ORIGINAL ARTICLE

Molecular Subtypes of Breast Cancer in Quetta - A Hospital-Based Study

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ABSTRACT

Objective: This study aimed to determine the molecular subtypes of breast cancer using immunohistochemistry and to assess their distribution in the study population.

Study Design: It was a retrospective observational study.

Place and Duration of Study: The study was done at Combined Military Hospital, Quetta, from January 2022 to May 2025.

Materials and Methods: The study included 105 patients with complete histopathological data. The data was analysed using SPSS version 20, and employing chi-square tests to evaluate their associations. Statistical significance was determined at p-value < 0.05.

Results: Among the 105 cases, Invasive Ductal Carcinoma (IDC) was most common (89.5%, n=94), followed by Invasive Lobular Carcinoma (ILC) (4.8%, n=5) and Mucinous Carcinoma (3.8%, n=4). Rarer types included Metaplastic carcinoma and Medullary carcinomas. IHC-based molecular subtyping revealed Luminal A as the predominant subtype (40%, n=42), followed by Luminal B (24%, n=26), Triple-Negative Breast Cancers, TNBCs (19%, n=20), and HER2-enriched (16.2%, n=17). These subtypes were correlated with the patient's age, histological grade, and proliferation index.

Conclusion: Luminal A emerged as the most common molecular subtype, typically presenting with a lower histological grade than Luminal B, HER2-enriched, and TNBCs. Most lobular carcinomas were Luminal A type. TNBCs were high-grade, more frequent in younger patients, while other subtypes were common in older age groups. Utilizing IHC markers for molecular subtyping can enhance prognosis and facilitate targeted therapies for improved patient outcomes.

Key Words*: Breast Cancers, Molecular Subtypes, Triple-negative breast cancers.*

Introduction

Breast cancer continues to be a major health concern globally. In 2018, approximately 2.1 million new cases were reported worldwide, resulting in 627,000 deaths.[1] In Asia, the burden of breast cancer has been increasing, with Pakistan reporting the highest incidence rate among Asian countries. One in every nine Pakistani women faces a lifetime risk of being diagnosed with carcinoma breast.[1] According to recent projections, the age-standardized death rate (ASDR) for breast cancer in South Asia is expected to increase by approximately 35% from 13.4/100,000 in 1990 to 18.1/100,000 in 2030.[2] Pakistan, in particular, is predicted to experience the highest percent change in ASDR between 1990 and 2030, with a staggering 62% increase.[2] A recent study of 9,766 Pakistani women showed low breast cancer knowledge, with only 42.7% aware of risk factors and 41.8% familiar with symptoms.[3] This lack of awareness leads to delayed diagnosis and poorer outcomes.

Prognostic factors are integral to the management and treatment decision-making process in breast cancer. Traditionally, key elements influencing patient outcomes include tumor size, histological grade, lymph node status, and hormone receptor expression.[4] The status of hormone receptors—specifically estrogen receptor and progesterone receptor positivity—is linked to improved outcomes and is vital for guiding targeted therapeutic strategies.[5] Additionally, HER2 status has emerged as

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a crucial factor with both prognostic and predictive value. Notably, HER2-low status is associated with favorable outcomes in hormone receptor-positive breast cancers.[5] The proliferation marker Ki67 is associated with poor prognosis when expressed at elevated levels.[6]

Molecular subtyping of breast cancer using gene expression profiling (GEP) is crucial for prognostication and treatment planning. Immunohistochemical markers such as ER, PR, HER2, and Ki-67 act as reliable surrogate markers, allowing practical classification of tumors, prediction of therapeutic response, and risk stratification, especially in settings where gene expression profiling is not routinely available.[7] These subtypes include Luminal A (ER+/PR+/HER2-/Ki-67 low), Luminal B (ER+/PR+/HER2- with high Ki-67 or HER2+), HER2-enriched (ER-/PR-/HER2+), and Triple-Negative Breast Cancers (ER-/PR-/HER2-).[8] Each subtype exhibits distinct clinical outcomes and treatment responses. For instance, Luminal A is associated with the most favorable prognosis, whereas Triple-negative (TNBCs) and HER2-enriched subtypes are linked to higher risks of locoregional recurrence.[8] Immunohistochemistry markers serve as effective surrogates for molecular subtyping, offering a cost-efficient alternative to more complex gene expression profiling methods.

