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Original Research Paper

Radio-Diagnosis

ULTRASONOGRAPHY AND MAMMOGRAPHIC FEATURES ACTING AS PROXY TO MOLECULAR SUBTYPING OF BREAST CARCINOMA: A CROSS SECTIONAL STUDY

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ABSTRACT

1. AIMS AND OBJECTIVES :

- To determine the correlation between and predict the molecular subtypes of breast carcinoma based on mammography and ultrasound findings.
- To correlate the different epidemiological features of breast cancer patients with the molecular subtypes of breast carcinoma.

2. Materials And Methods: In our cross-sectional study, 84 biopsy proven cases of breast carcinoma were referred to radiology department. After taking informed consent and in presence of a female attendant, ultrasonography and mammography of the patients were done. All these patients went for surgical excision of the neoplastic mass; and the mass were sent for IHC analysis. Epidemiological data, clinical features, mammographic features and sonographic features were correlated with IHC subtype of the mass and P-Values were found out. P-Value <0.05 was considered to be statistically significant.

3. Results:

- Among 84 patients, 22 were of Triple Basal Negative subtype, 17 were of Luminal-A subtype, 16 were of Luminal-B (Her-2 negative) subtype, 15 were of Her-2 enriched subtype and 14 out of 84 patients were of Luminal-B (Her-2 positive) subtype.
- IHC correlation between sex distribution, age distribution, age of menarche distribution, achievement of menopause and age of menopause distribution, parity distribution, history of breast feeding, family history of breast carcinoma, history of breast pain, breast ulceration, nipple discharge, quadrant location of breast mass and size of mass showed P-Value >0.05; which was statistically insignificant.
- IHC corelation with mammographic microcalcifications, shape of breast mass, margins of breast mass, posterior acoustic pattern of breast masses and vascularity of the breast mass showed P-Value <0.05; which was statistically significant.

4. Conclusion: Mammographic microcalcification and other features on sonography like shape of the mass, margins of the mass, posterior acoustic features and vascularity of the mass are strongly correlated in predicting the molecular subtype of breast carcinoma.

KEYWORDS : Mammography, Ultrasonography, Immunohistochemistry, Breast, Carcinoma.

INTRODUCTION

The incidence of breast carcinoma is ever increasing among the women population and is the most common cancer in Indian women and also globally. (1) Its epidemiology, clinical features, natural history and biological features are highly diversified and plays an important role in the treatment. Molecular subtyping of breast cancer plays an important role in treatment planning, assessing prognosis and for follow-up. (2) But, IHC analysis is an invasive methodology and is highly costly. This test is not affordable by many poor people in the country. On other hand, ultrasound and mammography are two non-invasive relatively cheaper and routine investigations done in assessment of a patient of breast carcinoma. So, if we can predict the molecular subtypes based on the ultrasonographic and mammographic features, then we can lessen the financial burden of the patients and do effective treatment of the patients who were otherwise not able to do the costly investigation for IHC analysis. A honest attempt has been made in this study to show the features of mammography and ultrasound which would predict the molecular subtype of breast cancer. According to the St. Gallen International Expert Consensus, IHC subtyping is done in the way which is depicted in Table 1. (3-8).

MATERIALS AND METHODS

1. ETHICS:

a. Approval from Institutional Ethics Committee (IEC) was sought. Informed written consent in Subject's vernacular language was taken before enrolment for study.

2. Selection Of Patients:

a. This a cross sectional study where the included patients are: Breast cancer patients who underwent mammography and/or USG and underwent surgical excision followed by IHC examination of the sample.

b. Inclusion And Exclusion Criteria:

Inclusion Criteria:

- Breast cancer patients who underwent mammography and/or USG and underwent surgical excision followed by IHC examination of the sample.

Exclusion Criteria:

- Patients with contralateral breast mass.
- Patients with distant metastasis.
- Patients with history of prior neoadjuvant chemotherapy.
- Patients with prior history of cancer treatment.
- Patients unable to undergo Histopathological Examinations (HPE) and Immunohistochemistry (IHC) examinations.
- Patients not willing to participate in the study.

3. Method Of Collection Of Data:

- Patients with biopsy proven breast carcinoma was included in our study after written informed consent, explaining procedure in detail to the patient.
- On the day of USG appointment, complete history and clinical evaluation of the patient was done. Patient was taken up for USG examination in presence of a female attendant. After USG examination, patient was taken up for mammography examination.

