Prediction of Histological Grade and Molecular Subtype of Breast Cancer by Ultrasound Imaging Findings

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ABSTRACT

Background: Molecular subtyping of breast cancer is a common practice for individualized cancer management, to understand prognosis of disease and avoid overtreatment. Breast ultrasound has an important role in diagnosis, and follow-up of patients with breast cancer, and it may also help to predict histological grade and molecular subtypes of patients with breast cancer for guiding treatment and better management.

Objective: To correlate breast ultrasound findings and histologic grading and molecular subtypes of breast cancer.

Patients and methods: Prospective study included 69 consecutive invasive breast cancer female patients were reviewed from the database according to the Breast Imaging and Reporting Data System (BI-RADS). Clinicopathological findings and breast ultrasound (US) findings were evaluated and correlate the findings among histological grade and molecular subtypes of breast cancer.

Results: Tumors with irregular shape, non-circumscribed margins and posterior acoustic shadowing were likely to be luminal A or B subtype of breast cancer and of low histological grade [P < 0.0001]. Larger size tumors with posterior mixed acoustic feature and microcalcifications were associated with HER2 enriched subtype [P<0.0001]. Tumors with oval shape, circumscribed margins and posterior acoustic enhancement were highly suggestive of triple-negative breast cancer and of high histological grade (P < 0.0001).

Conclusion: Ultrasonographic features are strongly associated with molecular subtype, histologic grade, and hormone receptor status of the tumor.

Keywords: breast cancer, Breast ultrasound, molecular subtypes, histological grades.

INTRODUCTION

Breast cancer is the most common cause of cancer deaths in women worldwide, and its incidence has been rising [1]. It is a diverse group of diseases with a variable natural course, histopathological subtypes, treatment response, and prognosis. The molecular classification of
breast cancer has become an essential requirement for treatment planning, and disease prognosis [2].

St. Gallen International Experts Consensus [3] recently classified breast cancer into four different molecular subtypes based on gene expression patterns: luminal A (LA), luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and triple-negative (TN) subtypes. According to the expression status of tumor markers these molecular subtypes are classified into: estrogen receptor (ER), progesterone receptor (PR), HER2neu overexpression, and Ki-67 index. Invasive breast cancer with positive ER and/or PR, and low Ki-67 index (ki-67 <14%) is considered LA type, ER and/or PR-positive with high Ki-67 index (Ki-67 ≥14%), and HER2-negative is LB subtype (HER2-), ER and/or PR-positive and HER2-positive are LB subtype (HER2+), ER and PR-negative and HER2neu overexpression is HER2-enriched, three receptors (ER/PR/HER2neu) are all negative Breast cancer is basal or triple-negative [4].

Immunohistochemistry (IHC) is the gold standard test for detecting hormone receptors (ER / PR), HER2 overexpression, and Ki-67 index, but it is invasive, expensive, and it is not widely available in many developing and underdeveloped countries [5].

Breast ultrasound (US) is widely used as a diagnostic method to evaluate suspected clinical or radiological abnormalities. It is an effective screening method to detect occult breast cancer hidden in dense breasts [6].

Thus, our study aims to find the relationship between breast ultrasound morphological findings such as (tumor shape, margin, orientation, boundary, echo pattern, posterior acoustic features, and calcifications), and prognostic indicators in breast cancer such as histological tumor grade, and molecular classifications.

PATIENT AND METHOD

This prospective study was approved by the institutional review board (IRB) of our institute and including all female patients presented with a breast mass referred to the diagnostic radiology department from clinical oncology and surgical departments during the period from January 2020 to January 2021, we examined a total of 112 patients. Informed consent was taken from all patients before the study.

Patients who had any of the following criteria were excluded from the study as pathologically proved benign breast tumors (n=24), recurrent breast cancers (n=3), history of prior neoadjuvant chemotherapy or previous cancer surgery (n=12), patients unable to undergo histopathological or IHC biomarkers (n=4).

The final included patients were 69 patients with malignant breast mass.

Breast ultrasound examination

Bilateral complete ultrasound examination by radial and antiradial scanning of the entire breast and axillary tail of both sides were obtained using a (Logiq P7, GE Healthcare) ultrasound system with straight linear array probe (7–12 MHz frequency).
Image interpretation

Ultrasound images were interpreted using the 5th edition of the American College of Radiology (ACR) breast imaging reporting and data system (BIRADS)US lexicon (2013) [7] by two experienced radiologists (more than 15 years) blinded to the histopathological data on a separate session. In each case, they asked to make comments on size, site, number (multifocality or multicentricity), shape (regular or irregular), margins (well-circumscribed or lobulated), orientation (parallel, not parallel), boundary (abrupt, halo), posterior acoustic features (shadowing, enhancement, mixed or none), Echogenicity (homogenous hypoechoic or heterogeneous), Presence or absence of calcification, and axillary LNs (shape, fat hilum and LN index).

