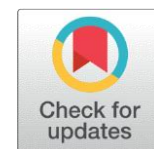


# MAMMOGRAPHIC PATTERNS REFLECTING MOLECULAR SUBTYPES IN BREAST CANCER: A CROSS-SECTIONAL STUDY

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## ABSTRACT

**Background:** Breast cancer is the most common invasive cancer in women worldwide. Its prognosis depends upon tumor biology and molecular type. Mammography is globally accepted as the main modality for screening and early detection of breast cancer. Mammographically demonstrated morphological features can also provide clues about tumor subtypes. Our study aimed to evaluate the association between mammographic morphological features and molecular subtypes of breast cancer.

**Results:** This cross sectional study was conducted on 378 women with histopathologically confirmed breast cancer. Mammographic features of these women were assessed as per American College of Radiology- BIRADS lexicon and findings were recorded. Breast cancers were classified as Luminal A, Luminal B, HER2- enriched, or triple negative using ER, PR, HER2 receptor status and Ki-67 scores. Invasive ductal carcinoma was the most common type and accounted for 89.6% of the cases. Mammographic features differed significantly between molecular subtypes. Irregular masses and spiculated margins were more common in Luminal A and B subtypes whereas circumscribed round masses were more common in triple negative cancers ( $p < 0.001$ ). HER2 enriched and triple negative cancers showed higher density masses ( $p < 0.001$ ). Pleomorphic microcalcifications were more common in Luminal A and HER2 enriched types ( $p < 0.001$ ). Logistic regression showed that round shape and circumscribed margins were associated with triple negative breast cancers. Microcalcifications and spiculated margins were associated with Luminal B and HER2 enriched cancers.

**Conclusion:** Mammographic features showed significant association with breast cancer subtypes. Hence mammography may provide useful clues about tumor biology and help support individualized diagnosis and management.

**Keywords:** Breast Carcinoma, Mammography, Molecular Subtypes, Calcifications, Hormonal Receptors

## 1. INTRODUCTION

Breast carcinoma (BC) is the most common invasive cancer among females, accounting for 30% of all women cancers. In 2022, approximately 2.3 million women were diagnosed with BC worldwide, and 670,000 died from the disease [1]. In India, BC accounted for 10.6% of cancer-related deaths and 13.5% of new cancer cases in 2020, making it the leading cause of cancer and mortality among Indian women [2]. Early detection significantly improves outcomes, and routine screening mammography has been associated with at least a 20% reduction in BC mortality [3].

Tumor receptor status remains one of the most reliable predictors of prognosis and treatment response in BC. It provides greater prognostic and therapeutic information than several traditional risk factors, including age, parity, and family history [4]. Immunohistochemistry (IHC) is used to assess the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu). These markers form the basis for classification into four major molecular subtypes: luminal A, luminal B, HER2-enriched, and triple-negative breast cancer (TNBC) [5]. This molecular classification has important implications for treatment selection and prognosis.

Hormone receptor-positive (luminal) tumors are generally responsive to endocrine therapy. In contrast, TNBC lacks expression of ER, PR, and HER2/neu and therefore does not benefit from endocrine or HER2-targeted therapy. TNBC may demonstrate a high pathological complete response rate following neoadjuvant chemotherapy; however, the risk of recurrence remains substantial because of the limited availability of effective targeted therapies. HER2 overexpression is associated with increased axillary lymphadenopathy and poorer prognosis [6,7]. Because molecular subtypes differ in biological behaviour, their mammographic appearances also vary. Accurate correlation of

imaging features with histopathology is therefore vital for comprehensive mammographic evaluation. Hence, our study aims to evaluate the association between mammographic morphological features and the molecular subtypes of breast carcinoma.

## **2. METHODOLOGY**

This cross-sectional study was conducted over two years (August 2022–July 2024) at a tertiary care center and included 378 women with breast carcinoma. Institutional Ethics Committee approval was obtained. All women undergoing screening or diagnostic mammography were enrolled, along with those with a prior mastectomy for BC presenting for contralateral breast surveillance. Patients with a history of prior breast surgery or recurrent carcinoma were excluded.

All participants underwent standard DM on a digital mammography unit (GE Healthcare Senographe Essential 54020/CESM1/SenoClaireA.6). DM was performed in standard craniocaudal (CC) and mediolateral oblique (MLO) views, with DBT acquired in the MLO view. Additional views were obtained when required. As per American College of Radiology (ACR) Breast Imaging Reporting and Data System (BI-RADS) lexicon, the descriptors of the mass including shape, margins, density, presence of calcifications, asymmetry and associated features including architectural distortion, skin retraction or thickening, nipple retraction and trabecular thickening were recorded. Each case received a BI-RADS assessment category based on clinical and mammographic findings.[8] Among the 574 lesions categorized as BI-RADS 4/5 and sampled with trucut biopsy, 404 were confirmed malignant on histopathological analysis.

ER, PR and HER2-neu status were available for 391 patients and were obtained from core biopsies or postoperative specimens. Ki-67 scoring was available in 378 patients; cases with >14% positive nuclei were categorized as high Ki-67 expression, and those with <14% as low expression. Tumors were classified into molecular subtypes based on receptor expression, reflecting their therapeutic and prognostic significance[9]. The four molecular subtypes were:

- **Luminal A** (ER+, PR+, HER2±) with low Ki 67 proliferation index,
- **Luminal B** (include ER+, PR+, HER2- with low Ki 67 proliferation index) or (ER+, PR+, HER2 regardless of Ki 67 proliferation index)
- **HER2-amplified** breast cancer refers to tumors that are explicitly positive for HER2 expression
- **Triple-negative** (ER–, PR–, HER2–)

Patients, whose immunohistochemistry (IHC) reports and Ki-67 scoring were not available, were excluded from the analysis. Hence, our final sample size was 378.

