Original Research Article

Molecular classification of breast cancer

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A R T I C L E   I N F O

A B S T R A C T

Background: Breast cancer is a heterogeneous disease with different biological and histological properties due to genetics and epigenetic changes with varying clinical features and treatment responses. This study was planned and carried out with the objective to classify breast cancers molecularly using surrogate markers, ER, PgR, Her2/neu and Ki67 and correlate the various types with conventional prognostic markers.

Materials and Methods: 70 cases of invasive breast carcinomas were subjected to routine staining and immunohistochemistry with estrogen receptors(ER), progesterone receptors (PgR), Her2/neu and Ki67and classified into molecular subtypes as defined in various studies. These were correlated with other conventional prognostic parameters and analyzed statistically.

Result: 70 cases of invasive breast cancer were classified into 18(22%) cases of luminal B, 16(25%) cases of Her2/neu and triple negative each and 14(22%) cases of luminal A subtypes. There was an even distribution of molecular subtypes varying from 22 to 28%. Luminal B and Her subtype were commoner in the Indian setting as compare to other studies. Luminal A and Luminal B subtypes were commoner in patients older than 50 years. Her2/neu and TNBC cases were more commonly of higher histological grade and pathological stage, while Luminal A and B subtypes showed lower grade and stages. Luminal B subtype and Her2/neu subtypes showed DCIS more often and Luminal B and TNBC subtypes, more frequent lymph node metastasis.

Conclusion: The molecular classification of breast cancer by IHC in this study population showed an almost equal distribution of the 4 subtypes. The association of tumor grade and LVI with the molecular subtypes showed a significant correlation.

1. Introduction

Breast cancer is a commonest and leading cause of deaths in women due to cancer, with more than 10,00,000 cases diagnosed worldwide annually.[1] According to the WHO there are 7.6 million deaths worldwide due to cancer every year, out of which 502,000 are caused by breast cancer alone. During the past two decades the mortality rate has decreased significantly due to early detection of disease and the use of aggressive multimodality treatment, including targeted therapy.

Prognostic information is important in determining the likely outcome of the disease and planning further management. Apart from clinical parameters like age, menopausal status and disease presentation, important prognostic indicators in histopathology are tumour size, histologic type, histologic grade and pathological stage. In addition, there are other factors which are not only prognostic but also predictive of response to therapy.[2] Some of these factors includes ER, PgR, HER2/neu and Ki67.

Breast cancers are conventionally classified as per WHO classification, the latest version of which is of 2012.[3] A major drawback of this classification is that the majority of all breast cancers belong to one of the two major

histopathological classes namely invasive carcinoma (NST) or invasive lobular carcinoma.[4] This implies that this classification is unable to actually define the much wider heterogeneity of breast cancer with different biological and clinical profiles. As the concept of heterogeneity in breast cancer has now become accepted a new molecular classification has been proposed which may provide better targets therapies. This was first proposed by Perou and Sorliein in 2000.[5]

Recently according to the 2011 St. Gallen consensus, molecular subtypes of breast cancer have been classified into Luminal A (ER+/PgR+/HER2-/low Ki67), Luminal B (ER+/PgR+/HER2-/+/high Ki 67), HER2 overexpression (ER-/PgR-/HER2+) and triple negative breast cancer/TNBCS (ER-/PgR-/HER2-). One additional subtype, Basal like, refers to TNBCs that are positive for basal markers (CK5/6).[6]

Today, the immunohistochemical demonstration of ER, PgR and HER2/neu on core biopsies is standardized through well-established protocols.[7] There is a need to classify breast cancer based on IHC expression of these surrogate molecular markers and understand the biological significance and relate these to the existing classification and prognostic parameters in the Indian context. This study will thus attempt to establish the relevance of molecular classification in this region.

2. Materials and Methods

A cross sectional prospective and retrospective study was conducted of 70 cases with carcinoma breast diagnosed in the Department of Pathology of Bharati Vidyapeeth Deemed to be University Medical College and Hospital, Pune, from August 2016 to July 2019. Cases who had received preoperative chemotherapy or radiotherapy for breast cancer and the cases who came with recurrence were excluded from the study.