The study of ER, PR, HER2, and Ki67 in breast cancer has gained increasing importance in recent times. These biomarkers are crucial in determining breast cancer subtypes, guiding treatment decisions, and predicting patient outcomes.[9] In Pakistan, where breast cancer incidence is on the rise, accurate assessment of these biomarkers is essential for optimal patient care. Such studies are limited in Pakistan, particularly in remote areas like Quetta. Therefore, determining ER, PR, HER2, and Ki67 expression is expected to significantly contribute to improving treatment outcomes and providing valuable prognostic information for breast cancer patients across the country.

Materials and Methods

This was a retrospective observational study which was conducted at Combined Military Hospital Quetta, on patients who presented with breast lesions between 01 Jan 2022 and 05 May 2025 (CMH QTA-IERB/112/2025).

The study population included adults of both genders aged 22 years to 88 years who presented with breast lumps and were diagnosed with a malignant diagnosis following trucut biopsies, incisional biopsies, and mastectomies. Also, the patients for whom immunohistochemical markers for hormone receptors ER, PR, HER2/Neu, and Ki67 were applied were included. Patients with complete clinical and histopathological data were included.

Patients with incomplete biopsy data or inadequate tissue samples for histological evaluation were excluded. Patients with benign diagnoses and on whom no Immunohistochemical markers were applied were also excluded. Poorly fixed, inadequate, and nonrepresentative specimens were also excluded.

A sample size of 105 was determined using the WHO sample size calculator, incorporating a 95% confidence interval, an anticipated population proportion of 47.2%,[10] and an absolute precision of 10%. Data was collected from hospital electronic records and histopathology archives. Ethical approval was obtained from the Institutional Review Board (CMH QTA-IERB/112/2025), Combined Military Hospital, Quetta. Data confidentiality was maintained.

All surgical specimens, including trucut, incisional biopsies and mastectomies, were reported following the guidelines recommended by the RCPath. Tumor grade was systematically assessed using the modified Bloom–Richardson grading system. For hormone receptor evaluation, the Allred scoring method was employed to quantify both the presence and activity of these receptors. This assessment involved determining the percentage of positively stained tumor cells and calculating the average intensity of the staining.[11] HER2/Neu staining was evaluated on a scale ranging from 0 to 3+. The proliferation marker Ki-67 was quantified as the percentage of cells showing positive staining among the total malignant cell count, subsequently categorized into low (≤15%), intermediate (15–30%), and high (>30%) expression groups. For analytical purposes, patients were stratified into three distinct age groups: I (less than 30 years old), II (31 to 60 years old), and III (over 60 years old)

The study aimed to investigate the interrelationships among various parameters, including patient age,

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histologic type, histologic grade, and the expression levels of Estrogen Receptor, Progesterone Receptor, HER2/neu, and the Ki-67 index. Based on the expression profiles of these key immunohistochemical markers, the tumors were classified into four main molecular subtypes: Luminal A, Luminal B, Triple-Negative Breast Cancers, and HER2-positive categories. Statistical analyses were performed using SPSS version 20. Frequencies were calculated for all relevant variables, including age demographics, hormone receptor status, HER2 status, and the distribution of histological and molecular subtypes, alongside Ki-67 results. Chi-square tests were used to determine the statistical significance of the aforementioned variables, with findings considered significant if the p-value was less than 0.05.

Results

Patients in our study ranged from 22 to 88 years in age. Their mean age was 48.93 years ±14.09. Most of the cases were between 30 and 55 years of age. Females comprised the predominant group, representing 99.1% (n=104) of the sample, while only one male accounted for 0.9% (n=1). The samples primarily consisted of trucut biopsies, 52.4% (n=55), and incisional biopsies, 27.6% (n=29) followed by modified radical mastectomy (MRM) specimens, 17.1% (n=18), and 2.9% (n=3) cases of wide local excision. Among all breast cancer cases, 89.5% (n=94) were classified as invasive ductal carcinomas, no special type (IDC NST), 4.8% (n=5) as invasive lobular carcinomas (ILC), 3.8% (n=4) as mucinous carcinomas, 1% (n=1) medullary carcinoma and 1% (n=1) as metaplastic carcinoma. The results of immunohistochemical markers ER, PR, and HER2/Neu indicated that 66.7% (n=70) of cases were ER positive, while 33.3% (n=35) were ER negative. Additionally, 54% (n=84) of cases were PR positive, and 44.8% (n=47) were PR negative. Her2/Neu showed positivity in 22.9% (n=24) cases and was negative in 75.2% (n=79) of cases, while 1.9% (n=2) cases were equivocal and were sent for confirmation by fluorescence in situ hybridization, as shown in Table I.