After data tabulation, statistical analysis was performed using SPSS. Frequencies of individual variables were calculated and summarized. Imaging features were compared with histological subtypes using the chi-square test or Fisher's exact test for qualitative variables, and the Kruskal–Wallis test for quantitative variables. Logistic regression was applied to identify imaging features predictive of specific subtypes. A p-value < 0.05 was considered statistically significant.

### 3. RESULTS

Among the 378 BC cases, IDC- no special type (NST) was the most common histological subtype across all molecular classes (89.6%). The distribution of histological types differed significantly among the four molecular subtypes ( $\chi^2$  test,  $p < 0.001$ ).

In the Luminal A group (n = 60), IDC-NST accounted for 75%, which was significantly lower than in Luminal B (89.2%), HER2-amplified (98.3%), and triple-negative breast cancer (TNBC) (95.1%) ( $p < 0.01$  for inter-group comparison). Ductal carcinoma in situ (DCIS) was markedly more frequent in

Luminal A (20%) compared with Luminal B (5.1%) and TNBC (1.2%) groups and was absent in HER2-amplified tumors ( $p < 0.001$ ). Luminal B tumors demonstrated the greatest histological diversity, showing small proportions of Invasive lobular carcinoma (ILC) (2.8%), mucinous carcinoma (0.56%), papillary carcinoma (1.1%), neuroendocrine carcinoma (0.56%), and metaplastic carcinoma (0.56%). The overall distribution of these non-NST variants was significantly different from the remaining subtypes ( $p = 0.02$ ). The HER2-amplified group had the highest proportion of IDC-NST (98.3%) with only a single ILC case (1.6%), and no other histological variants were observed, showing significantly lower heterogeneity compared with Luminal B ( $p < 0.05$ ). The TNBC subtype exhibited a high prevalence of IDC-NST (95.1%), but had the highest frequency of medullary carcinoma (2.4%) and neuroendocrine carcinoma (1.2%) among all groups ( $p = 0.03$ ). Overall, the prevalence of IDC-NST differed significantly across molecular subtypes ( $p < 0.001$ ), with Luminal A demonstrating the lowest proportion and HER2-amplified tumors the highest. Non-NST histological variants were uncommon but showed distinct subtype-specific enrichment, particularly DCIS in Luminal A and medullary carcinoma in triple-negative cancers. (Table 1)

**Table 1:** Distribution of various pathological types (n=378) in four molecular subtypes of breast cancer

| Molecular type (n=378)  | IDC (NST) (n= 339) | DCIS (n=22) | ILC (n=8) | Mucinous CA (n=2) | Invasive papillary (n= 2) | NEC (n= 2) | Medulla ry (n=2) | Metaplasti c (n=1) |
|-------------------------|--------------------|-------------|-----------|-------------------|---------------------------|------------|------------------|--------------------|
| Luminal A (n=60)        | 45 (75.0 %)        | 12 (20.0%)  | 2( 3.3%)  | 1( % 1.6)         | 0 (0 %)                   | 0 ( 0%)    | 0 (0 %)          | 0 ( %)             |
| Luminal B (n= 176)      | 157 ( 89.2%)       | 9 ( 5.1%)   | 5( 2.8%)  | 1( 0.56%)         | 2 ( 1.1%)                 | 1 (0.56 %) | 0 (0 %)          | 1 (0.56%)          |
| HER2-amplified (n= 60)  | 59 ( 98.3%)        | 0 ( 0%)     | 1( 1.6%)  | 0( 0%)            | 0 ( 0%)                   | 0 ( 0%)    | 0 (0 %)          | 0 ( 0%)            |
| Triple-negative (n= 82) | 78 ( 95.1%)        | 1 ( 1.2%)   | 0( 0 %)   | 0( 0 %)           | 0 ( 0 %)                  | 1 ( 1.2 %) | 2 ( 2.4%)        | 0 (0 %)            |

