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RESEARCH

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Mammographic parameters as predictors of molecular subtype of breast cancer: a prospective analysis

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Abstract

Background The prevalence of breast cancer is increasing globally and its early detection is the need of hour for giving the patient a long disease-free meaningful life. The latest management regimes depend upon the biological behavior of the breast cancer that itself relies upon expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her 2) neu status for its molecular subtyping.

Aim To determine the predictive value of mammographic parameters in identifying the estrogen and progesterone hormone receptor status, human epidermal growth factor receptor 2 (Her 2) neu expression and molecular subtypes of breast cancer.

Methods A prospective observational study was conducted from January 2021 to September 2022 in a tertiary care institute. The study enrolled 51 females with histopathologically proven invasive breast carcinoma. The patients underwent digital mammography followed by tissue biopsy. Mammographic parameters were based on Breast Imaging-Reporting and Data System (BI-RADS) imaging features. The molecular subtypes of breast cancer were grouped into four subtypes based on St. Gallen International Expert Consensus Panel 2013. The mammographic features were then statistically correlated with molecular subtypes of breast cancer.

Results Luminal type A was the most common molecular subtype in our study [17 (33.33%)] followed by triple negative type [10(19.61%)]. Tumors with non-circumscribed margins were predicted to be Luminal A or Luminal B subtype (p value < 0.02). Tumor with microcalcification was strongly predicted to be Her 2 subtype with a statistically significant association (p value < 0.001). Circumscribed tumors with absence of microcalcification were predicted to be triple-negative type of breast cancer.

Conclusions Key features in mammography were significantly associated with breast cancer molecular subtypes. Knowledge of such correlations could help clinicians stratify breast cancer patients according to their likely molecular subtypes, potentially enabling earlier, more effective treatment or aiding in therapeutic decisions in countries where immunohistochemical (IHC) hormone receptor and Her 2 testing is not readily available.

Keywords Breast cancer, Mammography, Microcalcification, Molecular subtype, Hormone receptor

Background

Breast cancer is an umbrella term given to a diverse group of tumor subtypes, each having its own natural history and survival. Since the incidence of this cancer is

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increasing a practical approach is required for the allocation of the available diagnostics and therapies to this disease.

The traditional classification of breast cancer is based on clinicopathological analysis of tumor that defines breast cancer by histopathological features and grading. The tumor size, lymph node involvement, local invasion into skin and chest wall and distant metastasis are used to decide treatment choices (Trop et al. 2014). During the past two decades, molecular subtyping of breast cancer has been the focus of much research (Cho 2016) and currently there are several classification systems that complement each other to better stratify risk in breast cancer patients. As the latest generation of anti- cancer agents are based on biological behavior of tumor, therefore an elaborate molecular subtyping of breast cancer has become an essential prerequisite in guiding clinical care. The recommendations for systemic therapies for breast cancer depend on the molecular subtype as per the St. Gallen International Expert Consensus Panel 2013. This panel has classified breast cancer into four molecular subtypes based on immunohistochemistry surrogate markers: luminal A-like (ER- and PR-positive, Her2-negative, Ki67 < 20%); Luminal B-like, subdivided into two types, luminal B-like Her2-negative (ER-positive, Her2-negative), luminal B-like Her2-positive (ER- and HER2-positive); Her2-positive (ER- and PR-negative, Her2-positive) and TNBC (ER, PR, Her2-negative) (Somal et al. 2023). Patients with hormone receptor positive (HR+) breast cancer will show good response to hormone therapy (Meisel et al. 2018). Triple negative breast cancer (TNBC) is a subcategory which is aggressive and lacks expression of all the three receptors, that is ER, PR and Her 2 (Mersin et al. 2008). They usually present at a younger age, exhibit higher histologic grade, have large size, higher chances of distant metastasis and recurrence rates therefore associated with poor prognosis (Dent et al. 2007, Rakha et al. 2007). So, this highlights early identification of ER, PR, Ki 67 index and Her 2 status of breast cancer since they have prognostic and predictive value (Kim et al. 2008).

The full proof method for identifying the intrinsic breast carcinoma subtype is gene expression profiling. However, as it is not readily available and cost effective, immunohistochemistry (IHC) remains the gold standard for detecting hormone receptor (ER/PR), Her 2 overexpression and Ki 67 status. Mammography and ultrasound (USG) on the other hand are primary imaging modalities used for screening and diagnosis of breast cancer, its staging, treatment response and follow up of the treated patients. These are non-invasive, cost-effective modalities that are readily available in the remote areas also. Mammography has an added advantage over USG in the elderly female population for the detection of the mass because of their breast composition. In the digital mammogram, the features that are assessed are mass shape, margins, presence or absence of suspicious microcalcifications and architectural distortion, as outlined in the current imaging criteria used in BI-RADS (Rao et al. 2016).