Lumpectomy and mastectomy specimens received were adequately fixed in 10% buffered formalin for 12 – 24 hours. Representative tissue sections were processed, and micro sections were stained with hematoxylin and eosin (H & E) as per standard protocol. Each case was classified as per WHO 2012 Classification of Breast Tumors. Additionally tumors were evaluated for grade using the Modified Scarff Bloom Richardson system, presence of lymph node metastasis and lymphovascular invasion(LVI) were recorded.

Microsections were subjected to immunohistochemistry (IHC) using antibodies to estrogen receptor(ER), progesterone receptor (PgR), HER2/Neu and Ki67. The scoring of ER, PR, HER2/Neu staining in the positive cases was done as per American society of Clinical Oncology and the College of American pathologist (ASCO/CAP) guidelines using Allred scoring system. The Allred score is the sum of proportion (proportion of stained nuclei of cells)

and the intensity score (intensity of stained nuclei). Positive interpretation requires at least 1% of tumor cells showing positive nuclear staining of any intensity.

For HER2/ Neu expression score of 0 and 1+ was considered as negative. Score of 2+ was considered as equivocal and 3+ was considered as positive. For interpretation of Ki67 staining, nuclear staining was considered as positive. Scoring involved counting of at least 500 malignant invasive cells and was expressed as a percentage of positively staining cells in hot spot. In this study we used a Ki67 scoring index of 14% for differentiating Luminal A and Luminal B subtypes.

Each case was then classified as per the Molecular classification of breast cancer. The molecular subtypes were correlated with histological types and all other demographic and prognostic parameters recorded earlier.

2.1. Statistical analysis

Association between molecular subtype with histological grade, tumor size, lymph node status evaluated by chi suare tests. In the present study, the significance level was set as $p < 0.05$. All the tabulations and statistical analysis were done using IBM SPSS 25.0 (Statistical Product for Services Solutions) data software.

3. Results

The present study, included 70 cases of carcinoma breast. The left breast was most commonly involved in 48 cases (69%). Painless lump was the most common presenting symptoms in 69% cases. Invasive carcinoma (NST) 47(66%) was the most common histological type identified.

ER positivity was seen in 37(52%) cases which were further graded and the majority of ER positive cases 35(95%) belonged to grade I and grade II. PgR positivity was seen in 30(43%) cases which were further graded and 29(96%) of PgR positive cases belonged to grade I and grade II. This correlation was statistically significant (p = 0.001). Her2/neu positivity was seen in 21/70 (30%) cases, 12/70 (17%) cases were Her2/neu negative and 6/70 (8%) cases were Her2/neu equivocal. Equivocal cases (2+) were advised FISH but this was not in the scope of this study and despite attempts to get information, these cases were lost to follow up. Her2/neu positive cases were further graded and 80% (17/21) of Her2/neu positive cases were in grade I or II. This association was not statistically significant (p = 0.37) Molecular subtyping was done in 64 cases excluding 6 Her2/neu equivocal cases using ER/PR, Her2/Neu and Ki67score. Luminal A cases were 14(22%), Luminal B cases were 18(28%), Her2/Neu and triple negative were 16(25%) each. 12 out of 18 cases of luminal B subtype were seen in age group > 50 years. 08 out of 16 (50%) cases of Her2/neu positive and 13 out of 16 (81%) cases

Table 1: Age, tumor size, lymph node metastasis, LVI in various molecular subtypes