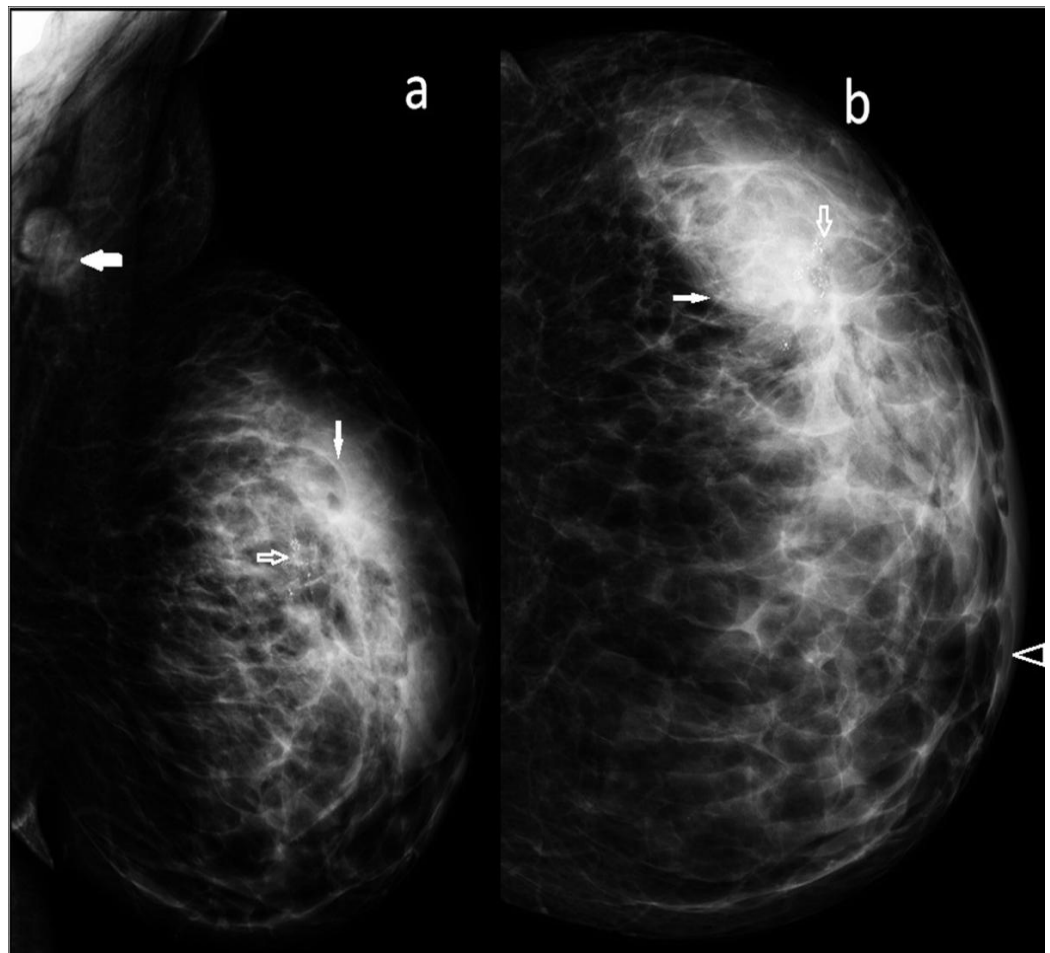
Mammographic features of different molecular subtypes of BC were also evaluated (Table 2).

**Table 2:** Mammographic features of different molecular subtypes of breast cancer (n=378)

| Mammographic feature           |                                                        |                             | Molecular subtype   |                      |                            |                | P value |        |
|--------------------------------|--------------------------------------------------------|-----------------------------|---------------------|----------------------|----------------------------|----------------|---------|--------|
|                                |                                                        |                             | Luminal A<br>(n=60) | Luminal B<br>(n=176) | HER2<br>enriched<br>(n=60) | TNBC<br>(n=82) |         |        |
| Masses<br>(n=344)              | Shape                                                  | oval                        | 1                   | 3                    | 0                          | 4              | <0.001  |        |
|                                |                                                        | round                       | 3                   | 10                   | 2                          | 13             |         |        |
|                                |                                                        | irregular                   | 48                  | 146                  | 53                         | 61             |         |        |
|                                | Margins                                                | circumscribed               | 1                   | 1                    | 2                          | 12             | <0.001  |        |
|                                |                                                        | obscured                    | 1                   | 6                    | 3                          | 10             |         |        |
|                                |                                                        | indistinct                  | 11                  | 17                   | 14                         | 11             |         |        |
|                                |                                                        | microlobulated              | 12                  | 41                   | 10                         | 17             |         |        |
|                                |                                                        | spiculated                  | 27                  | 94                   | 31                         | 28             |         |        |
|                                | Density                                                | high                        | 31                  | 110                  | 41                         | 62             | <0.001  |        |
|                                |                                                        | equal                       | 20                  | 47                   | 14                         | 16             |         |        |
|                                |                                                        | low                         | 1                   | 2                    | 0                          | 0              |         |        |
|                                |                                                        | fatty                       | 0                   | 0                    | 0                          | 0              |         |        |
|                                | Associated features                                    | microcalcifications (n=143) | 18                  | 58                   | 36                         | 24             | <0.001  |        |
|                                |                                                        | nipple retraction           | 11                  | 30                   | 17                         | 7              |         | <0.001 |
|                                |                                                        | trabecular thickening       | 5                   | 11                   | 18                         | 3              |         | 0.235  |
|                                |                                                        | architectural distortion    | 17                  | 38                   | 23                         | 14             |         | 0.100  |
|                                |                                                        | skin thickening             | 19                  | 42                   | 27                         | 11             |         | <0.001 |
|                                | Suspicious microcalcifications without a lesion (n=19) | amorphous                   | 2                   | 1                    | 0                          | 1              | <0.001  |        |
|                                |                                                        | coarse heterogenous         | 1                   | 4                    | 0                          | 2              |         |        |
|                                |                                                        | fine pleomorphic            | 1                   | 2                    | 2                          | 0              |         |        |
|                                |                                                        | fine linear and branching   | 0                   | 3                    | 0                          | 0              |         |        |
| Asymmetry (n=9)                |                                                        | 1                           | 5                   | 2                    | 1                          | 0.783          |         |        |
| Architectural distortion (n=6) |                                                        | 3                           | 2                   | 1                    | 0                          | 0.987          |         |        |

A mass was detected in 344 cases, and mass shape, margins, and density varied significantly among molecular subtypes based on chi-square testing (all  $p < 0.001$ ). Irregular masses predominated across subtypes but were most frequent in Luminal B and HER2-enriched cancers. (Figure1) Mass margins also differed

significantly ( $p < 0.001$ ), with spiculated margins occurring most commonly in Luminal A (Figure 2) and B tumors, whereas circumscribed margins predominated in triple-negative cancers. TNBC showed a significantly higher proportion of round and circumscribed masses compared with the other groups ( $p < 0.001$ ). (Figure 3) High-density masses were significantly associated with HER2-enriched and triple-negative tumors ( $p < 0.001$ ). (Figure 4) Quantitative parameters demonstrated no significant variation on Kruskal–Wallis testing.