With expanding knowledge of the various biological factors that affect breast cancer management and prognosis, more attention is needed towards imaging to determine whether certain types of tumor biological factors can be predicted by imaging. The use of non-invasive, less resource-intensive method has a practical significance in predicting type of breast cancer. The aim of this study was to investigate whether different mammographic imaging parameters like margin of the breast mass and presence of mammographic microcalcifications, their morphologic pattern and distribution were associated with hormone receptor status, Her 2neu expression, and molecular subtypes of breast cancer and also to calculate the predictive value of their correlation.

Methods

This prospective observational study was conducted for a period of one and a half year from January 2021 to September 2022 in Indira Gandhi Medical College and Hospital, Shimla, H. P., India which is a tertiary care institute. The approval of the institutional ethical committee was taken in accordance with the 1964 Helsinki declaration for the study protocol [IGMC, Shimla No. HFW (MC-II) B (12) ETHICS /2022/10399 dated 23–04-22] and informed consent was acquired before each scan. Female patients who had suspicious breast mass on screening mammography, who were 18 years and above and presented with a clinically suspicious breast lump or had already been diagnosed with invasive breast carcinoma on core or excision biopsy were included in the study. When the patient was less than 40 years of age and had undergone USG as an initial imaging modality, mammography was done later on as a complimentary modality when the diagnosis of malignancy was confirmed. The exclusion criteria included were patients with in-situ breast cancer, patients who underwent neoadjuvant chemotherapy or with recurrent breast cancer, lesions only identified on MRI examination, inadequate tissue sample for IHC examination and pregnant patients. Detailed history like patients' age, symptoms experienced by the patients, any significant past or family history were taken, and the patients who fit the inclusion and exclusion criteria were enrolled in the study.

Digital mammography

Digital mammography was done on Fujifilm AMULET Innovality digital mammography using 28–30 kVp and 45–50 mAs. Cranio-caudal (CC) and Mediolateral oblique (MLO) views were performed in all patients undergoing mammography. Mammographic features that were recorded based on analytical criteria of BI-RADS were breast composition, shape and margin of the mass, density of the mass, architectural distortion, suspicious morphology of calcifications and their distribution and axillary lymphadenopathy. For the purpose of the study, two categories were made as per the features of margin of the mass. Mass with well demarcated margins showing no or two—three macrolobulations were termed as circumscribed. Non- circumscribed mass included the ones with indistinct margin, microlobulations and spiculations. When the entire margin could not be seen, it was considered obscured and not grouped into these categories.

Pathologic analysis

The patients were then subjected to core or excision biopsy of the breast mass within a week of mammography and specimen sent for histopathological analysis. On IHC examination for ER and PR expression based on Allred scoring system as per the 2020 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines, tumor cell nuclear stain > / = 1% was taken as positive. Allred scoring included proportion of cells stained and intensity of staining. Score of more than 2 (proportion + intensity score) was taken as positive (Allison et al. 2020).

The assessment of Her 2 IHC slides was done using the ASCO/CAP 2023 and were graded as follows (Wolff et al. 2023):

> Positive: IHC 3+ (strong positive): tumor displays complete, intense circumferential membranous staining in > 10% of tumor cells (readily appreciated using a low power objective and observed within a homogenous and contiguous invasive cell population)
> Equivocal: IHC 2+: weak to moderate complete membrane staining observed in > 10% of invasive tumor cells
> Negative: IHC 1+: incomplete faint membrane staining and within > 10% of invasive tumor cells
> IHC 0: no staining observed or incomplete faint / barely perceptible membrane staining within ≤ 10% of invasive tumor cells

The score of 0 and 1 + were considered as negative (unamplified), score of 2 + as equivocal and score of 3 + as

Table 1 Distribution of mammographic parameters of breast masses

Parameters	Frequency (*n* = 51)	Percentage
Breast composition		
Type A	4	7.84%
Type B	32	62.75%
Type C	13	25.49%
Type D	2	3.92%
Shape		
No mass visualized (only microcalcification)	1	1.96%
Round	2	3.92%
Oval	10	19.60%
Irregular	38	74.51%
Margins		
No mass visualized (only microcalcification)	1	1.96%
Circumscribed	15	29.41%
Non-circumscribed (Indistinct, Microlobulated, Spiculated)	33	64.70%
Obscured	2	03.92%
Density		
High	43	84.31%
No mass visualized (only microcalcification)	1	1.96%
Equal	7	13.73%
Suspicious morphology calcifications		
Absent	24	47.06%
Amorphous	14	27.45%
Coarse heterogenous	2	3.92%
Fine linear	2	3.92%
Fine pleomorphic	9	17.65%
Distribution of calcification		
Grouped	22	81.48%
Regional	4	14.81%
Segmental	1	3.70%
Architectural distortion		
Absent	9	17.65%
Present	42	82.35%
Location of lesion		
Lower inner quadrant	4	7.84%
Lower outer and inner quadrant	1	1.96%
Lower outer quadrant	1	1.96%
Retro Areolar	11	21.57%
Upper inner quadrant	5	9.80%
Upper outer and inner quadrant	1	1.96%
Upper outer quadrant	28	54.90%
Axillary lymphadenopathy		
Absent	13	25.49%
Present	38	74.51%

positive (Wolff et al. 2023). Her 2-score 2 + (equivocal) were considered Her 2 positive if fluorescent in situ hybridization (FISH) showed Her 2 gene amplification. Since, FISH was not available in our institute, these cases were excluded from the study.