Ultrasound-guided core

True cut biopsy of the breast was performed for all cases using a 14-gauge needle. Multiple core biopsies were taken from different parts of lesions to analyze tumor type, grade, and hormonal receptors by histopathological examination, IHC for ER, PR, HER2, Ki-67 status assessment, and FISH for HER2 equivocal cases.

Histopathological and immunohistochemical analysis

Histologic grading of invasive duct carcinoma (IDC) was based on the modified Scarff-Bloom-Richardson system (8) and classified as: grade 1 (well-differentiated), grade 2 (moderately differentiated), and grade 3 (poorly differentiated). For the purpose of our study, grade 1 and 2 were considered as low grade, whereas grade 3 was considered as high grade.

Immunohistochemistry study was performed for all samples to detect the levels of ER, PR, HER2 oncogene overexpression, and Ki-67 index. Stained slides were examined by pathologists for nuclear ER or PR expression according to the College of American Pathologists guidelines (≥ 1% cutoff for positive).

In our study, Ki-67 index < 14% was considered as low expression, and ≥ 14% was considered high expression according to St. Gallen International Expert Consensus.

HER2 expression on IHC was based on the cell membrane staining pattern with grade 2+ considered equivocal, grade 3+ considered positive, and grade 1+ or 0 considered negative. All the equivocal samples were further analyzed with fluorescence in situ hybridization where FISH ratio higher than 2.2 or HER2 gene copy greater than 6.0 was considered positive.

Based on ER/PR/HER2 and Ki-67 expression status, breast cancers were categorized into four molecular subtypes in accordance with St. Gallen 2011 consensus surrogate definitions [3] of the molecular subtypes: LA subtype (ER- and/or PR-positive, HER2-negative, and Ki-67 < 14%), LB subtype (either ER- and/or PR-positive, HER2-negative, and Ki-67 ≥ 14% or ER- and/or PR-positive and HER2-positive), HER2-enriched type (HER2) (ER- and PR-negative and HER2-positive), Triple-negative type (TN) (ER, PR, and HER2-negative).

Statistical analysis

All data were collected, tabulated, and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium). Quantitative data were expressed as the mean ± SD & median (range), and qualitative data
were expressed as an absolute frequency (number) & relative frequency (percentage). Categorical data were compared using the Chi-square test or Fisher’s exact test when appropriate. p-value < 0.05 was considered statistically significant (S), p-value < 0.001 was considered highly statistically significant (HS), and p-value ≥ 0.05 was considered statistically insignificant (NS).

RESULT

This study consisted of 69 patients with a mean age of 48.23 years, 47.8% were ≤ 50 years, and 52.2% were > 50 years. As regard to the molecular classification, we had 29 patients (42%) LA subtype (Figure 1), 23 patients (34%) LB (Figure 2), 8 (10%) HER2/neu-enriched (Figure 3), and 9 (13%) had TNBC (Figure 4). According to the histological grade, low grade (I & II) tumors were more common 42 (60.5%) in comparison to high grade (grade III) 27 (39.5%) (Table 1).

Table (1): Demographic characteristics and pathologic data among the studied breast cancer patients:

<table>
<thead>
<tr>
<th>Pathological findings</th>
<th>The studied breast cancer patients (N=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (No.)</td>
</tr>
<tr>
<td><strong>Age group</strong></td>
<td></td>
</tr>
<tr>
<td>≤50 years</td>
<td>33</td>
</tr>
<tr>
<td>&gt;50 years</td>
<td>36</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>48.23 ± 9.96</td>
</tr>
<tr>
<td>Median (Range)</td>
<td>51 (25–69)</td>
</tr>
<tr>
<td><strong>Menopausal status</strong></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>25</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>44</td>
</tr>
<tr>
<td><strong>Grade of IDC</strong></td>
<td></td>
</tr>
<tr>
<td>Low grade(I &amp; II)</td>
<td>42</td>
</tr>
<tr>
<td>High grade (III)</td>
<td>27</td>
</tr>
<tr>
<td><strong>Molecular subtype</strong></td>
<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>29</td>
</tr>
<tr>
<td>Luminal B</td>
<td>23</td>
</tr>
<tr>
<td>HER2-Enriched</td>
<td>8</td>
</tr>
<tr>
<td>Triple negative</td>
<td>9</td>
</tr>
</tbody>
</table>
Categorical variables were expressed as number (percentage).