Ultrasound Imaging Protocols:

Patients were subjected to USG after history taking, clinical evaluation and local examination of bilateral breast

A) Equipment:
MACHINE PHILIPS GE LOGIQ S8.

Transducer For Local Breast Imaging:
- 9LD linear probe (2-8 MHZ)
- C1-5 Curvilinear Probe (1-5MHz)

B) Protocol:
Both the breasts were imaged in all the four quadrants in axial, sagittal and oblique views.

C) Ultrasound Based Imaging Criteria:
1. SIZE: Maximum dimension on Ultrasound.
2. MARGINS:CircumscribedorNon-Circumscribed
3. SHAPE: Oval/Round/Irregular.
4. POSTERIOR ACOUSTIC FEATURES:

Shadowing/Enhancement/Mixed(Both enhancement and shadowing) /No changes.
5. Vascularity: Adler's Degree: 0,1: low; 2,3: High

0:No Vascularity; 1: minimal vascularity; 2: Moderate Vascularity ; 3: Marked Vascularity.

2. Mammography Imaging Protocols:
Patients were subjected to mammography after USG with informed consent and in presence of female attendant.

A. Equipment:
Model: MAM-VENUS
SR NO.: 2K805010-M
"CLASS 1 EQUIPMENT WITH B TYPE HV GENERATOR"
MANUFUCTERED BY: ALLENGERS MEDICAL SYSTEMS LIMITED.

B. PROTOCOL:
Mammography imaging was taken in cranio-caudal and medio-latero- oblique views.

Mammography Based Imaging Criteria:
1. Microcalcifications: Present or absent.

Immunohistochemistry:
It was collected from follow-up study after the patients underwent IHC analysis. Data was collected from the pathology, radiotherapy and surgery departments.

Statistical Analysis
- Collected data was entered into Microsoft Excel software and coded
- Charts and tables were prepared using Microsoft word and excel software.
- Descriptive data was presented in frequency and percentage.
- The correlation between IHC findings, USG and Mammography findings; clinical history was performed by Chi2 test.
- P value <0.05 was considered as statistically significant.
- Statistical software SPSS 19.0v was used for data analysis.

RESULTS

1. Distribution Ofcases According To Immunohistochemistry Status:
Out of 84 patients, 22(26.19%) out of 84 patients were of Triple basal negative subtype; 17 (20.23%) out of 84 patients were of Luminal-A subtype; 16 (19.05%) out of 84 patients were of Luminal B (Her2 negative subtype); 15(17.86%) out of 84 patients were of HER-2 enriched type; and 14 (16.67%) out of 84 patients were of Luminal B (Her-2 positive) subtype. (TABLE 2)

2. Epidemiological Data And Clinical Featurs With Its Ihc Subtype Correlation:(table 2)
2 (2.4%) out of 84 patients were male; whereas 82 (97.6%) were female Majority - 33 (39.3%) belonged to age group below 50 years. 52 (63.4%) out of 82 female patients had their age of menarche between 13-15 years old. 59(72%) out of 82 female patients achieved menopause. 46 out of 59 patients had their age of menopause above 46 years old. 51(62.2%) out of 82 patients had their parity between 1 and 2. 65 (77.4%) out of 84 patients had history of breast feeding. 48 (57.1%) out of 84 patients had family history of breast carcinoma. 49 (58.33%) out of 84 patients presented with associated breast pain. 30 (35.71%) out of 84 patients presented with associated nipple discharge. Out of 84 patients, 17 patients had the breast mass in upper outer, upper inner, lower outer and central quadrant each. 16 patients had the breast mass in lower inner quadrant. Each of these parameters were correlated with IHC subtype of the breast cancer and P-Value was found to be >0.05; hence statistically insignificant.