Based on ER, PR, Ki67% (proliferative index) and Her 2-expression status, breast cancers were categorized into four molecular subtypes based on St. Gallen International Expert Consensus Panel 2013 (Somal et al. 2023).

1 Luminal A subtype: ER, PR positive, Her 2-negative and Ki 67 % < 20%.
2 Luminal B subtype (Her 2-negative): ER +, Her 2-negative and at least one of the following- Ki 67 % >/ = 20% and PR – / low (<20%).
Luminal B subtype (Her 2-positive): ER+, Her 2-positive, any Ki 67, any PR.
3 HER2-enriched type (HER2): ER, PR negative and Her 2-positive.
4 Triple-negative type (TN): ER, PR and Her 2-negative.

Statistical analysis

The presentation of the Categorical variables was done in the form of number and percentage (%). On the other hand, the quantitative data were presented as the means ± SD and as median with 25th and 75th percentiles (interquartile range). The following statistical tests were applied for the results:

1. The comparison of the variables which were quantitative in nature were analyzed using independent t test.
2. The comparison of the variables which were qualitative in nature were analyzed using Chi-Square test. If any cell had an expected value of less than 5 then Fisher's exact test was used.

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 25.0. For statistical significance, p value of less than 0.05 was considered statistically significant.

Results

The study enrolled 51 females with histopathologically proven invasive breast carcinoma. The age range of the patients was from 31 to 82 years with a mean value of 51.63 ± 10.5 years. The majority of patients [33(64.71%)] was from rural background. No significant family history was present in any of the patients. In majority

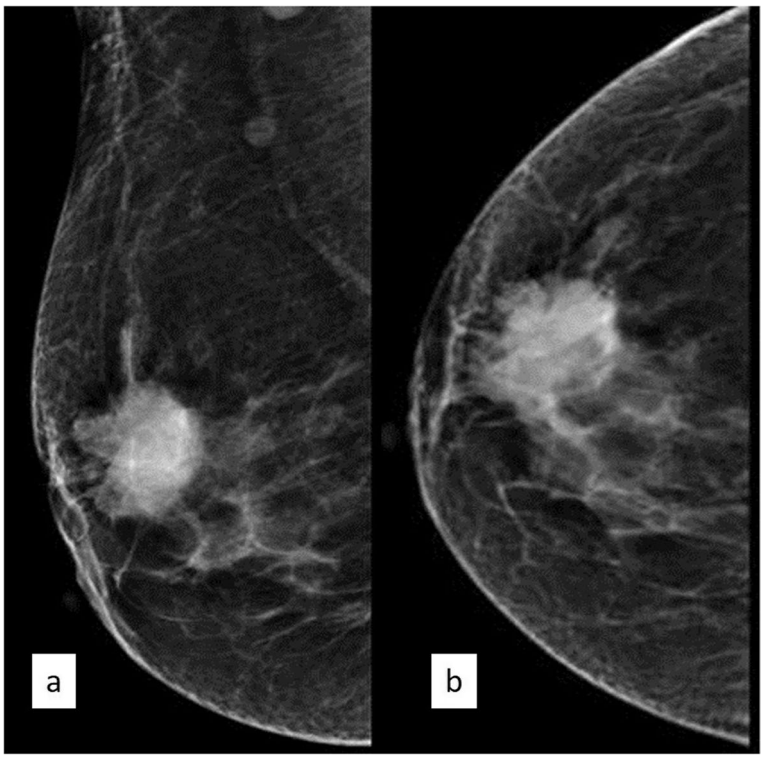

Fig. 1 A 47-year-old female with Invasive ductal carcinoma and Luminal type A molecular subtype of breast cancer. Mammography (**a**) MLO (**b**) CC views show ill-defined high- density mass with spiculated margins

[50(98.04%)], past history of breast cancer was absent with only one having past history of cancer in the contralateral breast. Palpable lump was present in right breast in 27 out of 51 patients (52.94%). No other relevant complaints like nipple discharge or breast pain were present in any of the patients.