Molecular types	Age (years) n(%)		Tumor size (cm) n(%)			Lymph node metastasis n (%)		LVI n(%)	
	< 50	>50	<= 2 cm	>2-5 cm	> 5 cm	Positive	Negative	LVI seen	LVI not seen
Luminal A	06(18)	08(25)	03 (50)	07 (20)	04 (18)	05(16)	08(36)	04(16)	09(32)
Luminal B	06(08)	12(38)	00 (00)	12 (33)	06 (28)	10(31)	06(27)	05(20)	11(40)
Her2 positive	08(24)	08(27)	01 (17)	10 (27)	05 (22)	07(22)	06(27)	05(20)	08(28)
Triple negative	13(42)	03(10)	02 (33)	07 (20)	07 (32)	09(31)	02(10)	11(44)	00(00)
Total	33 (100)	31 (100)	06 (100)	36 (100)	22 (100)	31(100)	22 (100)	25(100)	28(100)
p value	p = 0.139		p = 0.4			p = 0.05		p = 0.001	

1) Age and tumor size correlation was done in 64 cases excluding the Her2/neu equivocal cases.
2) Lymph node and LVI correlation was done in 53 cases after excluding Her2/neu equivocal and trucut biopsies cases.

Table 2: Tumor grade wise distribution of various molecular type

	Luminal A n (%)	Luminal B n (%)	Her2/Neu n (%)	Triple negative n (%)	Total n (%)
Grade I	09(65)	05(28)	02(13)	00(00)	16(25)
Grade II	05(35)	11(61)	10(62)	10(62)	36(56)
Grade III	00(00)	02(11)	04(25)	06(38)	12(19)
Total	14(100)	18(100)	16(100)	16(100)	64(100)
p value			p = 0.001		

Note:- After excluding the 06 Her2/neu equivocal cases correlation was done in 64 cases

Table 3: Molecular classification: Comparison with other studies

Molecular subtype	Onitilo AA et al.[8] n= 1134	Setyawati et al.[6] n= 247	Geethamala K et al.[9] n= 100	Walke et al.[10] n= 47	Ambroise et al.[11] n= 321	Present study 2019 n= 70
Luminal A	68.9% Luminal A and B	41.3%	54% Luminal A and B	21%	48%(luminal A and B)	22%
Luminal B		13.8%		08%		28%
HER2/Neu positive	17.7%	19.4%	26%	19%	27%	25%
TNBC	13.4%	25.5%	20%	51%	25%	25%

of triple negative subtypes were seen in women less than 50 years of age. However this association was not statistically significant (p=0.139). (Table 1)

Out of 70 cases 14 cases were trucut biopsies (03 cases of true cut biopsies were Her2/ neu equivocal) and 03 were Her2/neu equivocal, so lymph node metastasis was noted in 53 cases. Tumor size correlation was done in 64 cases excluding the 06 Her2/ neu equivocal cases. The majority of cases had tumor sizes of 2-5 cm in (36/64) 56%, followed by 34% (22/64) cases with tumor size > 5 cm and 06(09%) cases with tumor size < 2 cm. In luminal A and luminal B 10(71%) and 12(66%) cases respectively had a tumor size of 5 cm or less. 14 out of 16 triple negative cases (87%) and 15 out of 16 cases (93%) Her2/ neu cases showed tumor size of > 2cm. This correlation was not statistically significant (p = 0.4).

Lymph node metastasis was observed in total of 58% (31/53) cases. The highest percentage of nodes positivity was observed in luminal B subtype 19%(10/53) followed by

triple negative in 17% (9/53), 13% (7/53)of Her2/neu had lymph node metastasis. Luminal A had the largest number of lymph node negative cases in 15%(8/53). This association was not statistically significant (p = 0.05). (Table 1)

Tumor grade revealed that the majority of cases were grade II 56% cases (36/53), followed by grade I and grade III 16(25%) and 12(17%) cases. When tumor grade was correlated with molecular classes, majority of luminal A 09 out of 14 (65%) cases belonged grade I, 16 out of 18 cases of luminal B subtype were either in grade I or grade II. 14 out of 16 cases of Her2/neu and all 16 triple negative cases were in grade II or grade III. (Table 2) LVI was seen in 25 out of 53 cases (47%) out of which most of the cases 11(44%) were seen in triple negative molecular subtype. (Table 3)

