (n = 48 assessable cases)

Node Status	n (of 107)	Percentage (%)
N0 (node-negative)	21	19.6
N1a (1–3 positive nodes)	8	7.5
N1 (not further classified)	4	3.7
N2a (4–9 positive nodes)	10	9.3
N3a (≥10 positive nodes)	5	4.7
Not applicable	59	55.1
Total	107	100.0

Details the stratification of regional axillary lymph node involvement observed across the surgical excision specimens.

Table VII. IHC Marker Expression Profile (n = 107)

Marker	Positive n (%)	Negative n (%)
ER	51 (47.7%)	56 (52.3%)
PR	40 (37.4%)	67 (62.6%)
HER2 (3+)	29 (27.1%)	78 (72.9%)
Ki-67 ≥ 14% (High)	[n] ([%])	[n] ([%])

ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2. The separate biomarker positivity rates assessed via standardized immunohistochemistry are summarized.

Table VIII. Molecular Subtype Distribution (n = 107)

Molecular Subtype	n	Percentage (%)
Triple-Negative (TNBC)	33	30.8
Luminal A	28	26.2
HER2-Enriched	23	21.5
Luminal B	18	16.8
Luminal B (HER2-positive)	5	4.7
Total	107	100.0

TNBC: triple-negative breast cancer. The comprehensive distribution profile of surrogate molecular classifications according to St. Gallen definitions is mapped in.

Table IX. Association Between Molecular Subtypes and Clinicopathological Parameters

Parameter	Luminal A n (%)	Luminal B n (%)	HER2-Enriched n (%)	TNBC n (%)	Luminal B (HER2-positive) n (%)	p-value
Age group						
< 30 years (n=7)	3 (42.9)	0 (0.0)	2 (28.6)	2 (28.6)	0 (0.0)	0.525
31–50 years (n=72)	15 (20.8)	14 (19.4)	17 (23.6)	21 (29.2)	5 (6.9)	
> 50 years (n=28)	10 (35.7)	4 (14.3)	4 (14.3)	10 (35.7)	0 (0.0)	
Tumour size						
≤ 2 cm (n=4)	0 (0.0)	1 (25.0)	1 (25.0)	1 (25.0)	1 (25.0)	0.428
2.1–5 cm (n=72)	23 (31.9)	12 (16.7)	11 (15.3)	24 (33.3)	2 (2.8)	
> 5 cm (n=10)	4 (40.0)	2 (20.0)	2 (20.0)	2 (20.0)	0 (0.0)	
Tumour grade						
Grade I (n=24)	12 (50.0)	4 (16.7)	7 (29.2)	1 (4.2)	0 (0.0)	0.004
Grade II (n=49)	13 (26.5)	7 (14.3)	8 (16.3)	16 (32.7)	5 (10.2)	
Grade III (n=23)	2 (8.7)	4 (17.4)	5 (21.7)	12 (52.2)	0 (0.0)	
LN **status**						
N0 (n=21)	10 (47.6)	3 (14.3)	0 (0.0)	7 (33.3)	1 (4.8)	0.035
N1a (n=8)	4 (50.0)	1 (12.5)	0 (0.0)	3 (37.5)	0 (0.0)	
N2a (n=10)	3 (30.0)	3 (30.0)	3 (30.0)	2 (20.0)	0 (0.0)	
N3a (n=5)	2 (40.0)	0 (0.0)	3 (60.0)	0 (0.0)	0 (0.0)	
N1 (n=4)	0 (0.0)	0 (0.0)	1 (25.0)	3 (75.0)	0 (0.0)	
LVI status						
Present (n=31)	12 (38.7)	3 (9.7)	4 (12.9)	10 (32.3)	2 (6.5)	0.582
Absent (n=4)	1 (25.0)	0 (0.0)	2 (50.0)	1 (25.0)	0 (0.0)	

TNBC: triple-negative breast cancer; LN: lymph node; LVI: lymphovascular invasion; Chi-square or Fisher's exact test used. evaluates the cross-tabulations and statistical correlations between the intrinsic molecular subtypes and key diagnostic parameters.

Table X. Association Between Patient Age and Tumour Grade (p = 0.402)

Age Group	Grade I n (%)	Grade II n (%)	Grade III n (%)
≤ 30 years (n=7)	1 (14.3)	5 (71.4)	1 (14.3)
31–50 years (n=62)	17 (27.4)	33 (53.2)	12 (19.4)
> 50 years (n=27)	6 (22.2)	11 (40.7)	10 (37.0)

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Illustrates the non-significant statistical relationship observed between the chronological age brackets of patients and their corresponding biological tumor grade.