Analysis of our obtained ultrasound findings we found that although TNBC were more common in premenopausal females (66.7%), there were no significant differences in age ($P = .654$) among tumor subtypes.

The median size of mass lesions on US was larger in HER2-enriched tumors. However, there was no significant difference in tumor size among different molecular subtypes ($P = .598$). Oval mass shape was strongly correlated with TN breast cancer (7/9, 78 %) compared to luminal A (4/29, 13.8 %) and luminal B (3/23, 13%) cancers ($P < .001$). The Irregular shaped mass was more frequent in luminal A (25/29, 86.2%), and luminal B lesions (20/23, 87%) than TN lesions (2/9, 22%) ($P < .001$). There was a significant difference in mass margins among breast cancer subtypes ($P < .001$). TN breast cancer more frequently had circumscribed margins (7/9, 78%) compared to luminal A (4/29, 13.8%), and luminal B lesions (3/23, 13%). Irregular or Speculated margins was frequently associated with luminal A breast cancers (25/29, 86.2%) compared to TN lesions (2/9, 22%). HER2-enriched breast cancers were more likely to be multifocal (4/8, 50 %), LB lesions were multifocal in (4/23, 17.4%), and multicentric in (3/23, 13 %).

According to the tumor boundary, we found that the mass with abrupt boundary was more common in TNBC (100%) while the mass with halo boundary was more common in LA (62.1%), LB (52.2%), and HER2 (75%). However, there was no statistical significance between tumor boundary, and different molecular subtypes ($P = .072$).

There was a statistically significant difference in orientation pattern ($P < .001$) of different molecular subtypes, parallel orientation was more common in TNBC (78%) and non-parallel orientation was more common with LA(86.2%) and (100%) in LB and HER2.

Posterior acoustic features analysis suggested that tumors with posterior shadowing were more likely to be LA or LB type, tumors with mixed posterior acoustic feature were significantly associated with HER2+ overexpression, and tumors with posterior enhancement were strongly associated with TNBC ($P = .001$). Ultrasonography detected microcalcifications were strongly associated with HER2 overexpression ($P < .001$).

Axillary lymph node involvement was seen most frequently in TNBC (6/9, 66.6%). There was no significant axillary lymph node involvement among tumor subtypes ($P = .289$).

There was a statistically significant difference in histological grade ($P = .012$) associated with different molecular subtypes, Low histological grade was suggested to be associated with Luminal subtypes (86.2%) in LA and (69.6%) in LB, while high histological grade was more commonly associated with HER2 (75%) and TNBC (78%). (Table 2).

Table (2): US characteristic of breast cancer molecular subtypes among the studied patients
Categorical variables were expressed as number (percentage); a: Chi-square test; p-value < 0.05 is significant; Sig.: Significance

Analysis of our obtained ultrasound characteristics of histological grade of IDC we found that there was no significant difference for tumor size with histological grade the median tumor size of mass lesions on US was larger in high grade tumors compared with other subtypes (P = .389). Irregular shape of mass was more common in low histological grade (42/42, 100%) compared to (11/27, 40.7%) in high-histological grade (P = .002). There was significant difference in mass margin among breast cancer histological grades (P = .002). The irregular mass margin was associated with low-grade breast cancers (42/42, 100%) compared to high-grade (11/27, 40.7%).
As regards the tumor boundary, we found that there was a statistically significant between the tumor boundary and different histological grades (.001). The abrupt boundary was more common with high grade (81.5%) while halo boundary was more common with low grade (73.8%).

There was a strong relationship between orientation pattern, and different tumor grades (P = .027). Non-parallel orientation was more common with low tumor grade(95.2% ) compared to (66.7%) in high tumor grades.

Posterior acoustic features analysis revealed that tumors with posterior shadowing were more common with tumor of low histological grade (69%) compared to (48.1%) in tumor of high histological grade. There was no significant association between posterior acoustic characteristics and histological grading of tumors( P=.140). Involvement of axillary lymph nodes (LNs) was frequently noted in high-grade tumors (14/27,51.9%). However, the results were not significant (P = .158) (table 3).

Table (3): Ultrasound characteristic of histological grades of breast cancer among the studied patients
Categorical variables were expressed as number (percentage); a: Chi-square test; p-value < 0.05 is significant; Sig.: Significance.

We recorded sensitivity and specificity of US in prediction of histological grade of invasive duct carcinoma was (66.66%, 86.95%) respectively (table 4).

Table (4): Diagnostic performance of ultrasonography in differentiation between low grade carcinoma and high-grade carcinoma.