On mammography, type B (scattered areas of fibro glandular density) was the most common type of breast composition seen in 32 out of 51 patients. In one of the patients, mammography revealed only calcification with suspicious morphology and no mass could be delineated which was then correlated with the ultrasound findings. Irregular shape of mass was the most common seen in maximum number of patients [38 (74.51%)]. Non circumscribed margin of mass was seen in 33 out of 48 patients (68.75%) while circumscribed margin was seen in 15 (31.25%). No mass but only microcalcification was seen in one case and in rest of the two cases, the breast density was BIRADS 4, making it difficult to precisely comment upon the margins. Density of mass was high in most of the patients. Microcalcification was absent in 24(47.06%) out of 51 patients and when present,

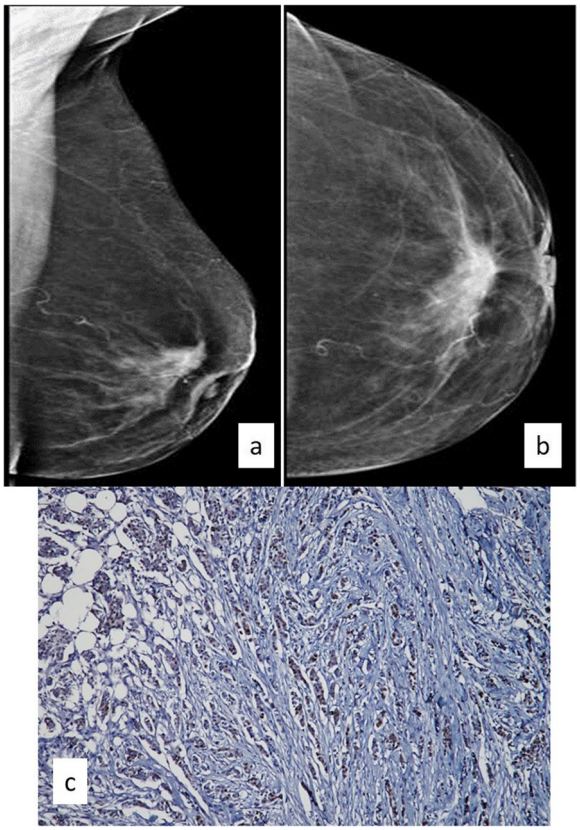

Fig. 2 A 65 – year-old female with Invasive lobular carcinoma and Luminal type B Her 2 negative molecular subtype of breast cancer. Mammography (**a**) MLO (**b**) CC views show ill-defined high- density mass with spiculated margins (**c**) Immunohistochemistry picture shows Ki 67% > 20%

Table 2 Distribution of pathological type, receptor status and molecular subtype of breast cancer

Type of tumor	**Frequency**	**Percentage**
Invasive ductal carcinoma (NOS)	48	94.12%
Invasive lobular carcinoma	2	3.92%
Mucinous carcinoma	1	1.96%
Total	51	100.00%
Receptor status	**Frequency**	**Percentage**
Estrogen receptor	35	68.63%
Progesterone receptor	28	54.90%
Her-2Neu receptor	18	35.29%
Molecular subtype	**Frequency**	**Percentage**
Her-neu enriched type	9	17.65%
Luminal type A	17	33.33%
Luminal type B (Her -ve)	6	11.76%
Luminal type B (Her + ve)	9	17.65%
Triple negative type	10	19.61%
Total	51	100.00%

amorphous calcification was the most common type [14(27.45%)]. In majority of patients, distribution of calcification was grouped [22(81.48%)]. Architectural distortion of the breast fibroglandular tissue was present in 42(82.35%). The location of lesion was upper outer quadrant in 28(54.90%) patients. Axillary lymphadenopathy was present in majority of patients (Table 1).

Invasive ductal carcinoma was the most common tumor type in our study. Luminal type A was the most common molecular subtype in our study [17 (33.33%)] (Fig. 1) followed by triple negative type [10(19.61%)]. Luminal type B, Her 2 negative was seen in 6 (11.76%) patients (Fig. 2). Nine patients (17.65%) showed Luminal type B, Her 2 positivity and similar number showed Her-2-neu enriched expression (Table 2). Non circumscribed margin was significantly correlated (p value 0.02) with non-triple negative breast cancer (Luminal type A, Luminal type B Her 2-ve, Luminal type B Her 2 + ve, Her 2 enriched) while circumscribed margin was more related to triple negative breast cancer (TNBC) (Table 3).

The ER positivity was seen in 35 patients and PR positivity was seen in 28 patients. No statistically significant association was seen between presence of calcification

Table 3 Association of tumor margin with molecular subtype

Margin	**Her-neu enriched type**	**Luminal type A**	**Luminal type B Her 2 -ve**	**Luminal type B Her 2 + ve**	**Triple negative type**	**Total**	***P* value**
Circumscribed	2 (13.33%)	4 (26.66%)	2 (13.33%)	1 (6.67%)	6 (40%)	15 (100%)	0.02*
Non-circumscribed	7 (19.44%)	13 (36.11%)	4 (11.11%)	8 (22.22%)	1 (03.03%)	33 (100%)	
Total	9 (17.65%)	17 (33.33%)	6 (11.76%)	9 (17.65%)	7 (14.58%)	48 (100%)	