Discussion

This cross-sectional study provides comprehensive molecular characterisation of invasive breast carcinoma in a Bangladeshi cohort using IHC surrogate markers according to St. Gallen criteria. The principal finding is the high prevalence of triple-negative breast cancer (TNBC) at 30.8%, which emerged as the most common molecular subtypea pattern strikingly different from Western populations.

Molecular Subtype Distribution: Global and Regional Context

The predominance of TNBC (30.8%) in this cohort substantially exceeds the global average of 15–20% and Western rates of 10–15%.[15] This finding is consistent with recent South Asian and African data. Belachew et al. (2023) reported TNBC prevalence of 33.1% in an Ethiopian cohort,[16] while Bangladeshi studies have reported TNBC rates of 22.5–33.3%,[17] lending convergent validity to our observations. The inversion of the traditional subtype hierarchywherein Luminal A typically predominates in Western populations at 40–50%,[18] but ranked second in our cohort at 26.2% carries profound therapeutic consequences. TNBC lacks expression of ER, PR, and HER2, rendering it unresponsive to endocrine therapy and HER2 targeted agents.[19] Until the recent regulatory approval of pembrolizumab and PARP inhibitors, cytotoxic chemotherapy remained the only systemic treatment modality.[20] The high TNBC burden in this population therefore mandates robust chemotherapy infrastructure and equitable access to emerging targeted agents.

The HER2-enriched subtype constituted 21.5% of this cohortnotably higher than the global average of 10–15%.[21] This finding aligns with reports from other Asian populations and may reflect genetic or environmental factors influencing HER2 amplification rates.[22] The clinical significance is substantial: trastuzumab-based dual HER2 blockade regimens achieve pathological complete response rates exceeding 60% in the neoadjuvant setting, transforming HER2-positive disease from one of the worst prognostic categories to one of the most therapeutically responsive.[23] Ensuring equitable access to these agents is a priority healthcare policy issue.

Luminal B tumours accounted for 16.8% of cases, and Luminal B (HER2-positive) tumours for 4.7%. Combined hormone receptor-positive subtypes (Luminal A + Luminal B + Luminal B HER2-positive) constituted 47.7% of casesmarkedly lower than the 70–75% reported in Western populations.[24] This implies that fewer than half of patients in this cohort are candidates for endocrine therapy as the primary treatment modality, contrasting with practice patterns in high-income countries.

Age at Presentation

The mean age of 45.4 ± 10.6 years observed in this study, with 67.3% of patients presenting between 31–50 years, is consistent with established epidemiological patterns of earlier breast cancer onset in South Asia.[25] Rahman and Karim (2018) reported a mean age of 43.95 years in a Chittagong cohort,[17] and studies from Morocco and Pakistan report means of 45 and 47.55 years respectively in sharp contrast to the early 60s typical of Western populations.[26] This younger age profile likely reflects premenopausal hormonal milieu, population demographic pyramids, genetic predisposition in South Asian women, and potentially a role for reproductive and lifestyle factors. The non-significant association between age group and molecular subtype ($p = 0.525$) is noteworthy: it indicates that aggressive phenotypesparticularly TNBCare not confined to older age groups, emphasising that young premenopausal women in this population face disproportionate exposure to high-risk subtypes.

Tumour Grade and Molecular Subtypes

The highly significant correlation between tumour histological grade and molecular subtype (p = 0.004) provides biological validation of the St. Gallen IHC classification system. Grade III tumours demonstrated striking enrichment for TNBC (52.2%), while Grade I tumours showed predominance of Luminal A (50%). This inverse relationship aligns with extensive published literature documenting the association between TNBC and high-grade morphology.[27]At the molecular level, TNBC exhibits high proliferation indices, genomic instability, and loss of differentiation regulatory mechanisms, which manifest morphologically as poorly differentiated tumours with high nuclear pleomorphism, increased mitotic activity, and pushing borders.[28] Luminal A tumours, characterised by low Ki-67 (< 14%), strong ER signalling, and relatively stable genomes, predictably exhibit well-differentiated morphological features.[29] The correlation between grade and molecular subtype has a practical implication in resource-limited settings: careful histological grading can provide initial prognostic information and guide the urgency of molecular testing when IHC resources are constrained.