4. Discussion

Carcinoma of breast is a commonly occurring cancer in clinical practice and is one of the leading causes of death in women due to cancer. The mortality due to cancer has
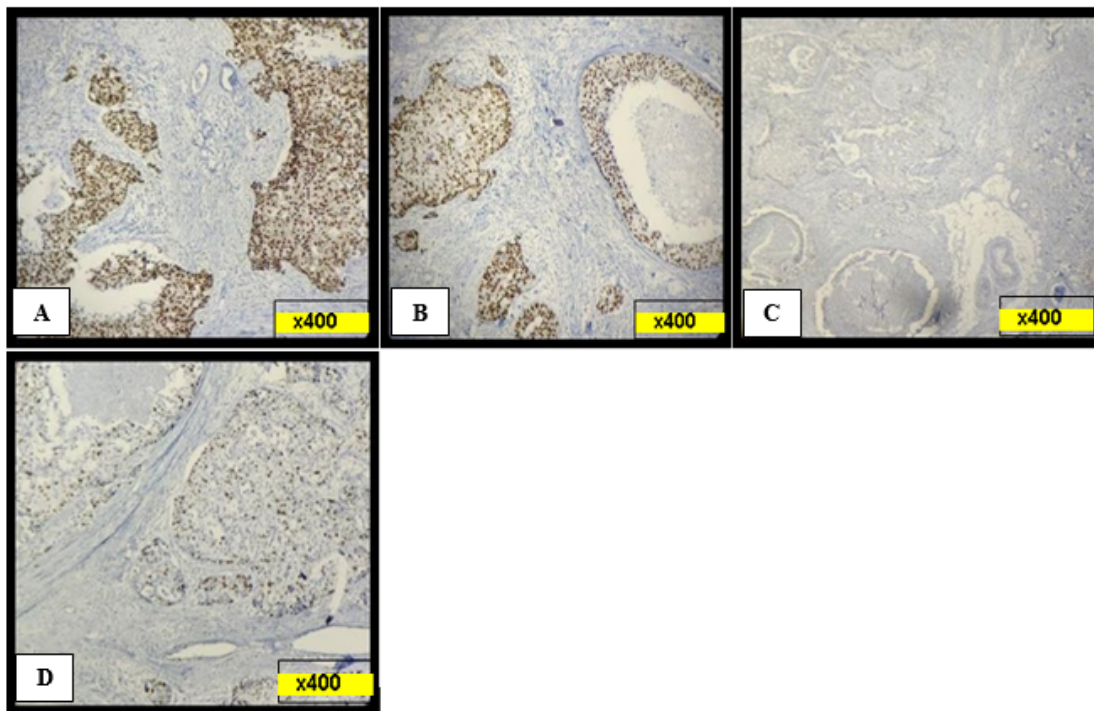
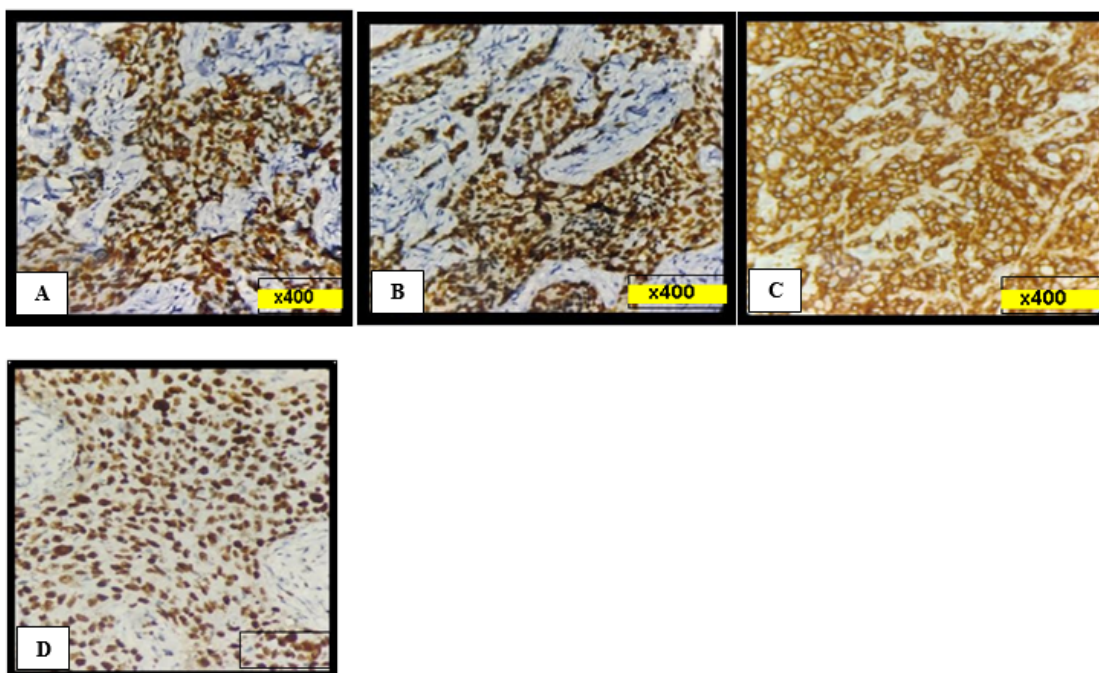**Fig. 1:** Luminal A type of breast cancer **A:** ER positive, **B:** PR positive, **C:** Her2/neu negative, **D:** Ki67 with low L.I