3. Mammographic And Sonographic Features Of Breast Cancer Patients With Its Ihc Correlation: (table 3 And 4)
- Out of 84 patients who underwent mammography; 28 (33.33%) patients had microcalcifications whereas 56 (66.66%) patients did not have micro-calcifications. Out of 28 patients who had micro-calcifications in the mammographic images of breast parenchyma, 11 (40.74%) patients turned out to be HER-2 enriched and 10 (35.71%) patients turned out to be Luminal B (HER-2 positive) subtype. P-Value of this analysis was <0.001 (<0.05). Hence the correlation is statistically insignificant. So, we can say that the patients who had microcalcifications in their mammographic images are more likely to have HER-2 positive status. (Figure 1)
- Out of 84 patients, 71(84.5%) patients presented with breast masses of size more than 20 mm. 13(15.48%) patients had breast masses equal to or less than 20 mm. P-Value of this analysis was 0.816 (>0.05). Hence the correlation is statistically insignificant.
- Out of 84 patients, 30 (35.71%) patients presented with round shaped mass; 36 (42.86%) patients presented with oval mass; and 18 (21.43%) patients presented with irregular shaped mass. Out of 30 patients who had round shaped mass; 13 (43.33 %) patients had Luminal-A subtype and 12(40%) patients had Luminal-B (Her2-Negative) subtype. Out of 36 patients who had oval shaped mass; 13 (36.11%) patients had Luminal B (Her-2 positive) subtype; 14 (38.89%) patients had Her-2 enriched subtype.Out of 18 patients who had irregular mass, 16 (88.89%) patients had triple basal negative subtype. P-Value of this analysis was <0.001 (<0.05). Hence the correlation is statistically significant. (Figure 2)
- Out of 84 patients, 64(76.2%) patients had non-circumscribed masses; whereas 20 (23.8%) patients had well circumscribed masses. Out of 20 patients who had well-circumscribed margins, 12 (60%) patients had triple basal negative cancer. P-Value of this analysis was 0.003 (<0.05). Hence the correlation is statistically significant. (Figure 3)
- Out of 84 patients, 35(41.67%) patients showed posterior acoustic shadowing; 27 (32.14%) showed posterior acoustic enhancement; 15 (17.86%) showed mixed pattern and 7 (8.33%) showed no changes. Out of 35 patients who show posterior acoustic shadowing, 13 (37.14%) patients have Luminal-A subtype; 14 (40%) patients have Luminal-B (Her-2 negative) subtype. Out of 27 patients who show posterior acoustic enhancement, 19 (70.37%) patients have triple basal negative subtype. Out of 15 patients who show mixed pattern, 7 (46.67%) patients have HER-2 enriched subtype and 6 (40%) patients have Luminal B (Her-2 positive) subtype. P-Value of this analysis was <0.001 (<0.05). Hence the correlation is statistically significant. (Figure 4)

- Out of 84 patients with breast malignant neoplastic masses, 38 (45.24%) patients presented with low vascularity and 46(54.76%) patients presented with high vascularity. Out of 38 patients with low vascularity, 17 (44.74%) patients had Luminal-A and Triple Basal Negative subtype each. Out of 46 patients with high vascularity, 14 (30.43%) patients had Luminal B (Her-2 positive and negative each); and 13 (28.26%) patients had HER-2 enriched subtype. P-Value of this analysis was <0.001 (<0.05). Hence the correlation is statistically significant. (Figure 5)

DISCUSSION:

IHC subtype determination based on the receptor status of the particular tumour is particularly important for the current practice of breast cancer management. It requires sample for testing and is the gold standard test for molecular subtyping. However, the aim of this study was to show that certain non-invasive radio-imaging modalities like mammography and ultrasound would improve the potential for pre-surgical treatment strategies. In this study, we correlated the findings of microcalcification mammographically; size, shape, margin, posterior acoustic pattern and vascularity with that of the immunohistochemistry subtype of the breast cancer and found out whether they are statistically significant or not. We compared the study with the study made by Rashmi, S. Kamala and S. Sudha Murthy and concluded whether the findings were corroborative or not. (9)

Tables And Figures

Table 1: Table Determining The Different Ihc Status With Respect To The Absence Or Presence Of Different Molecular Receptors.

IHC status	ER	PR	HER-2	Ki-67
Luminal-A	Positive	Positive	Negative	Low
Luminal-B (Her-2+ve)	Positive	Positive	Positive	High/Low
Luminal-B (Her-2-ve)	Positive	Positive	Negative	High
Her-2 Enriched	Negative	Negative	Positive	High
Basal Like	Negative	Negative	Negative	High

Table 2: Table Showing The Different Epidemiological Features, Clinical Features And Sonographic Criteria Which Were Corelated With Ihc Subtype And Their P-value Came Out To Be >0.05; Hence Statistically Insignificant

