HISTOMORPHOLOGY AND IMMUNOHISTOCHEMICAL SURROGATE MOLECULAR PROFILE OF CARCINOMA BREAST- A ONE-YEAR STUDY

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ABSTRACT

BACKGROUND

Being the most common cancer in female's worldwide, breast cancer is the most important cause of cancer deaths. There are different subtypes of carcinoma breast. In this study, we have tried the surrogate classification of carcinoma breast based on Immunohistochemical profiling. We wanted to analyse the histomorphological features of carcinoma breast in all the modified radical mastectomy and TruCut biopsy specimens received in our department for a period of one year and classify the breast cancers according to the immunohistochemically markers- ER, PR, Her2neu. We also wanted to evaluate the association between the molecular subtypes and the Modified Scarf Bloom Richardson Grading System of carcinoma breast.

METHODS

This is a retrospective cross-sectional study, in which, we had included 256 confirmed cases of carcinoma breast, by histopathological examination. The H & E stained sections were studied using various parameters like, age, histological subtype, which were graded according to the Modified Scarf Bloom and Richardson grading system. The breast carcinoma cases were classified into 4 molecular subtypes according to ER, PR, and HER2/neu status.

RESULTS

According to our study, invasive ductal carcinoma was the most common subtype of breast cancer diagnosed in the institution. Of the three grades of carcinoma, Modified Bloom Richardson's Grade 2 was most commonly seen. Surrogate classification based on IHC was done and showed the dominance of triple negative cancers in our study group.

CONCLUSIONS

Upon statistical analysis, there was significant association between the molecular classification and the histological grade.

KEYWORDS

Breast Cancer, IHC, Molecular Profiling, Triple Negative Cancers

BACKGROUND

Cancer of breast is a heterogeneous disease which has variable clinical outcomes, histomorphological features and epidemiological characteristics. It is the most commonly diagnosed cancer worldwide, among females and second most common malignancy in India after carcinoma cervix. The familial history of breast cancer and mutation of tumour suppressor genes BRCA1, BRCA2 and p53 increases risk of breast cancer. According to the fourth edition of World Health Organization (WHO) classification, breast cancer can be classified into 21 distinct histological types on the basis of cell morphology, growth and architecture patterns, the most common type being, Invasive Ductal Carcinoma Of No Special Type.[1] Based on gene expression profiling studies, breast carcinomas have been classified into intrinsic molecular subtypes, luminal A and B, HER-2 and basal like tumours. In areas where gene expression profiling is not feasible, surrogate classifications based on Immunohistochemistry (IHC) have been proposed.[2] The expert panel of the St. Gallen in 2013 proposed to classify tumours for therapeutic purposes based on such "surrogate intrinsic subtypes."[3]

We wanted to analyse the histomorphological features of carcinoma breast in all the Modified Radical mastectomy and TruCut biopsy specimens received in our department for a period of one year and classify the breast cancers according to the immunohistochemically markers - ER, PR, Her2neu. We also wanted to evaluate the association between the molecular subtypes and the Modified Scarf Bloom Richardson Grading System of carcinoma breast.

METHODS

This is a retrospective cross-sectional study of all the confirmed cases of carcinoma breast, in mastectomy and TruCut biopsy specimen received in the Department of

Pathology, Government Medical College, Kozhikode. We had conducted the study in the specimen obtained in the department for one-year period (2010-2011) at Kozhikode medical college.

All cases of carcinoma breast who had come to the department of pathology, Kozhikode medical college, in the study period, which were histologically confirmed and immunohistochemically profiled were included in the study. The TruCut biopsy specimen which were inadequate for immunohistochemical analysis and cases of carcinoma breast examined in our department which were previously given chemotherapy and radiotherapy were excluded from the study.

Methodology

The study was conducted in 256 histologically confirmed cases of carcinoma breast. We obtained the following parameters for the study - age at diagnosis, whether premenopausal or post-menopausal age groups, histological subtype, which were graded according to the Modified Scarf Bloom and Richardson grading system. The formalin fixed paraffin embedded tissue sections were stained by haematoxylin and eosin. The sections were analysed and verified independently by two qualified pathologists, and the diagnosed cases were subjected to immunohistochemical analysis. Immunohistochemical staining of ER and PR and HER2 neu in the invasive malignant cells was done with microwave aided antigen retrieval and standard immunoperoxidase technique, as per the standard operating procedures.