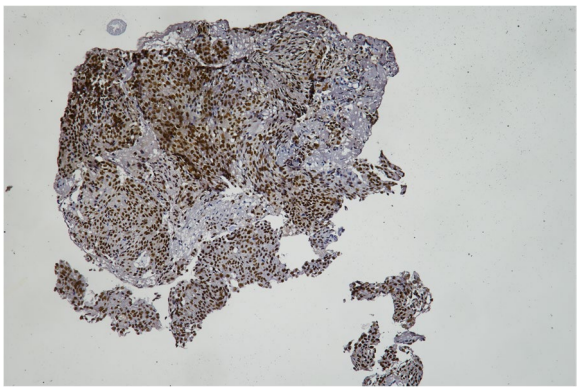

Fig. 3 Immunohistochemistry picture shows positive Estrogen Receptor (ER)

and ER positive (Fig. 3) or PR positive (Fig. 4) status. In our study, out of 18 patients with positive Her-2-neu expression, 17(62.96%) patients had presence of calcifications, this association showed statistical significance (p value < 0.001) (Table 4) (Fig. 5).

No statistically significant association was seen with ER, PR or Her-2-neu positivity and morphology and distribution of calcification (Tables 5 and 6). Proportion of patients with triple negative molecular subtype was significantly higher in the group without calcifications (33.33%) (Fig. 6) in comparison to group with calcifications (7.41%) (p value < 0.0001). No significant correlation was seen between the morphological type and distribution of calcification with the molecular subtype also (Table 7). Hence, cases with non- circumscribed margins without calcification showed Luminal type A and Luminal type B, Her 2 negative molecular subtype while cases with circumscribed margins devoid of calcification revealed triple negative status. Calcification was statistically correlated with Her 2 neu positivity and seen in Luminal type B Her 2+ve and Her 2 neu enriched molecular subtypes of breast cancer.

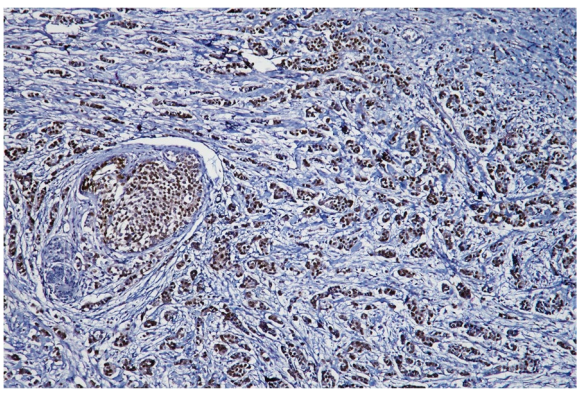

Fig. 4 Immunohistochemistry picture shows positive Progesterone Receptor (PR)

Table 4 Correlation of calcifications with estrogen, progesterone and Her 2 neu receptor

Calcifications	Absent	Present	Total	*P* value
ER negative (*n* = 16)	8 (33.33%)	8 (29.63%)	16 (31.37%)	0.776[†]
ER positive (*n* = 35)	16 (66.67%)	19 (70.37%)	35 (68.63%)	
Total	24 (100%)	27 (100%)	51 (100%)	
PR negative (*n* = 23)	9 (37.50%)	15 (62.50%)	23 (45.10%)	0.304[†]
PR positive (*n* = 28)	14 (51.85%)	13 (48.15%)	28 (54.90%)	
Total	23 (45.10%)	28 (54.90%)	51 (100%)	
Her2 neu negative (*n* = 33)	23 (95.83%)	10 (37.04%)	33 (64.71%)	< 0.0001
Her2 neu positive (*n* = 18)	1 (4.17%)	17 (62.96%)	18 (35.29%)	
Total	24 (100%)	27(100%)	51 (100%)	