Lymph Node Metastasis and Molecular Subtypes

The significant association between nodal status and molecular subtype (p = 0.035) adds to the literature on differential metastatic behaviour across subtypes. Luminal A tumours showed relatively higher representation among node-negative cases (47.6% of N0 cases), consistent with their generally more indolent biology, lower proliferation rates, and maintained cellular adhesion mechanisms.[30] Conversely, HER2-enriched tumours dominated cases with extensive nodal involvement (N3a, 60%), reflecting the aggressive metastatic propensity characteristic of HER2positive disease.[31] The heterogeneous distribution of TNBC across nodal stages may reflect the known biological diversity within the TNBC categoryencompassing basal-like, claudin-low, immunomodulatory, and luminal androgen receptor subtypeseach with distinct metastatic patterns and preferences for lymphatic versus haematogenous spread.[32] The integration of both anatomical (TNM staging) and biological (molecular subtype) information thus optimises prognostication and treatment planning.

Tumour Size and Lymphovascular Invasion

The absence of significant associations between molecular subtype and tumour size (p = 0.428) or LVI (p = 0.582) may reflect methodological constraints rather than a true biological null finding. The narrow size range of this cohortwith 67.3% of tumours in the pT2 categorylimits statistical power to detect subtype-related size differences. The non-significant LVI association is likely influenced by the small number of cases where this parameter was evaluable (n = 35), predominantly from surgical specimens. Furthermore, the strikingly high LVI prevalence among assessable cases (88.6%) severely restricts discriminatory power across subtypes.

Strengths

This study has several strengths. It represents one of the larger single-institution IHC molecular classification studies from Bangladesh, with standardised protocols and comprehensive clinicopathological correlation. Classification according to internationally recognised St. Gallen criteria enables valid comparison with global data. Dual independent histopathologist review with consensus on discordant cases enhanced scoring reproducibility. The breadth of clinicopathological parameters examinedincluding age, tumour size, grade, LVI, and nodal statusprovides a comprehensive biological characterisation.

Limitations

Several limitations must be acknowledged. First, the cross-sectional design precludes assessment of long-term disease-free and overall survival outcomes. Second, the single-institution nature may limit generalisability to the broader Bangladeshi population, though institutional findings align with multi-site Bangladeshi literature. Third, HER2

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equivocal (2+) cases were classified as negative in the absence of FISH confirmation, potentially underestimating true HER2-positive rates by 5–10% [43]. Fourth, Ki-67 assessment carries known interobserver variability, and the 14% cutoff, while internationally endorsed, remains contested.[33] Fifth, IHC-based classification approximates but does not perfectly replicate gene expression profiling (concordance approximately 80–85%).[34] Sixth, the limited tissue in core biopsies precluded LVI assessment in 67.3% of cases, reducing power for this clinicopathological association.

Clinical and Translational Implications

To mitigate the high burden of unfavorable molecular subtypes in resource-limited settings like Bangladesh,[20] expanding molecular diagnostic services (such as IHC and HER2 testing) is essential for precision oncology. However, as our prior work identified substantial gaps between breast health knowledge and regular self-examination practices among local Bangladeshi women,[35] diagnostic availability alone is insufficient. Therefore, integrating routine IHC profiling with grassroots health-education initiatives will be imperative. Establishing population-based early screening programs in tandem with accessible subtype profiling represents a vital two-pronged strategy to promote early detection, guide targeted therapies, and ultimately improve overall breast cancer survival outcomes.[36]

Conclusion

This cross-sectional study of 107 invasive breast carcinoma patients in Bangladesh reveals a distinctive molecular landscape characterised by high prevalence of TNBC (30.8%) as the predominant subtype, followed by Luminal A (26.2%), HER2enriched (21.5%), Luminal B (16.8%), and Luminal B (HER2-positive) (4.7%) subtypes. The high proportions of hormone receptor-negative and HER2positive tumours diverge markedly from Western patterns and underscore a substantially different biological and therapeutic context. Statistically significant associations between molecular subtype and histological grade ($p = 0.004$) and lymph node metastasis ($p = 0.035$) validate the clinical utility of St. Gallen IHC classification in this setting. These findings mandate strengthened chemotherapy services, sustainable access to HER2-targeted therapies, and integration of newer immunotherapeutic agents for TNBC, alongside population-specific early detection strategies. Future longitudinal studies correlating molecular subtypes with treatment response and survival outcomes in Bangladeshi cohorts are essential to guide evidence-based oncology practice in this underserved population.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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