The breast carcinoma cases were classified into 4 molecular subtypes according to ER, PR, and HER2/neu status: luminal A (ER and/or PR positive and HER2/neu negative), luminal B (ER and/or PR positive and HER2/neu positive), HER2-positive (ER and PR negative and HER2/neu positive), and triple negative (ER, PR, and HER2/neu negative).[4,5]

Statistical Analysis

Qualitative variables were presented as frequencies and percentages. The association between the different grades of the malignancies and molecular subtypes were analysed using Chi square test and p value calculated.

RESULTS

A total of 256 cases were studied, of which 190 cases were mastectomy specimen and 66 specimens, TruCut biopsies. Among the study group, 2 cases were males and the remaining 254 cases females. Maximum number of breast cancers came under the age group of 40-49 yrs. Carcinoma breast was more among post-menopausal women, n=155 (60.5%). Among the histological subtypes, 243 cases (94.9%) were invasive ductal carcinoma, No Special Type. When the cancers were graded according to Modified Scarf Bloom Richardson Grading System, majority belonged to grade 2 (n= 110, 42.9%). On immune profiling, ER was positive in n=94 (36.7%) of the cases, PR was positive in

n=80 (31.3%) cases and Her 2 neu in n=63 (24.6%) cases. Upon molecular subtyping, 41.4% (n=106) cases belonged to the basal like (triple negative) category, 31.6% (n=81) cases belong to luminal A type, 15.6% (n=40) belong to her 2 enriched category and 7.8% (n=20) cases were of luminal B type. Of these, 9 cases were considered inconclusive, because of the focal and incomplete staining pattern obtained in the sections.
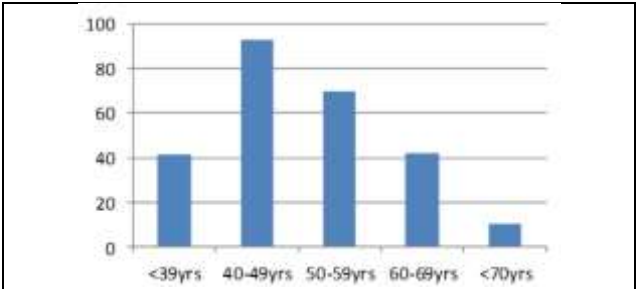
**Graph 1. Age Distribution of Confirmed Cases of Carcinoma Breast**
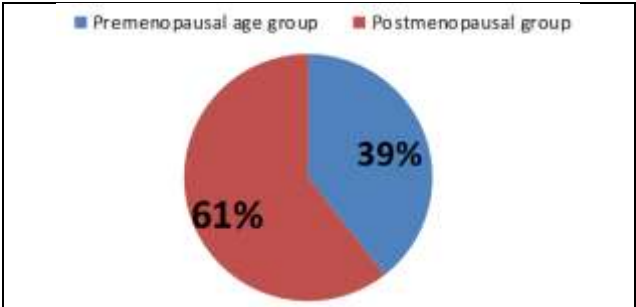
Graph 2. Distribution of Carcinoma Breast among Premenopausal & Post-Menopausal Women

Modified Scarff Bloom Richardson Grade	Number of Cases (Percentage)
Grade 1	94 (36.7%)
Grade 2	110 (42.9%)
Grade 3	52 (20.3%)

Table 1. Distribution of Cases in Percentages According to the Grades

Histological Subtype	Frequency (Total=256)
Invasive ductal carcinoma	243(94.9%)
Metaplastic carcinoma	3(1.1%)
Mucinous carcinoma	3(1.1%)
Intracystic papillary carcinoma	2(0.7%)
Lobular carcinoma	2(0.7%)
Medullary carcinoma	1(0.3%)
Apocrine carcinoma	1(0.3%)
Invasive papillary carcinoma	1(0.3%)