<table>
<thead>
<tr>
<th>Echo pattern</th>
<th>Low grade carcinoma</th>
<th>High grade carcinoma</th>
<th>SN (95%CI)</th>
<th>SP (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoechoic</td>
<td>60</td>
<td>36</td>
<td>86.9% (38.38 – 88.17)</td>
<td>88.9% (66.41 – 97.22)</td>
</tr>
<tr>
<td>Complex</td>
<td>9</td>
<td>6</td>
<td>13.1%</td>
<td>14.3%</td>
</tr>
<tr>
<td>Posterior acoustic features</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shadowing</td>
<td>42</td>
<td>29</td>
<td>60.9%</td>
<td>69%</td>
</tr>
<tr>
<td>Mixed</td>
<td>4</td>
<td>2</td>
<td>5.8%</td>
<td>4.8%</td>
</tr>
<tr>
<td>Enhancement</td>
<td>5</td>
<td>0</td>
<td>7.2%</td>
<td>0%</td>
</tr>
<tr>
<td>No change</td>
<td>18</td>
<td>11</td>
<td>26.1%</td>
<td>26.2%</td>
</tr>
<tr>
<td>Lymph node</td>
<td>42</td>
<td>29</td>
<td>60.9%</td>
<td>69%</td>
</tr>
<tr>
<td>Negative</td>
<td>42</td>
<td>29</td>
<td>60.9%</td>
<td>69%</td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td>13</td>
<td>39.1%</td>
<td>31%</td>
</tr>
</tbody>
</table>

SN: Sensitivity; SP: Specificity; CI: Confidence Interval
Figure (1): A 51-year-old woman with hormone receptor-positive (luminal A subtype) invasive ductal carcinoma (low grade). US image shows speculated hypoechoic mass with non-circumscribed margin and posterior acoustic shadowing in the right upper outer quadrant of breast.

Figure (2): A 55-year-old woman with LB (her2-) invasive ductal carcinoma (low grade). US image shows non-circumscribed irregular hypoechoic mass with posterior acoustic shadowing.
**Figure (3):** A 49 years-old woman with HER2-enriched-type: IDC of high grade, US shows micro-lobulated mass with non-circumscribed margins, microcalcifications, and posterior mixed acoustic pattern.

**Figure (4):** A 38-year-old woman with TNBC (high grade IDC), US shows oval hypoechoic mass with circumscribed margin and posterior enhancement.
DISCUSSION

Breast cancer is a widespread disease and has variable genotypic and phenotypic subtypes [8]. The histopathologic features of tumors have been used to determine the prognosis and line of treatment of breast cancer. Although, they do not obtain sufficient information due to tumor heterogeneity [9]. So, the identification of breast cancer subtypes has an important role in directing the type of treatment [10].

This study aimed to correlate the relationship between the histological grade of invasive duct carcinoma (IDC) and molecular subtypes of breast cancer with imaging features obtained by breast ultrasound.

We found that although Luminal A subtype was more common in the older age group (postmenopausal state) (68.8%), and TNBC was more common in premenopausal female patients (66.7%), the value was statistically non-significant. This was in line with Dogan et al., & Fan et al., [11,12] they were reported that there was no significant correlation between age and different breast cancer molecular subtypes, however, Costantini et al., [13] found that the younger age group was significantly related to the TN subtype.

We reported that the mean size of tumor was larger with HER2-positive (75%) compared to LA (37.9%), LB (47.8%), and TNBC (33.3%). However, there was no significant difference of tumor size among different molecular subtypes. This was in line with Fan et al., [12] who found that the largest diameter was associated with the HER2-positive subtype, and Temiz et al., [14] who showed that a larger diameter is associated with hormone receptor-negative tumors.

As regards the shape of breast mass, our study revealed that there was a significant relationship between the mass shape and the histological grade, and molecular subtypes. The irregular shape was related to LA (86.2%), LB (87%), and low histological grade (100%), while the oval shape of the mass was more common in high histological grade (59.3%), and was related to TNBC (78%).

Our study revealed that Luminal A and Luminal B tumors were more common to have an irregular/ speculated margin (86.2 & 87%) respectively and low histological grade (100%). While the well-circumscribed margin of the mass was significantly associated with TNBC (78%) and high histological grade tumors (59.3%).

Our result agrees with Algazzar et al., and Rashmi et al.,[15,5] who found that hormonal-receptor-negative tumors were statistically different from hormonal-receptor-positive tumors regarding the mass margins, as the hormonal-receptor-negative tumors were well-circumscribed on ultrasound. Also similar to Shin et al., [16] who reported that the microlobulated or circumscribed outlines with posterior acoustic reinforcement were associated with high-grade and HR-negative tumors.