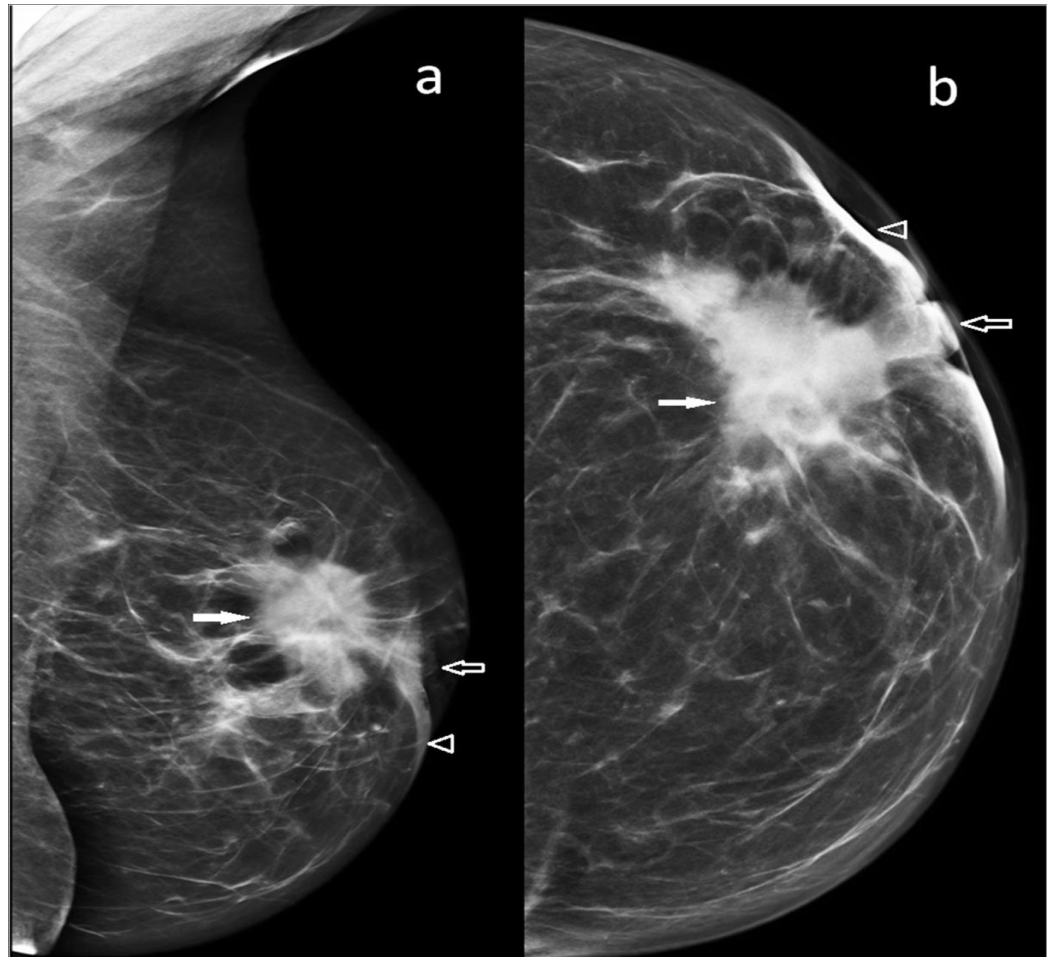


**Figure 1.** Digital Mammogram in MLO (a) and CC (b) views show a large irregular, high density lesion with indistinct margins (solid arrow) and pleomorphic microcalcifications (open arrow) in upper outer quadrant. Associated skin thickening (arrowhead) and enlarged axillary lymph nodes (short solid arrow) are also visible. Biopsy was suggestive of Luminal B breast cancer.

Associated features, including micro-calcifications ( $p < 0.001$ ), nipple retraction ( $p < 0.001$ ), and skin thickening ( $p < 0.001$ ), differed significantly across molecular classes. Micro-calcifications were most prominent in Luminal B and