Table 2. Distribution of Histological Subtypes of Carcinoma Breast
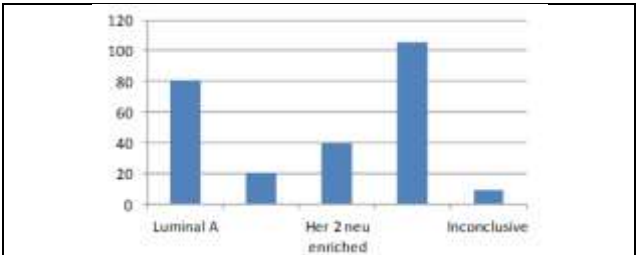
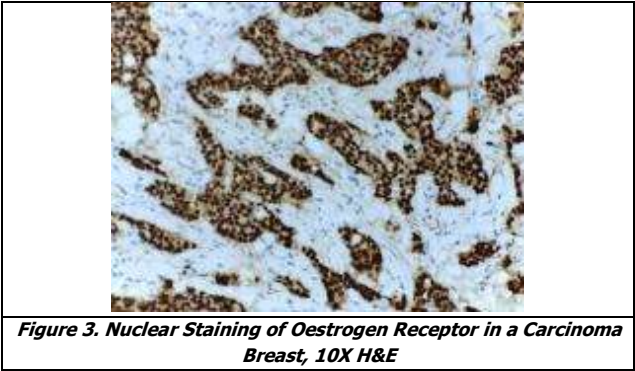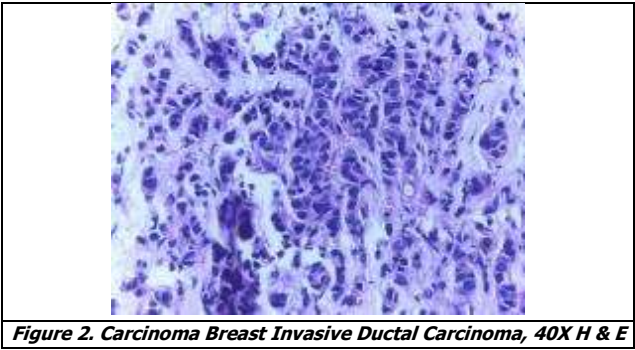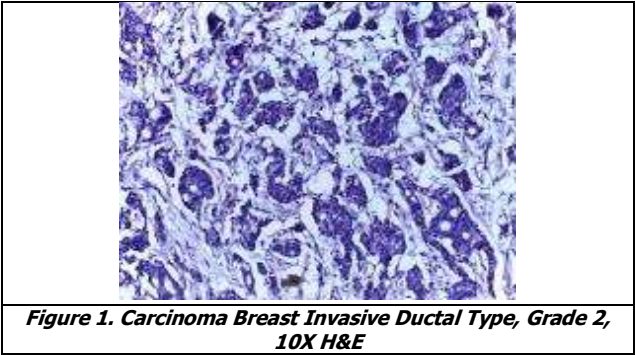**Graph 3. Distribution of Molecular Subtypes of Carcinoma Breast**

Characteristics	Luminal A	Luminal B	Her 2 Positive	Triple Negative	Inconclusive	Total
Total	81(31.6%)	20(7.8%)	40 (15.6%)	+106 (41.4%)	9	256
Histology	76(93.8%)	20(7.8%)	39(97.5%)	101(95.2%)	7	243(94.9%)
Ductal	5(6.1%)	0	1(2.5%)	5(4.7%)	2	13(5.1%)
Others Grade						
1	57(70.3%)	13(65%)	9(22.5%)	12(11.3%)	3	94(36.7%)
2	23(28.4%)	5(25%)	20(50%)	58(54.7%)	4	110(42.9%)
3	1(1.2%)	2(10%)	11(27.5%)	36(33.9%)	2	52(20.3%)

Table 3. The Distribution of Histopathological Characteristics according to Molecular Subtypes

Modified Bloom Richardson Grade	Luminal-A	Luminal-B	Her 2 Neu Positive	Triple Negative	Inconclusive
Grade 1	57	13	9	12	3
Grade 2	23	5	20	58	4
Grade 3	1	2	11	36	2

Table 4. ER, PR, Her2 Neu Expression with the Grades of Carcinoma Breast, as per Modified Bloom Richardson Grading System

Chi-square test done; p value= 0.001

Figure 1. Carcinoma Breast Invasive Ductal Type, Grade 2, 10X H&E

Figure 2. Carcinoma Breast Invasive Ductal Carcinoma, 40X H & E

Figure 3. Nuclear Staining of Oestrogen Receptor in a Carcinoma Breast, 10X H&E

Figure 4. Nuclear Staining of Progesterone Receptor in Ductal Carcinoma Breast, 10X H&E

Figure 5. Her2 Neu Staining of the Cytoplasm and Cytoplasmic Membrane, 40X H&E

The association between molecular subtypes and different grades of carcinoma breast was assessed using the chi-square test; and was found to be statistically, significant (p value=0.001)