Molecular subtyping was conducted utilizing immunohistochemical markers, which identified Luminal A as the most prevalent subtype, accounting for 40% (n=42) of cases, followed by Luminal B at 24% (N=26), triple-negative breast cancers (TNBCs) at 19% (n=20), and Her2-enriched at 16.2% (n=17), as illustrated in Figure 1. Most of the triple-negative cancers were high-grade invasive ductal carcinoma, comprising 19.1% (n=18) of cases. Additionally, both Medullary carcinoma and Metaplastic carcinoma were also classified as triple-negative.

The majority of cases were classified as grade II tumors (52.4%, n=55), followed by grade I (26.1%, n=27) and grade III (21%, n=22). According to the Ki-67 index, most cases were categorized as low grade (38.1%, n=40) and high grade (37.1%, n=39). Ductal carcinoma in situ (DCIS) was identified in 12.5% (n=13) of cases, while lymphovascular invasion (LVI) was identified in 11.4% (n=12) of cases.

ER, PR positivity was more prevalent in age groups II and III compared to age group I. HER2/Neu positivity was predominantly observed in perimenopausal females, 87.5% (n=21), and was mostly negative in postmenopausal females, 82.6% (n=19). Tumors that were ER and PR positive and HER2-negative exhibited low histological grade with a low Ki-67 index. ER and PR positive cases demonstrated a lower Ki-67 proliferation index than ER and PR negative cases. The results indicated the lowest proliferation rate in Luminal A tumors (97.6%, n=41). The majority of Luminal B tumors exhibited moderate to high Ki-67 (42.3% and 53.8%), suggesting a higher proliferation rate than Luminal A. TNBCs and HER2-enriched tumors are highly proliferative groups, underscoring their aggressive nature. In TNBCs, 70% have a Ki-67 index greater than 30%, indicating very high proliferation and high grade. The significant p-value (0.001) provides strong statistical evidence for differences in Ki-67 expression among these subtypes, as illustrated in Table II.

The majority of Luminal A (64.3%), Luminal B (80.8%), and HER2-enriched tumors (88.2%) were observed in age group II. The majority of Triple Negative Breast Cancers (TNBCs) were also found in age group II, accounting for 55% (n=11), followed by age group I at 25% (n=5). The data indicate that Luminal A is most prevalent in age group II (31-60 years), followed by age group III (>60 years).

TNBC is most prevalent in age group I (25%), which is significantly higher than in other subtypes, suggesting a higher frequency of TNBC in younger

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patients. HER2-enriched tumors are predominantly found in age group II (31–60 years). These findings suggested a significant association between age groups and molecular subtype, with a p-value of 0.019, as presented in Table III.

The association between Ki-67 proliferation and age groups was not significant statistically (p=0.157). However, these findings indicated that a high Ki-67 level (>30%) was more prevalent in age group I (13.5%). Additionally, a single case of male breast carcinoma was observed, characterized by a triple-negative status and a low proliferative index (Ki-67 <15%).

Table I: ER, PR and Her2/Neu expression and its association with Ki67 (n=105)

IHC Markers	Ki67			Total	P-value
	<15%	15-30%	>30%		
ER Positive Negative	 41 (58.6%) 1 (2.9%)	 14 (20%) 12 (65.7%)	 15 (21.4%) 22 (62.9%)	 70 (66.7%) 35 (33.3%)	0.001
PR Positive Negative	 39 (67.2%) 3 (6.4%)	 11 (19%) 15 (31.9%)	 8 (13.8%) 29 (61.7%)	 58 (55.2%) 47 (44.8%)	0.001
Her2/Neu Positive Negative Equivocal	 0(%) 42 (53.2%) 0	 11 (45.8%) 13 (16.5%) 2	 13 (54.2%) 24 (30.4%) 2 (100%)	 24 (22.9%) 79 (75.2%) 2 (1.9%)	0.001