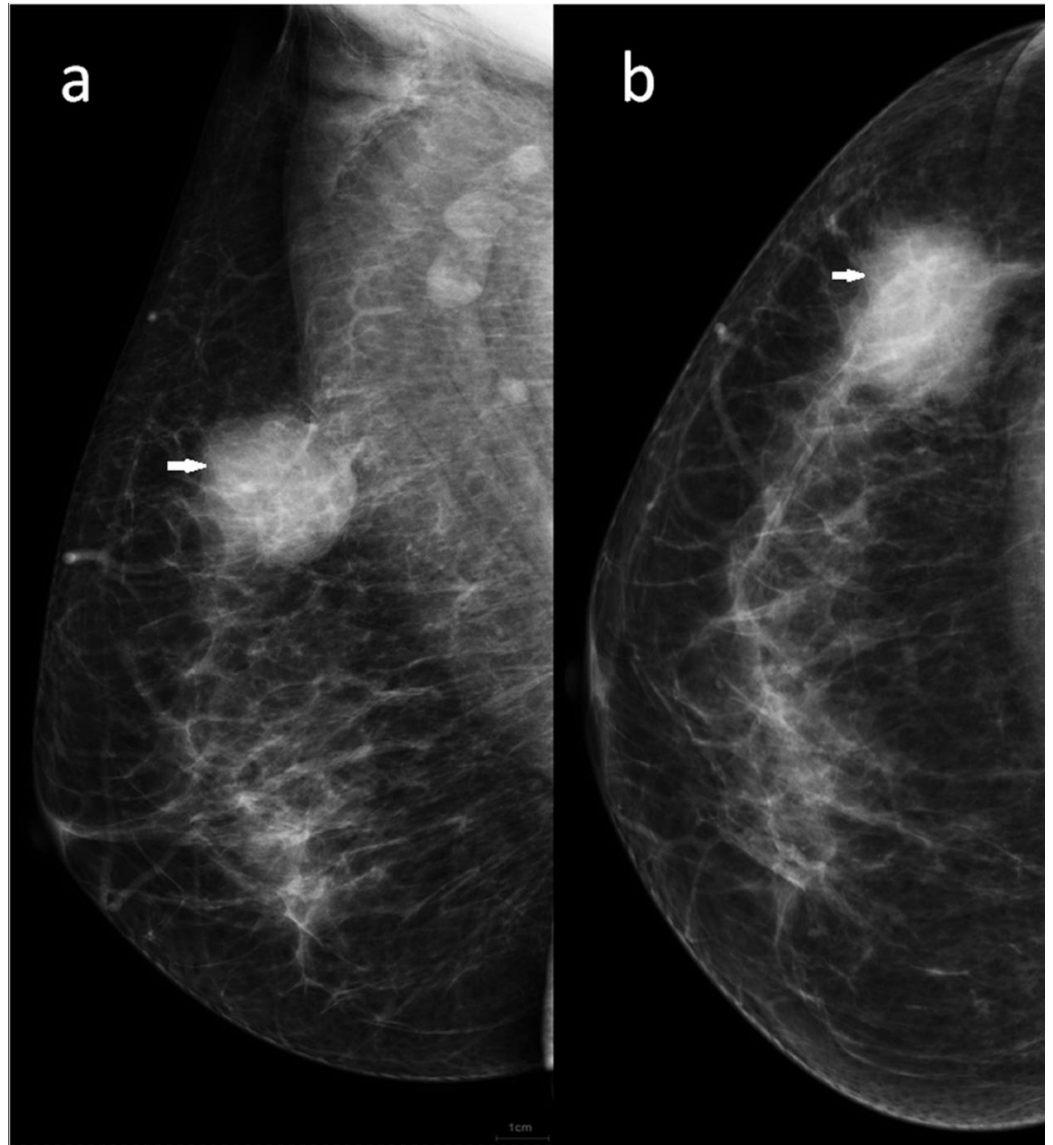
HER2-enriched tumors, whereas skin thickening was most frequently observed in HER2-enriched cancers. (Figure 5,6) In contrast, trabecular thickening ( $p = 0.235$ ) and architectural distortion ( $p = 0.100$ ) did not show statistically significant differences among subtypes.

Suspicious microcalcifications without an associated mass ( $n = 19$ ) exhibited significant subtype-dependent variation ( $p < 0.001$ ), with amorphous patterns occurring most in Luminal A, while pleomorphic and fine linear/branching patterns were more common in Luminal B and HER2-enriched cancers. (Figure 7) Asymmetry ( $p = 0.783$ ) and isolated architectural distortion ( $p = 0.987$ ) demonstrated no significant subtype association.



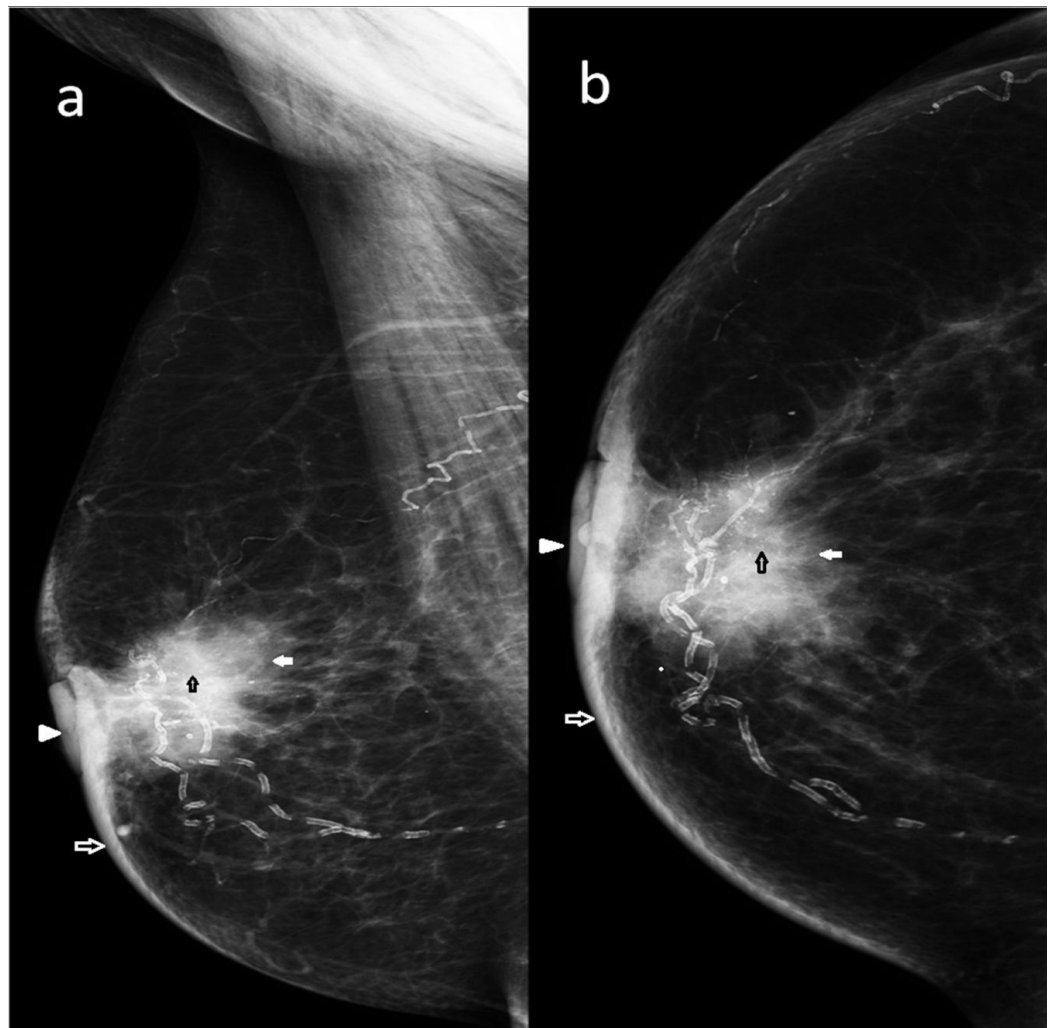
**Figure 2.** Digital Mammogram in MLO (a) and CC (b) views show an irregular spiculated high density lesion (solid arrow) in central region causing nipple retraction (open arrow) associated with skin thickening (arrowhead). Biopsy was suggestive of Luminal A breast cancer.