[†] Chi square test

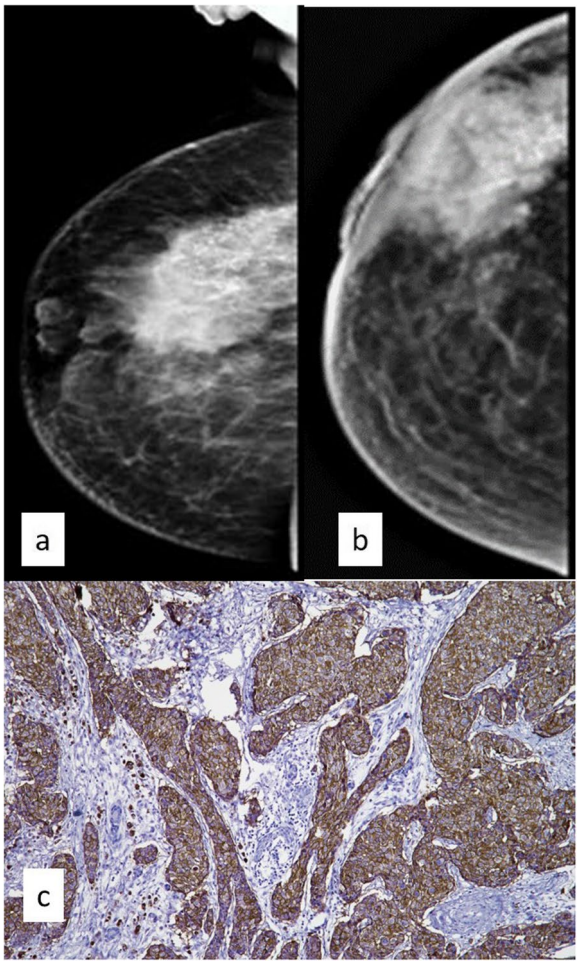

Fig. 5 A 52-year-old female with Invasive ductal carcinoma and HER-2-NEU enriched type molecular subtype of breast cancer. Mammography (**a**) MLO (**b**) CC views show ill-defined high- density mass with regional pleomorphic and linear calcifications (**c**) Immunohistochemistry picture shows positive HER-2-neu receptor (3 +)

Table 5 Association of suspicious morphology calcifications and distribution of calcification with Estrogen (ER) and Progesterone (PR) receptors

Variables	ER Negative ($n=8$)	ER positive($n=19$)	Total	*P* value	PR Negative ($n=14$)	PR positive($n=13$)	Total	*P* value
Suspicious morphology calcification								
Amorphous	6 (42.86%)	8 (57.14%)	14 (100%)	0.466*	8 (57.14%)	6 (42.86%)	14 (100%)	0.46*
Coarse heterogenous	0 (0%)	2 (100%)	2 (100%)		1 (50%)	1 (50%)	2 (100%)	
Fine linear	0 (0%)	2 (100%)	2 (100%)		2 (100%)	0 (0%)	2 (100%)	
Fine pleomorphic	2 (22.22%)	7 (77.78%)	9 (100%)		3 (33.33%)	6 (66.67%)	9 (100%)	
Distribution of calcification								
Grouped	6 (27.27%)	16 (72.73%)	22 (100%)	0.693*	11 (50%)	11 (50%)	22 (100%)	0.596*
Regional	2 (50%)	2 (50%)	4 (100%)		3 (75%)	1 (25%)	4 (100%)	
Segmental	0 (0%)	1 (100%)	1 (100%)		0 (0%)	1 (100%)	1 (100%)	

* Fisher's exact test

Discussion

Breast cancer is a diverse group of diseases which is characterized by a wide spectrum of imaging appearance, different histopathologic and molecular profiles, and different disease courses of the various molecular subtypes. The different molecular types of breast cancer have different biological behaviors and treatment response at the cellular level, which affect the rapidity of infiltration and destruction of the surrounding tissue, subsequently governing the macroscopical appearance of the tumor on imaging. Hence, suitable classification is needed for appropriate individual management (Viale 2012, Elsawaf et al. 2013, Rotstein and Neerhut 2005). Currently due to the limited prognostic power and predictive accuracy of existing classifications, a modified classification according to molecular characteristics of breast cancer was defined by the St. Gallen Breast Cancer Conference to categorize breast cancers into molecular subtypes (Somal

Table 6 Association of suspicious morphology calcifications and distribution with Her-2Neu receptor

Suspicious morphology calcifications	Her2 neu negative($n=10$)	Her2 neu positive($n=17$)	Total	*P* Value
Amorphous	4 (28.57%)	10 (71.43%)	14 (100%)	0.491*
Coarse heterogenous	0 (0%)	2 (100%)	2 (100%)	
Fine linear	1 (50%)	1 (50%)	2 (100%)	
Fine pleomorphic	5 (55.56%)	4 (44.44%)	9 (100%)	
Total	10 (37.04%)	17 (62.96%)	27 (100%)	
Distribution of calcification	**Her2 neu negative($n=10$)**	**Her2 neu positive($n=17$)**	**Total**	***P* Value**
Grouped	8 (36.36%)	14 (63.64%)	22 (100%)	0.764*
Regional	2 (50%)	2 (50%)	4 (100%)	
Segmental	0 (0%)	1 (100%)	1 (100%)	
Total	10 (37.04%)	17 (62.96%)	27 (100%)	