Fig. 2: Luminal B type of breast cancer **A:** ER positive, **B:** PR positive, **C:** Her2/neu positive, **D:** Ki67 with high L.I
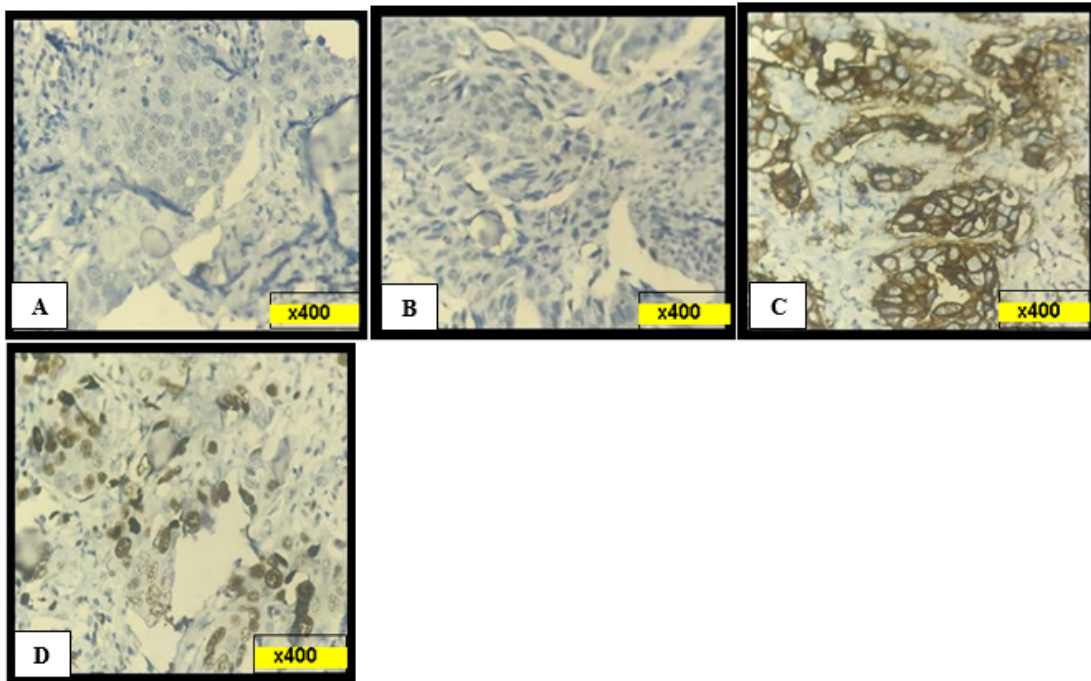
Fig. 3: Her2/Neu type of breast cancer **A:** ER negative, **B:** PgR negative, **C:** -Her2/neu strongly positive, **D:** Ki67 with high L.I
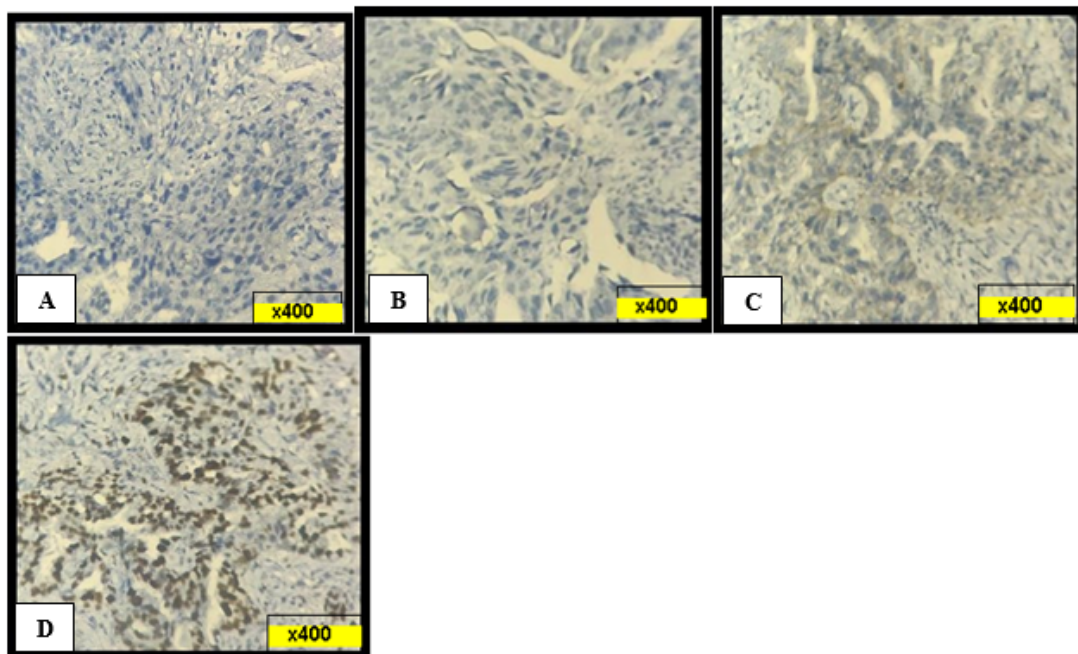