Epidemiological criteria/Clinical Feature/Sonographic Criteria	P-Value
Distribution of Sex of Patients	**0.53**
Distribution of Age of Patients	**0.215**
Distribution of Age of Menarche of female patients	**0.171**
Status of achievement of Menopause	**0.858**
Distribution of Age of menopause of the patients	**0.904**
Status of parity of the patients	**0.084**
History of breast feeding	**0.578**
Family History of breast carcinoma	**0.761**
History of breast pain	**0.34**
Breast Ulceration	**0.363**
Nipple discharge	
Quadrant wise location of breast mass	**0.054**
Size of breast mass below or above 20 mm	**0.816**

Table 3: Table Showing The Different Mammographic And Sonographic Criteria Which Were Corelated With Ihc Subtype And Their P-value Came Out To Be <0.05; Hence Statistically Significant

Mammographic/Sonographic Feature	P-Value
Presence or absence of mammographic microcalcifications	**<0.001**
Distribution of shape of breast mass	**<0.001**
Distribution of margin of breast mass	**0.003**
Distribution of posterior acoustic features of breast mass	**<0.001**
Distribution of vascularity status of breast mass	**<0.001**

Table 4: Table Showing The Different Combination Of Sonographic And Mammographic Features With Possible Outcome Characteristics Of Ihc Subtype With Associated P-value.

Multivariate analysis	Outcome Characteristics	P Value
Posterior Acoustic Mixed pattern + Mammographic Microcalcification	**Her-2 enriched**	**0.001**
Circumscribed Margin + Posterior Acoustic Enhancement	**Triple basal negative cancer**	**<0.001**
Non-circumscribed Margins + Posterior Acoustic Shadowing	**Luminal-A/Luminal-B**	**<0.001**
Non-circumscribed Margins + Posterior Acoustic Shadowing+High Vascularity	**Luminal-B(Her-2-ve)**	**<0.001**

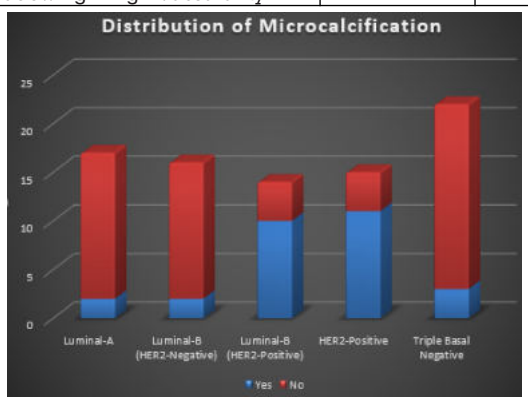


Figure 1: Bar Diagram Showing Distribution Of Microcalcification With Ihc Analysis

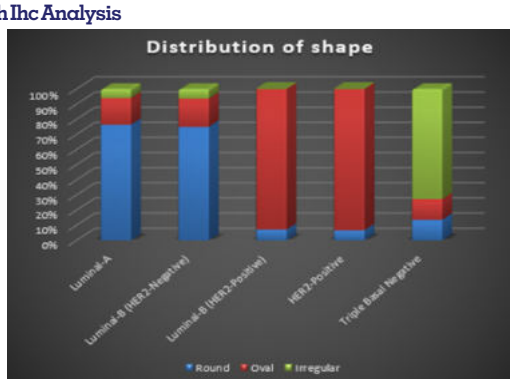


Figure 2: Bar Diagram Showing Distribution Of Shape Of The Breast Mass With Ihc Analysis

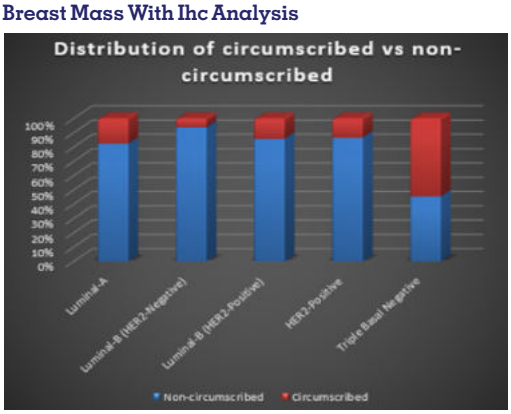


Figure 3: Bar Diagram Showing Distribution Of Margin Of Breast Mass With Ihc Analysis