* Fisher's exact test

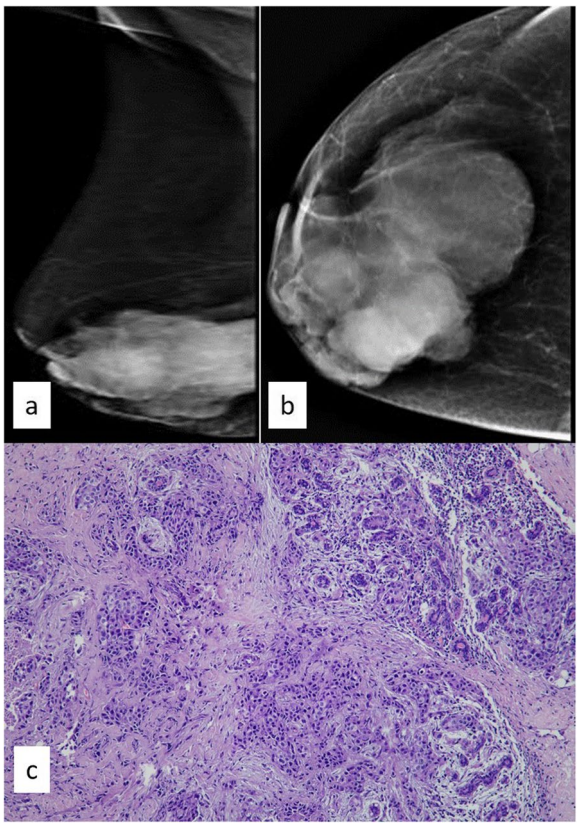

Fig. 6 A 50-Year-old female with Invasive ductal carcinoma and Triple negative molecular subtype of breast cancer. Mammography (**a**) MLO (**b**) CC views show well circumscribed high-density mass (**c**) Histopathology specimen shows invasive ductal carcinoma

et al. 2023). Definitions of the intrinsic molecular subtypes depend upon hormonal receptor status and Her 2neu overexpression. This is usually done by gene expression profiling. Studies have shown molecular subtypes to be an independent prognostic factor in breast cancer. So, the treatment can be tailored so that the resources can be meaningfully utilized. In the current breast cancer targeted regimens, hormonal therapy is routinely used for hormone-receptor positive tumors (Luminal A/B), while targeted therapy, such as Herceptin, is available for cancers which overexpress Her 2 (Luminal B/Her 2-enriched). On the other hand, TNBC tumors are more aggressive (due to poor dedifferentiation). Since these tumors lack ER, PR and Her 2 expressions, targeted therapeutic strategy is limited for the treatment of TNBC, leaving non-targeted chemotherapy as the mainstay in the treatment. The options available are immunotherapy, deoxyribonucleic acid (DNA)-interfering agents, and targeted therapies. PARP inhibitors are recommended for TNBC patients with BRCA mutations and anti-PD1 immunotherapy are available with metastatic PD-L1 positive TNBCs. In addition, patients with metastatic HER2-low TNBCs can receive trastuzumab-deruxtecan (Obidiro et al. 2023). But still, these tumors have a higher recurrence rate and metastatic potential.

The reliability of these hormone receptor status is dependent on tissue handling and processing. These steps can lead to false-negative results if quality control is not sufficient. The issues related to pathology services in low-middle income countries include limited financial resources, limited equipment, as well as inadequate numbers of expert pathologists and technologists (Saghir et al. 2011, Shyyan et al. 2006). Due to these adverse factors, hormone receptors are not routinely determined. An improved understanding of how the imaging features of breast cancer correlate to molecular subtype would aid in tailoring the treatment strategy for patients who are cost constrained.

In this study we attempted to find the correlation of mammographic parameters of breast cancer with molecular subtypes. Maximum number of our patients were in the 41–50 years of age group. Mean value of age(years) of study subjects was 51.63 ± 10.5 with median (25th-75th percentile) of 49 (45–60). This finding was consistent with the previous study done by Khalaf and Herdan et al. 2020.

Irregular shape and spiculated margins of the mass were the most common mammographic parameters. Spiculated margins were positively correlated with hormone receptor positive status (Luminal A or B). TNBC cancers were more likely to have circumscribed margins (Boisserie-Lacroix et al. 2013, Celebi et al. 2015). As hypothesized by earlier research, as luminal cancers tend to be of lower grade and grow at a slower rate, they provoke a desmoplastic reaction, resulting in radiologic findings of spiculated margins.

High risk microcalcifications detected on mammogram are associated with HER2-enriched cancer (Cen et al. 2017). Similar to our results, calcifications were shown to be characteristics of the HER2 subtype by Yang et al. (Yang et al. 2007). In their study, 88.8% of the HER2/neu-positive tumors had calcifications ($P < 0.001$). This goes in concordance with Boisserie-Lacroix et al. (Boisserie-Lacroix et al. 2013) who stated that the presence of calcifications in the mammogram may predict a HER2/neu- positive status when the HER2 score is equivocally 2+.

TNBC has been known to be associated with considerable differences in clinical, radiological, and pathological features (J-wei et al. 2018; Yang et al. 2015). This is the subtype most discussed in the literature and it was the second most commonly identified subgroup in our study. Imaging features that are distinctive to TNBC are similar to those that are also peculiar to benign tumors like noncalcified masses with well-circumscribed margins. Our findings were supported by studies by Lee et al. (Trop et al. 2014) and Lin et al.