Overall, histological and mammographic features showed clear patterns across molecular subtypes. Luminal A tumors showed less aggressive imaging features. Luminal B tumors exhibited the greatest morphological variation. HER2-enriched tumors were more often associated with high-density masses and extensive calcifications, and triple-negative cancers displaying more circumscribed or round masses with fewer calcifications.

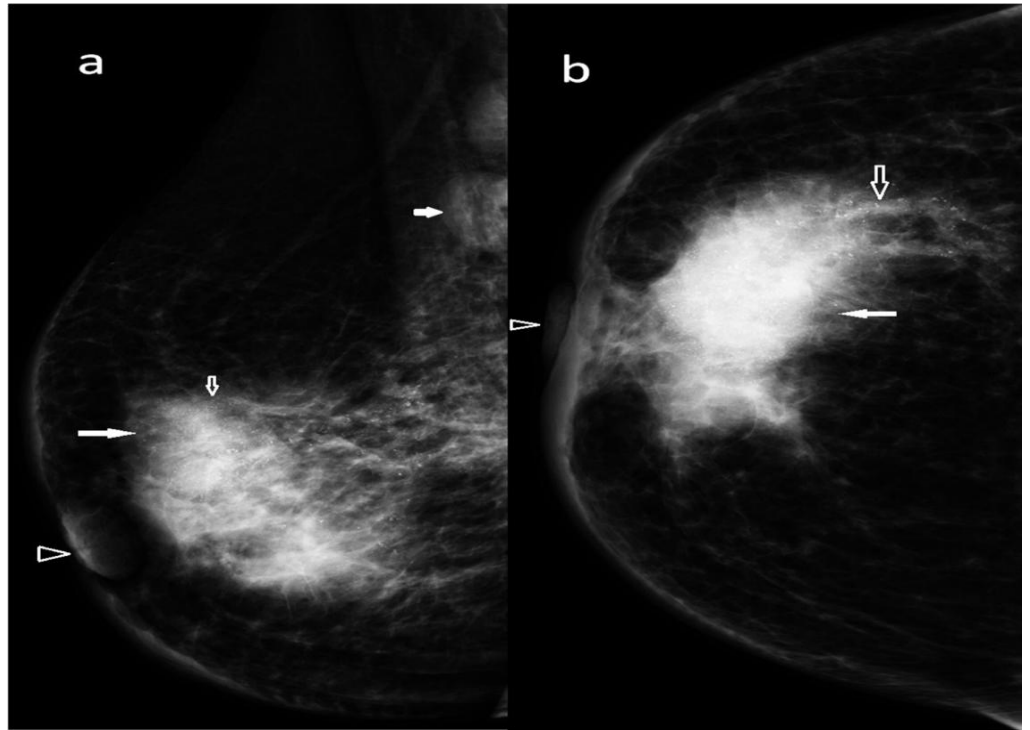


**Figure 3.** Digital Mammogram in MLO (a) and CC (b) views show a round, high density lesion (solid arrow) with microlobulated margins in upper outer quadrant. Biopsy was suggestive of TNBC.

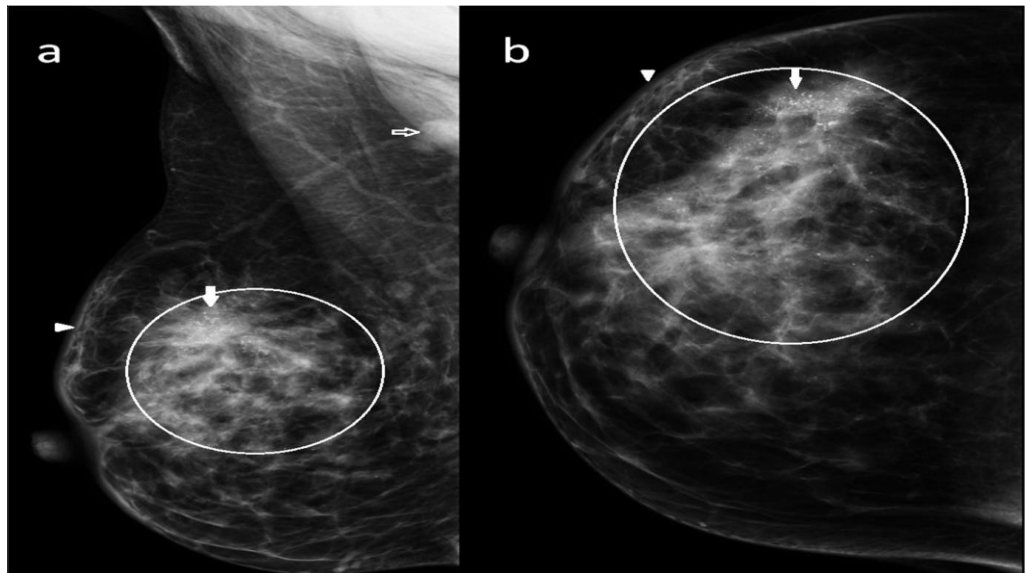
Binary logistic regression identified several imaging variables predictive of specific molecular subtypes. Circumscribed margins and round mass shape were independently associated with triple-negative cancers, while spiculated margins and the presence of microcalcifications were significant predictors of Luminal B tumors. High-density masses and skin thickening showed strong predictive value for HER2-enriched cancers. All reported associations were statistically significant at  $p < 0.05$ .