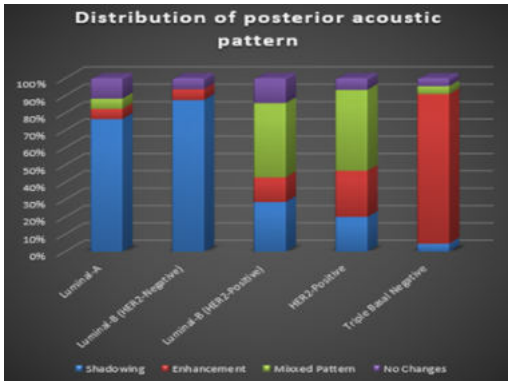


Figure 4: Bar Diagram Showing Distribution Of Posterior Acoustic Pattern With Ihc Analysis

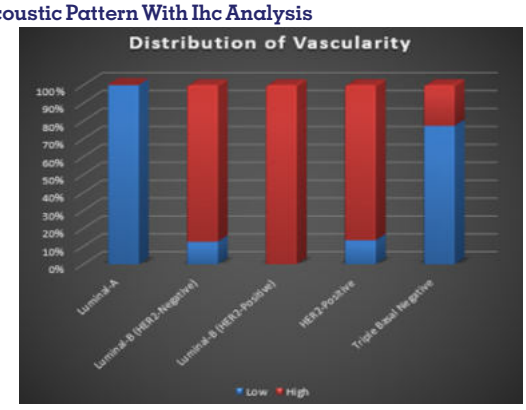
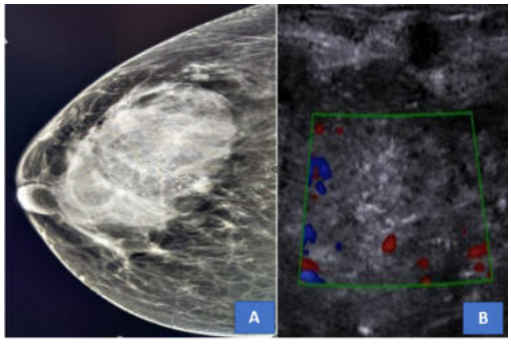
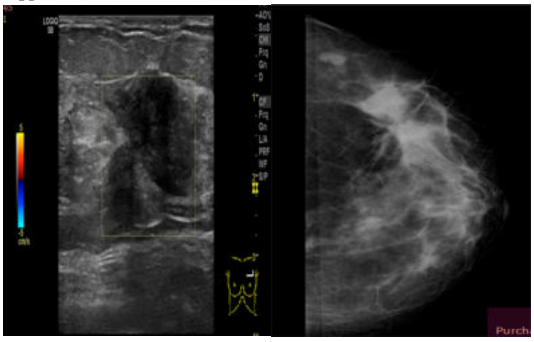


Figure 5: Bar Diagram Showing Vascularity Pattern With Ihc Analysis Cases



Case 1: Cranio-caudal View Of Mammography B: Usg Picture Of Breast Mass In Right Breast Parenchyma: Reveals An Oval Circumscribed Mass With Moderate Internal Vascularity And Mammographic Microcal cifications. Ihc Analysis Of This Patient Turned Out To Be Her-2 Neu Enriched Subtype.



Case 2: Usg Picture Of Breast Mass In Left Breast Parenchyma: Reveals an irregular non circumscribed mass with low internal vascularity and posterior acoustic shadowing in upper and outer quadrant of left breast. Cranio-caudal and Mediolateral oblique views of the mammogram reveals no microcalcification. IHC analysis of this patient turned out to be Luminal-A subtype.

Abbreviations

LA-Luminal-A; LB-Luminal-B; HER-2-Human Epidermal Growth Factor-2; TNBC-Triple Negative Breast Cancer; IHC: Immunohistochemistry; ER: Estrogen Receptor; PR: Progesterone Receptor; Ki-67: Kiel-67

CONCLUSION:

- Mammographic microcalcification and other features on sonography like shape of the mass, margins of the mass, posterior acoustic features and vascularity of the mass are strongly correlated in predicting the molecular subtype of breast carcinoma.
- Tumours with posterior acoustic shadowing and non-circumscribed margins are predicted to be of LA or LB subtype.
- Tumours with posterior acoustic shadowing, non-circumscribed margins and high vascularity are predicted to be of LB z(Her-2 negative) subtype.
- Tumours with presence of mammographic microcalc ification and mixed posterior acoustic pattern are predicted to be Her-2 positive subtype.
- Tumours with circumscribed margin and posterior acoustic enhancement and absence of mammographic microcalcification is predicted to be triple basal negative cancer subtype.

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