Fig. 4: Triple negative type of breast cancer **A:** ER negative, **B:** PgR negative, **C:** Her2/neu negative, **D:** Ki67 with high L.I

Table 4: Molecular classification: Comparison with result of other countries

Number of patients	Yang et al [12] Portland n = 804	Cheng at al [13] China n = 628	Carey et al [14] Carolina (USA) n = 196	Carolina (USA) n = 300	Present study India n = 70
Years	2006 – 2010	2000 - 2003	1993 – 1996		2017 - 2019
Luminal A	34.7%	46.5%	47.8%	54.0%	22%
Luminal B	15.9%	17.0%	12.7%	17.3%	28%
HER2/Neu	24.1%	15.0%	8.2%	5.6%	25%
TNBC	25.3%	21.5%	31.6%	23.0%	25%

significantly decreased in recent decades. Carcinoma of breast is now considered a heterogeneous disease and is comprised of different biological entities. These entities show many distinct clinical and pathological characteristics of which the more important ones are hormonal receptor status and histological grades. These entities have been shown to exhibit distinct behavioral patterns and different treatment strategies have evolved for this.

The St. Gallen consensus 2011, has brought out a molecular sub classification of breast cancer based on exhibition of ER, PgR, HER2/Neu and Ki67 markers and this sub classification would further enhance the therapeutic decision making. The conventional prognostic and predictive markers have been well discussed in literature in which the important ones include tumour stage, histological grade, immunohistochemical markers such as ER, PgR, Her2/Neu and Ki67 in addition to other pathological features.[6]

The present study comprised of 70 cases of carcinoma of breast diagnosed at the Department of Pathology of this institution. Molecular classification was carried out using the recent St. Gallen consensus 2011criteria, and the results of this study were compared with other studies as shown in Table 3.

In this study the majority of the cases were of Luminal B subtypes (28%), followed by equal number of cases of HER2/Neu and TNBC subtypes, 25% each, and 22% cases of Luminal A subtype. The results of this study were higher than the figures of Walke et al. in all subtypes except TNBC subtype. But the figures of this study were comparable to other studies for Luminal A and B subtypes of cancers.[10] (Table 3)

Results of this study were not comparable with the studies done in other countries. All other studies had higher number of Luminal A cases as compared to this study while the cases of Luminal B and HER2/Neu were higher in this study. TNBC cases was comparable with the study of Yang et al. and Carey et al. but was higher than the study of Cheng et al. (Table 4)

51% of the cases in this study occurred in women <50 years of age. This was comparable to the findings of Alnegheimish et al.[15] and lower than the figures of Shukla et al.[16] The strongest association in this study was between TNBC subtype and age < 50 years which was comparable

with the findings of Alnegheimish et al.[15] and Shukla et al.[16]

In all the studies it was noted that most of the tumours were >2 cm in size and largest number were seen in >2-5 cm group. These findings were comparable with the studies of Shukla et al.[16] and Walke et al.[10]

The histological grade was compared with molecular subtypes and it was observed that the majority of cases of luminal A and luminal B subtypes[14/14(100%) and 16/18 (88%)] were histological grade I and II. Similarly, the majority of cases of HER2/neu and TNBC subtypes i.e 14/16(87%) and 16/16 (100%) were histological grade II and grade III, suggesting the tumour aggressiveness of these two molecular subtypes. Findings of this study were comparable with that of Shukla et al.[16] 61% of the cases of Luminal A subtype was in grade I category, 51% of the cases of luminal B were in grade II category, while 46% Her2/Neu and 31% of TNCs were in grade III category. However Setyawati et al. have found that all molecular subtypes were predominantly of grade III suggesting that the diagnosis of breast cancer in this Indonesian study was delayed.[6]

Lymph node metastasis was seen in 41% (22/53) of the cases in this study. This was comparable to the study by Shukla et al.[16] (40%) but was lower than the figures quoted by Walke et al.[10] and Setyawati et al.[6] There was no significant difference in molecular subtypes, when correlated with lymph node metastasis. Walke et al.[10] however found a large amount of HER2/Neu and TNBC types with lymph node metastasis. Our findings correlate best with those of Setyawati et al.[6] who also did not find any significant correlation.

Lymphovascular invasion was seen in 47% of the cases in this study of which 44% were of triple negative subtype. Liao et al. has suggested that Luminal A subtype has small tumour size, less LVI and more lymph node involvement was seen in luminal B and other subtypes.[17]

The limitations of this study were the nonavailability of FISH to clarify on status of HER2/Neu equivocal (score 2+) cases. Also when distributed in groups the number of cases were small and hampered assessment of statistical significance. Non inclusion of CK 5/6 in the scope of this study did not permit complete molecular classification into basal and non basal subtypes. It is also mentioned in the literature that a discrepancy of upto 39% exists between

molecular classification by IHC and by gene expression.

5. Conclusion

The molecular classification of breast cancer by IHC in this study population showed an almost equal distribution of the 4 subtypes. The association of tumour grade with the molecular subtype was statistically significant with majority of Luminal A and B subtypes seen in grades I and II, while majority of HER2/Neu and TNBC subtypes in grade II and III. Most of the breast cancers in this study were >2 cm in dimension suggesting a need to proactively screen for the early diagnosis. There is also a need to study the relationship of molecular subtypes with risk factors in a large population across the country in multiple region.

6. Source of Funding

None.

7. Conflict of Interest

None.

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