Our study revealed that masses of non-parallel orientation were more common with the luminal group and low histological grade. While masses of parallel orientation were more likely to be associated with TNBC, and high histological grade. This result agrees with Kim et al[17].

In our study, we noted that the masses with halo boundary were significantly associated
with tumors of low histological grade (73.8\%), while the masses with abrupt boundary were significantly related to tumors of high histological grade (81.5\%).

In contrast to, Kim et al.[17] who reported that not circumscribed margin, an abrupt boundary, and a hypoechoic or complex echo pattern were more frequent in grade 3 invasive cancers than in grade 1 & 2 invasive cancers.

We reported that multifocality or multicentricity were more frequent in HER2-enriched (50\%) cancers, and also noted at LB (with her2 +ve) 17.4\% were multifocal and 13\% were multicentric, otherwise, TN breast cancers were uniquely unifocal (100\%), this is a concordance to Elias et al [18], reported that the multifocality was related to increased HER2 overexpression. This was similar to Grimm et al [19], who reported that multifocal or multicentric disease was significantly more frequent in luminal B, and HER2-positive subtypes.

As regards the posterior acoustic characteristics, we revealed that the posterior acoustic shadowing was frequently correlated with Luminal A (62.1\%) & Luminal B (87\%). Although posterior acoustic shadowing was more common in tumors of low histological grade (69\%), it was no significant difference between posterior acoustic shadowing and tumor histological grade. Posterior acoustic reinforcement was significantly correlated with TNBC (66.7\%). The result agrees with Celebi et al. who found that the tumors with posterior shadowing were non-TN subtype, low histologic grade, and had at least one HR-positive status. Also, Shin et al., Kojima et al., and Blaichman et al., [16,20,21] showed that mass with circumscribed margins and posterior acoustic reinforcement was associated with high-grade and HR-negative status.

These findings were similar to the results of Anupama et al.,[22] who reported that tumors with posterior shadowing were more commonly related to HR-positive status. Irshad et al.,[23] also found that tumors with posterior acoustic shadowing had greater than 9 times higher association with HR-positive status.

Conversely, Rotstein and Neerhut, [24] noted that grade III invasive ductal carcinoma shows the classic finding of acoustic shadowing. These studies did not use the BI-RADS lexicon to categorize sonographic characteristics.

But in our study there was no statistically significant association between posterior acoustic feature and histologic grade, so we differ from Blaichman et al.,[21] who reported that the presence of shadowing was strongly associated with low grade.

In the current study, expression of the HER-2/neu oncogene was correlated with the calcifications on breast ultrasound. However, there was no significant difference in calcifications among different molecular subtypes. The same is confirmed by Kim et al., Seo et al., and Zhang et al.,[17,25] who found that calcifications were more common in HER2-enriched tumors than the other subtypes. In the study of Rashmi et al.,[5] LA was the next most frequent molecular subtype after HER2-enriched to have microcalcifications.

In contrast to Cen et al.[26] who revealed that coarse heterogeneous calcifications were commonly associated with LA subtype.

In the current study, we noted that the presence of axillary lymph node involvement was more frequent in high histological grade tumors (51.9\%) than low histological grade tumors (31\%). However, the result was statistically non-significant (\(P\) value= .158). Our results
were in line with Temiz et al [14], who found a relationship between the presence of axillary pathological lymph node and larger tumor diameter, high histopathological grade, and high Ki-67 index

This study was the first to assess the diagnostic performance of breast ultrasound in predicting the tumor histological grade and we recorded sensitivity (66.66%), specificity(86.95%) in predicting the histological grade of IDC.

Our study had many limitations as, the small sample size of our study, and we did not assess if there is a relation between tumor vascularity by color doppler study and breast cancer molecular subtypes.

CONCLUSION:

This study showed that certain ultrasound findings such as tumor shape, margins, posterior acoustic features, and microcalcification are strongly correlated in predicting the histological grade and molecular subtype of breast cancer and thus may further expand the role of conventional breast imaging for more precise diagnosis of breast cancer. Tumors with non-circumscribed margins and posterior shadowing are predicted to be LA or LB subtype, hormone receptor-positive, and low-grade tumors. Tumors with a larger size, microcalcifications, and posterior mixed acoustic features are strongly predicted to be HER2 subtype. Oval shaped tumors with circumscribed margin, posterior enhancement, and absence of microcalcifications are predicted to be TN type and high-grade histopathology of breast cancer.

References:


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