DISCUSSION

Breast cancer is the most common cancer in female population worldwide and incidence has increased at the rate of 3-4% in developing countries which often gets diagnosed at late stage.[6] Most of our cases belonged to the age group 40-49 yrs. A similar study by Acharya et al observed the most common age group to be diagnosed with breast cancer was 41 to 55 yrs.[7] Bennis et al. Reported mean age of 45 years in a similar study of 366 cases in Morocco.[8] Akbar et al. found mean age of 47.55 years in a study of 60 cases in Pakistan.[9] The age group in our study corresponds to these studies. Of the 256 cases studied, 2 patients were males (0.7% cases). The result was in accordance to a study conducted by Rai et al who found 0.5% incidence of male breast cancer.[10] Among women, carcinoma was more among post-menopausal age group (60.5%). Of all the various subtypes, invasive ductal carcinoma came out as the most common variant. It accounted 243 cases (94.9%). A clinical database study conducted by Wang et al reported highest 90.1% of breast carcinoma was invasive ductal type similar to our study.[11] A study by Reddy et al observed 85.05% cases with duct cell carcinoma NOS type which correlates with our study.[12] Histological grading of breast carcinoma are based on Nottingham modification of the Scarf Bloom and Richardson's grading system. The extent of tubule formation, nuclear size and pleomorphism, and mitotic rate are the parameters measured. Each of the three elements was assigned with a score 1 to 3 and the final grade was identified from the sum of each individual scores.

Depending on the degree of differentiation, well differentiated (grade I) scores 3 to 5, moderately differentiated (grade II) scores 6 to 7 and poorly differentiated (grade III) scores 8 to 9.[13] In the present study, majority of the cases belonged to grade 2, moderately differentiated category (42.9%), followed by grade 1 and then grade 3. This was found correlating the results of the studies conducted by Acharya et al and Reddy et al.[7,12] On immuno profiling the, ER was positive in n=94 (36.7%) of the cases, PR was positive in n=80 (31.3%) cases and Her 2 neu in n=63 (24.6%) cases. The incidence of ER, PR expression in our study was comparable with other studies conducted in India and other Asian countries. In a study conducted by Gogoi et al, IHC evaluation of breast cancers showed ER+in 40.62%, PR+35.77%, Her2/Neu+18.69%.[14] Azizun-Nisa et al in a study done in Pakistan in 2008 also found similar results with only 32.7% ER and 25.3% PR positivity.[15]

This study classified tumours into molecular subtypes based on the protein expression pattern in IHC. 41.4% (n=106) cases belonged to the basal like (triple negative) category, 31.6% (n=81) cases belong to luminal A type, 15.6% (n=40) belong to her 2 enriched category and 7.8% (n=20) cases were of luminal B type. Of these, 9 cases were considered inconclusive, because of the focal and incomplete staining pattern obtained in the sections. Triple negative breast cancers refers to the immunophenotype that is immunologically negative to ER, PR, and Her2/Neu. Dent et al. found 11.2% and Rakha et al. found 16.3% of tumours were TNBC type.[16,17] The list comparing the TNBC data in Indian setting and Western setting significantly differ. All Indian studies showing a higher proportion of TNBC except one study.[18] Concurrent to our study findings, about half the cases (52.8%) in Tamimi et al's study were triple negative, with luminal tumours comprising only 28.5%.[19] In the present study, we found significant association between the grade of the cancer and the molecular phenotype. Most of the triple negative cancers belonged to the grade2 and 3 categories. These results are found similar in other literatures.[20,21] The role of mammography to detect the different molecular subtypes has been suggested in one study.[22] This showed that, HER2-positive tumours and triple negative tumours were less likely to be detected by mammography.

CONCLUSIONS
Being a heterogeneous disease, breast cancer exhibits various histopathological and biological features. Invasive ductal carcinoma NOS was the most common subtype in our study. According to the Modified Bloom Richardson Grading System, Grade 2 tumours were more common. Classification by immunohistochemistry revealed that, in our community, triple negative category was more common, followed by luminal A type. Upon statistical analysis, significant association between the grade of the cancer and the molecular phenotype, was found, as most of the triple negative tumours belonged to grade 2 and grade 3 categories.

Limitations
In our study, there was unavailability of Ki67, a cellular marker of proliferation that differentiates non-HER2 expressing luminal B from luminal A tumours.[23] We were also short of cytokeratin 5/6, which helps in detecting Basal-like tumours, a subset of triple negative tumours.[24] There is always a discrepancy rate of 39% between the two methods- molecular classification by immunohistochemistry, and by gene expression studies.

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