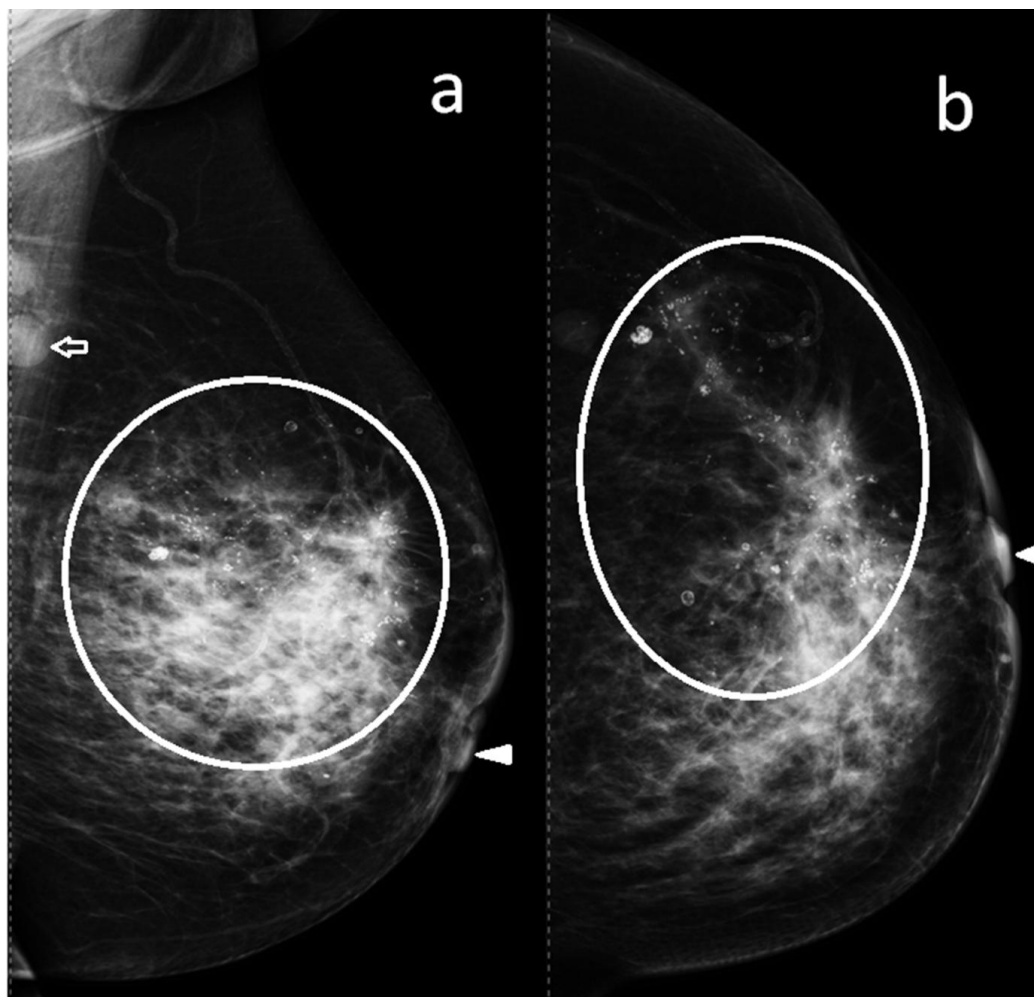
**Figure 4.** Digital Mammogram in MLO (a) and CC (b) views show an irregular high density lesion with indistinct margins (solid arrow) in central region containing numerous pleomorphic microcalcifications (black open arrow). The lesion is causing nipple retraction (arrowhead) associated with skin thickening (open arrow)). Biopsy was suggestive of HER2 amplified breast cancer.



**Figure 5.** Digital Mammogram in MLO (a) and CC (b) views show a large irregular, high density lesion with indistinct margins (solid arrow) containing numerous pleomorphic microcalcifications (open arrow). Nipple retraction (arrowhead), skin thickening enlarged axillary lymph nodes (short solid arrow) are also visible. Biopsy was suggestive of Luminal B breast cancer.



**Figure 6.** Digital Mammogram in MLO (a) and CC (b) views show a large area of pleomorphic calcifications (circle) along with an irregular, high density lesion with indistinct margins (solid arrow) in upper outer quadrant. Associated skin thickening (arrowhead) and enlarged axillary lymph nodes (open arrow) are also visible. Biopsy was suggestive of HER2 amplified breast cancer with grade 2 DCIS.