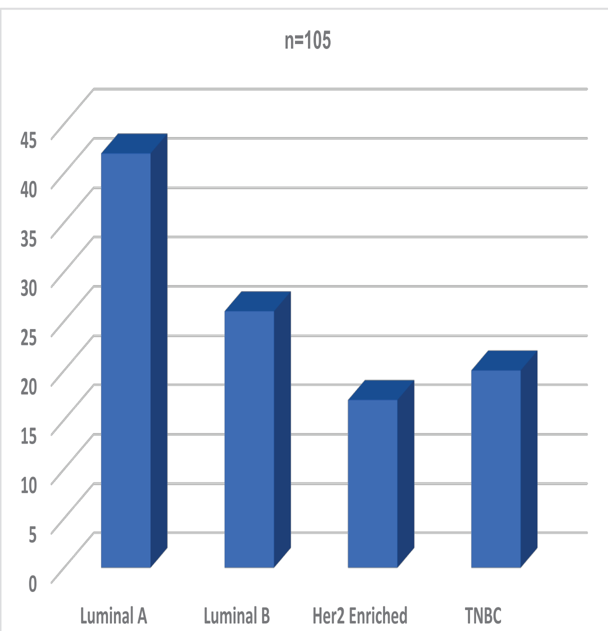

Figure-1: Molecular subtypes of breast cancer

Table II: Association of Molecular subtypes with Ki67 proliferation Index

IHC Markers	Ki67			Total	P-value
	<15%	15-30%	>30%		
Luminal A	41 (97.6%)	1 (2.4%)	0	42 (40%)	0.001
Luminal B	1 (2.5%)	11 (42.3%)	14 (53.8%)	26 (24.8%)	
Her2 Enriched	0	8 (47.1%)	9 (52.9%)	17 (16.2%)	
TNBC	0	6 (30%)	14 (70%)	20 (19%)	
Total	42 (40%)	26 (24.8%)	37 (35.2%)	105 (100%)	

Table III: Association of Molecular Subtypes with Age groups and Proliferation Index

IHC Markers	Age Groups			Total	P-value
	<30	31-60	>60		
Molecular Subtypes					
Luminal A	2 (4.8%)	27 (64.3%)	13 (31%)	42(40%)	0.019
Luminal B	1 (3.8%)	21 (80.8%)	4 (15.4%)	26(24.8%)	
Her2 Enriched	0	15 (88.2%)	2 (11.8%)	17(16.2%)	
TNBC	5 (25%)	11 (55%)	4 (20%)	20(19%)	
Total	8 (7.6%)	74 (70.5%)	23 (21.9%)	105 (100%)	
Ki67 Index					
Low grade (<15%)	2 (4.8%)	27(64.3%)	13(31%)	42 (40%%)	0.157
Intermediate grade (15-30%)	1(3.8%)	22 (84.6%)	3(11.5%)	26(24.8%)	
High grade (>30%)	5(13.5%)	25(67.6%)	7(18.9%)	37(35.2%)	
Total	8 (7.6%)	74 (70.5%)	23 (21.9%)	105(100%)	

Discussion

Breast cancer stands as the most frequently diagnosed malignancy globally, with over 2.26 million new cases reported in 2020.[12] This disease comprises various histological subtypes, each characterized by distinct clinical and pathological features. The most common form is invasive carcinoma of no special type, constituting approximately 80% of all breast cancer cases, followed by invasive lobular carcinoma at 10-15%.[13] Other less common subtypes include mucinous, tubular, medullary, metaplastic, and micropapillary carcinomas, each exhibiting unique characteristics concerning tumor size, lymph node involvement, and molecular subtypes.[14] In our study, invasive ductal carcinoma, NST (89.5%), emerged as the most prevalent subtype, accounting for the majority of cases, followed by invasive lobular carcinoma. These findings align with those of Mouabbi et al. and Jindal A et al., who reported IDC-NST in 80% and 83.09% of cases, respectively.[13,15] In our study Ductal carcinoma in situ (DCIS) was observed in 12.5% (n=13) of cases, and LVI was present in 11.2% (n=12) of cases.

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Immunohistochemical analysis in our cohort revealed estrogen receptor positivity in 66.7% (n=70) of cases, progesterone receptor positivity in 54% (n=84), and HER2/Neu positivity in 22.9% (n=24).