Table 7 Association of calcifications, suspicious morphology and distribution of calcifications with molecular subtype

Calcifications	**Her-neu enriched type (*n* = 9)**	**Luminal type A (*n* = 17)**	**Luminal type B (Her -ve) (*n* = 6)**	**Luminal type B (Her + ve) (*n* = 9)**	**Triple negative type (*n* = 10)**	**Total**	***P* value**
Absent	1 (4.17%)	11 (45.83%)	4 (16.66%)	0 (0%)	8 (33.33%)	24 (100%)	< .0001*
Present	8 (29.63%)	6 (22.22%)	2 (07.40%)	9 (33.33%)	2 (7.41%)	27 (100%)	
Total	9 (17.65%)	17 (33.33%)	6 (11.76%)	9 (17.65%)	10 (19.61%)	51 (100%)	
Suspicious morphology calcifications	**Her-neu enriched type (*n* = 8)**	**Luminal type A (*n* = 6)**	**Luminal type B (Her -ve) (*n* = 2)**	**Luminal type B (Her + ve) (*n* = 9)**	**Triple negative type (*n* = 2)**	**Total**	***P* value**
Amorphous	5 (35.71%)	3 (21.43%)	0	5 (35.71%)	1 (7.14%)	14 (100%)	0.369*
Coarse heterogenous	1 (50%)	0 (0%)	0	1 (50%)	0 (0%)	2 (100%)	
Fine linear	0 (0%)	0 (0%)	0	1 (50%)	1 (50%)	2 (100%)	
Fine pleomorphic	2 (22.22%)	3 (33.33%)	2 (22.22%)	2 (22.22%)	0 (0%)	9 (100%)	
Total	8 (29.63%)	6 (22.22%)	2 (7.41%)	9 (33.33%)	2 (7.41%)	27 (100%)	
Distribution of calcification	**Her-neu enriched type(*n* = 8)**	**Luminal type A(*n* = 6)**	**Luminal type B (Her -ve) (*n* = 2)**	**Luminal type B(*n* = 9)**	**Triple negative type(*n* = 2)**	**Total**	***P* value**
Grouped	6 (27.27%)	5 (22.72%)	2 (09.09%)	8 (36.36%)	1 (4.55%)	22 (100%)	0.378*
Regional	2 (50%)	1 (25%)	0	0 (0%)	1 (25%)	4 (100%)	
Segmental	0 (0%)	0 (0%)	0	1 (100%)	0 (0%)	1 (100%)	
Total	8 (29.63%)	6 (22.22%)	2 (07.40%)	9 (33.33%)	2 (7.41%)	27 (100%)	

* Fisher's exact test

(J-wei et al. 2018) who determined that benign "pseudo fibroadenoma" type features can often be seen in TNBC. TNBC are also known to lack the presence of suspicious microcalcifications on mammogram (Trop et al. 2014, Dogan and Turnbull 2012). However, Lin et al. (J-wei et al. 2018) believed that there are wide variations in imaging features for TNBC. We also studied that TNBC can less commonly share imaging features similar to non-TNBC, i.e., mass with irregular margins. In our study, we postulate that close resemblance of its imaging features with benign tumors warrants improving diagnostic performance for early suspicion and recognition of malignancy.

The limitation of our study was small sample size. Due to the relatively small sample size, some of the sub analysis could have lacked statistical power to detect a significant difference in imaging features across molecular subtypes.

Conclusions

Tumor margins and microcalcification detected on mammography are strongly correlated in predicting the molecular subtype of breast cancer, and thus may further expand the role of conventional breast imaging. It could add in a similar benefit as IHC to identify molecular profiles and define therapy, specifically in low-income countries where IHC is not available. However, larger multicenter studies are recommended for the validation.

Abbreviations

HR +	Hormone receptor positive
TNBC	Triple negative breast cancer
IHC	Immunohistochemistry
BI-RADS	Breast Imaging-Reporting and Data System
ASCO	American Society of Clinical Oncology
CAP	College of American Pathologists
FISH	Fluorescent in situ hybridization
USG	Ultrasonography
MRI	Magnetic Resonance Imaging

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Authors' contributions
Study concept and design: Dr. Nidhi Rana, Dr Shruti Thakur. Data acquisition: Dr. Nidhi Rana, Dr Anchana Gulati, Dr. Arun Chauhan. Data analysis: Dr. Shruti Thakur, Dr Sushma Makhaik. Drafting of manuscript: Dr Shruti Thakur, Dr. Arun Chauhan. Critical revision of the manuscript: Dr. Vijay Thakur, Dr Sushma Makhaik.

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Data availability
The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The approval of the institutional ethical committee was taken in accordance with the 1964 Helsinki declaration. An informed consent was taken from each participant before the commencement of the study.

Consent for publication
Yes.

Competing interests
The authors report no conflict of interest.

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