**Figure 7.** Digital Mammogram in MLO (a) and CC (b) views show a large area of coarse heterogeneous and pleomorphic calcifications (circle) in upper outer quadrant. Associated nipple retraction (arrowhead), skin thickening and suspicious rounded axillary lymph nodes (open arrow) are also visible. Biopsy was suggestive of HER2 amplified breast cancer with grade 2 DCIS. Lymph nodes showed reactive hyperplasia.

#### 4. DISCUSSION

Breast cancer is a biologically heterogeneous disease comprising multiple molecular subtypes that differ in their morphology, imaging appearance, clinical behaviour, and response to therapy. With the evolution of precision oncology, correlating imaging phenotypes with tumour biology has become an essential research focus. Mammography is a first-line imaging modality for breast cancer diagnosis. It shows morphological features that may reflect the tumor microenvironment and molecular subtype. This study evaluated the association

between mammographic morphological features and molecular subtypes of breast cancer.

In the present study, Infiltrating ductal carcinoma- No specific type (IDC-NST) was the most common histological type across molecular subtypes, consistent with global epidemiological data [10]. Luminal B tumors showed the widest histological diversity, including IDC, DCIS, ILC, mucinous carcinoma, and papillary carcinoma, whereas HER2-enriched and TNBC were predominantly IDC-NST. This finding is also consistent with Erber R et al. (2020), who stated that luminal B cancers often show mixed pathological patterns. HER2-positive and TNBC subtypes more commonly show high-grade IDC morphology [11]. Mass shape and margins were significantly associated with molecular subtype ( $p < 0.001$ ). Irregular masses were the most common finding across all subtypes. They were particularly frequent in luminal B and HER2-enriched tumors. Similar findings were reported by Lo Gullo R et al., who found that irregular masses were strongly associated with biologically aggressive tumors, including HER2-positive cancers [12]. Circumscribed masses were more common in TNBC. Previous studies have also reported this finding. TNBC may show pushing rather than infiltrative margins due to rapid tumour growth and central necrosis. These features can mimic benign lesions and may make TNBC more difficult to diagnose on mammography [13,141].

Spiculated margins are well-recognised indicators of invasive carcinomas with stromal desmoplasia. Their predominance in luminal A and B tumours in this study aligns with previous studies, which concluded that low-proliferative ER-positive cancers tend to elicit strong fibrotic responses, manifesting as spiculations on mammography. In contrast, HER2-enriched and TNBC subtypes displayed higher proportions of indistinct or microlobulated margins, consistent with their aggressive biological profiles [15,16]. High lesion density in HER2-enriched and TNBC groups in our study mirrors recent evidence linking radiographic mass density with high cellularity and proliferation indices characteristic of aggressive subtypes. Meta-analysis by Zhang and colleagues and more recent radiomics reviews have described similar associations between

high mammographic/tumour density and elevated proliferative markers or aggressive phenotype [17,18].

Microcalcifications were strongly associated with HER2-enriched tumours in our cohort (36/60) and were also common in luminal B cases (58/176). This concurs with multiple contemporary reports that HER2-positive cancers frequently present with suspicious pleomorphic or fine linear calcifications, reflecting intraductal components and comedo-type necrosis; luminal B cancers with substantial DCIS components also often demonstrate calcifications. Recent single-centre and multicentre studies have identified mammographic calcifications as predictors of HER2 positivity [18,19]. In contrast, TNBC showed fewer microcalcifications in our study, consistent with previous reports. A large cohort study by Boisserie-Lacroix M et al. similarly reported that TNBC less commonly exhibits calcifications, likely due to their basal-like phenotype and predominantly solid invasive nature [20]. Calcification morphology in cases without a mass was also significantly associated with molecular subtype ( $p < 0.001$ ). Luminal A and B cancers demonstrated a mixture of amorphous, coarse heterogeneous, and fine pleomorphic patterns, whereas HER2-enriched cases showed predominantly fine pleomorphic calcifications. This association may reflect the high-grade intraductal component commonly seen in HER2-positive tumors [21].

Skin thickening was significantly associated with molecular subtype ( $p < 0.001$ ). It was most frequent in HER2-enriched cancers (27/60). This may reflect their aggressive local behaviour and lymphovascular invasion, which can cause dermal oedema. Nipple retraction was more common in luminal B and HER2-positive tumours. This may be related to their central location and ductal involvement. Architectural distortion was not significantly different across subtypes in our study. However, its presence in luminal tumours is consistent with previous studies linking it to stromal desmoplasia in hormone receptor-positive cancers [10,18].

A major strength of this study is its robust sample size and systematic assessment of mammographic features. This allowed detailed correlation of

imaging features with molecular subtypes. The main limitation is its single-centre design, which may introduce selection bias. Breast density was also not analysed as an independent variable, although it can affect mammographic interpretation. Future multicentre studies should incorporate digital breast tomosynthesis, radiomic analysis, and genomic profiling. These approaches may help better define and validate radiogenomic signatures in breast cancer. The integration of imaging features with tumour biology is an evolving field known as radiogenomics. Our findings support the potential role of mammographic features as non-invasive imaging biomarkers of molecular subtypes. This may be particularly useful in resource-limited settings where advanced imaging and comprehensive molecular testing are not always available.

## 5. CONCLUSION

This study demonstrated significant associations between mammographic features and molecular subtypes of breast carcinoma. Circumscribed, round masses were more common in TNBC, while pleomorphic microcalcifications were associated with HER2-enriched and luminal B tumours. These findings suggest that mammographic morphology may provide useful non-invasive clues to tumour biology and may complement molecular assessment in personalised breast cancer management.

## 6. DECLARATIONS

**Competing interests:** The authors declare that they have no competing interests.

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