Molecular subtyping of breast cancer, typically determined through gene expression profiling, is vital for understanding disease heterogeneity and guiding personalized treatment strategies, offering insights into tumor behavior and therapeutic responses. Recent studies have shown prognostic significance of interferon-γ signatures in basal-like and luminal B breast cancers.[16] As gene expression profiling is not widely available, Immunohistochemistry (IHC) remains common for molecular subtyping due to its cost-effectiveness. A 2020 study found that IHC-based classifications for distinguishing between luminal A and B subtypes showed poor correlation with genomic subtyping by PAM50 assay, suggesting potential tumor misclassification.[17] However, a 2021 study proposed a deep learning framework for automatic molecular subtyping using IHC images, showing high concordance with pathologist assessment.[18]

Research consistently indicates that Luminal A and B tumors generally exhibit a lower histological grade, whereas HER2-positive and Triple-Negative Breast Cancers are often associated with higher grades. Our study corroborated this, showing ER positivity in 66.7% and PR positivity in 54% of cases, with HER2/Neu positive in 22.9%. Cases that were ER and PR positive exhibited a lower Ki67 index compared to those that were ER and PR negative. Utilizing IHC in our study, Luminal A was identified as the most common subtype at 40% (n=42), followed by Luminal B at 24% (n=26), TNBCs at 19% (n=20), and Her2 enriched at 16.2% (n=17). This pattern aligns with a study from KSA, which also found Luminal A to be the most prevalent (58.5%), followed by TNBCs (14.8%).[19] A study from India by Jindal et al. indicated that TNBCs and Luminal A were the most prevalent molecular subtypes.[15] The higher incidence of Luminal B and TNBCs in our population is likely attributable to low awareness, lack of screening, and inadequate healthcare facilities, resulting in the late presentation of patients with high-grade tumors. Despite both being hormone receptor-positive, Luminal A and Luminal B breast cancers possess distinct characteristics and clinical outcomes. Luminal A tumors, characterized by lower Ki67 expression, signify lower proliferation and a more favorable prognosis compared to Luminal B tumors.[17] TNBCs account for 12-20% of all breast cancer cases. TNBC appears more common among South Asian women compared to Western populations, which may affect prognosis and treatment.[20] In India, TNBC rates have increased to 31% of breast cancer cases.[21] Our study found 19% (n=20) cases were TNBCs, correlating with international studies. Most TNBCs were high-grade Invasive ductal carcinoma, NST 19.1% (n=18), with 70% (n=14) showing Ki67 >30%. Our study showed one case of medullary carcinoma and one case of metaplastic carcinoma, which were both triple-negative. Triple-negative breast cancers are recognized for their aggressive nature, poor outcomes, and limited treatment options, with metastatic TNBCs having only a 12% five-year survival rate.[22] While chemotherapy was previously the only treatment, targeted therapies now include PARP inhibitors for BRCA1/2 mutations and immune checkpoint inhibitors with chemotherapy for PD-L1 positive cases.[23] Higher TNBC prevalence among our patients may be due to early onset age, diet, obesity, multiparity, socioeconomic status and screening behaviours.

In our study, the Luminal A subtype was most frequently observed in age group II (31-60 years), followed by age group III (>60 years). Conversely, TNBCs were most prevalent in age group I, a significantly higher proportion than other subtypes. This finding aligns with international studies, which also demonstrate a consistent association of TNBC with younger age groups compared to other breast cancer subtypes. Research conducted by Thakur et al. (2021) highlighted this pattern, revealing that TNBC represents about 12-24% of all breast cancer cases, with a higher prevalence among younger women.[24]

Molecular typing of breast cancer through immunohistochemistry (IHC) holds significant importance in remote regions like Balochistan, Pakistan. A study on the health poverty index indicates that Balochistan is the poorest province in Pakistan, with 62% of its population experiencing health deprivation.[25] This highlights the urgent need for efficient and cost-effective diagnostic tools in the area. IHC-based molecular subtyping offers essential insights for the prognosis and targeted therapy of breast carcinoma, the most prevalent malignancy among women.[19] Additionally, large-scale, population-specific genomic studies from Pakistan and South Asia are also limited, emphasizing the necessity for regionally focused research to validate and potentially adapt molecular classifications developed in Western cohorts. This approach could expedite the subtyping process and reduce associated costs.

Conclusion

Immunohistochemistry (IHC) molecular typing of breast cancer is pivotal in precision oncology and personalized treatment strategies. The IHC-based classification, especially for ER, PR, HER2, and Ki67, remains the primary approach for identifying breast cancer subtypes and guiding therapeutic decisions. However, despite these advancements, the limitations of IHC-based subtyping underscore the necessity for ongoing research and the potential integration with more advanced genomic techniques to achieve accurate breast cancer classification and personalized treatment.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.