ORIGINAL RESEARCH PAPER
VOLUME-6 | ISSUE-7 | JULY-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CORRELATION OF Ki-67 EXPRESSION WITH MORPHOLOGICAL PROFILE AND HORMONE RECEPTOR STATUS IN BREAST CANCER

Pathology

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ABSTRACT

Introduction: Data on various receptor and histological in cases of breast carcinoma in Indian population is quite limited as receptor status is not routinely carried out for every patient. Therefore in present study to study all the cases of breast cancer with reference to biosocial profile along with relevant clinical details and receptor status was analyzed and it was correlated with morphological prognostic parameters.

Methods: Sample consisted of 105 specimens of breast carcinoma received in Department of Pathology, Hind Institute of Medical Sciences situated in Barabanki district of Uttar Pradesh. Northern India from year 2014 and 2016.

Results: About 57.1% of cases were either of 50 years or younger. Out of the 105 cases, 96 cases (91.00%) were invasive ductal carcinoma, followed by 4 cases (4%) of mucinous carcinoma. Histological grading showed majority of the cases to be grade II (42.8%). ER expression, PR expression positive, Her2/Neu positive were 33.3%, 32.4% and 29.5% respectively. About 49% cases were "triple" negative (estrogen receptor, progesterone receptor, HER/neu negative). Relationship of molecular classification with tumour size was significant. ($p<0.05$) The relation between molecular classification and histological grade was statistically significant ($p<0.05$).

Conclusion: Breast cancer in Indian population usually presents at younger age. The results of the study revealed about half proportion of triple-negative breast cancers. The association between tumor size and molecular classification was statistically significant. Diagnosing and treating the disease timely is increasing important so as to reduce mortality associated with it.

KEYWORDS:

Breast cancer, estrogen receptor, HER-2/neu, progesterone receptor

Introduction:

Breast carcinoma is the most common malignant tumour and the leading cause of cancer death in women, with more than ten lakhs cases are occurring worldwide annually. [1] Over 1,40,000 new breast cancer patients are estimated to be diagnosed annually in India with an age standardised incidence rate of 25.8 cases per 1,00,000 women per year, making it the most common cancer amongst Indian women. [2] Data from all urban and rural population-based cancer registries in India suggest a rising incidence of breast cancer in India. [3] Usually the three biomarkers, estrogen receptor (ER), progesterone receptor (PR), and HER-2/neu are routinely assessed and used to approximate the molecular category of breast cancer. Gene expression profiling has identified five major patterns of gene expression: Luminal A, Luminal B, Normal breast-like, Triple Negative, and HER2 positive. These molecular classes correlate with prognosis and response to therapy, and thus have taken on clinical importance. [4] Presence of both ER and PR is related to better prognosis and responsiveness to hormonal therapy. [5] A humanized monoclonal antibody, trastuzumab, targeting the HER-2/neu gene product is another example of targeted therapy in breast cancer. [6] Ki67 is an independent factor to predict tumour grade, thus the use of this proliferation marker in routine approach to patients with breast cancer is recommended. [7] Its prognostic value in breast cancer is still being explored under various circumstances to include it as a routine assessment for these further studies are needed. We have classified breast carcinoma based on the expression patterns of ER, PR, HER2/neu and correlated it with proliferative activity using Ki-67, histological grade, tumour size, lymph node status and patient age. Thereby, the data would help in planning the prognostic and therapeutic approach in patients with breast cancer. The purpose of the present work was to study all the cases of breast cancer with reference to biosocial profile and the relevant clinical details, to correlate the histopathology with the hormone receptor status and to determine the aggressive nature of tumour by studying ki-67 expression.

Material and Methods

Source of data: Cases of radical mastectomy received for routine Histopathological evaluation from the Department of Surgery, Hind Institute of Medical Sciences Barabanki, from May 2014 to June 2016 will form the source of data for the study.

Method of collection of data: The detailed clinical history and results of relevant investigations done were collected from the patients' case files. For study of prospective cases, the mastectomy and lymph node dissection specimen was received in the Pathology department in 10% formalin. In every case the standard protocol for surgical grossing of radical mastectomy specimens was followed. After a detailed specimen description, multiple sections were taken from the tumour, surgical margins, nipple and areola, non-neoplastic breast, and all the lymph nodes. After conventional processing, paraffin sections of 5µm thickness were stained by haematoxylin and eosin (H & E) for histopathological study. additional 4µm sections were taken from single paraffin block of tumour tissue on 4 glass slides coated with adhesive (polyLycine) for immunohistochemistry (IHC) to detect ER, PR, HER2/neu overexpression and Ki-67 proliferative index. For study of retrospective cases, the histopathology reports, slides and paraffin blocks were retrieved from the archives. Sections were taken from the paraffin blocks in a same manner.

The technique for IHC will include antigen retrieval in Tris buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibody (Biogenex), linking with rabbit anti mouse secondary antibody (Biogenex), enzyme labelling with streptavidin- horseradish peroxidase (Biogenex), developing chromogen with deaminobenzidine (DAB) and counterstaining with haematoxylin .

Positive and negative controls will be run with each batch of slides. The H & E stained slides were studied for the tumour histology, type, Scarff-Bloom-Richardson (SBR grade), lymph node metastasis etc.The immunostained slides was examined for nuclear staining in case of ER, PR and Ki-67, and membrane staining in case of HER2/neu. In each case, the proportion of positive staining tumour cells (expressed in percentage) and the average intensity of staining (expressed as 0, 1+, 2+ or 3+) will be evaluated. The relationship between various parameters such as age, menopausal status , duration of disease presentation, mammography findings, tumour size, tumour extent, histologic type, histologic grade, lymph node status, the expression of ER, PR, HER2/neu and Ki-67 index are studied.

Inclusion criteria: Radical mastectomy specimens from female patients of all ages, with breast carcinoma, are included in the study.

Exclusion criteria: Cases where only a trucut biopsy or limited surgery has been done , as in such cases all the parameters was not taken for assessment and cases where there is extensive tumour necrosis without sufficient viable tumours cells for accurate evaluation of the immuno-histochemical results.

Sample size determination: From the different studies done, expected proportion of the level of Ki-67 with relation to ER, PR and HER2/neu status, amongst the breast cancer cases has been assumed to be 60% with a relative precision of 15% and desired confidence level of 90%. The estimated number of cases to be included for the study works out to be 100-110 cases. Although generally the desired confidence level is taken as 95%, in view of constraints such as time period and cost of test etc, the desired confidence level in the present study has been kept as 90%. Nature of study will be a cross sectional study, both retrospective and prospective, taking the past 1 ½ year into consideration.

Statistical analysis: The data was analysed using SPSS Software Version 16 and the results were transferred to preformed tables in accordance to aims and objectives. Quantitative parameters such as age, size of tumour etc were summarised in terms of mean. Percentage expression for positivity of ER, PR, HER2/neu and Ki-67 has been estimated. The differences in the positivity for different independent variables were tested through independent t-test for quantitative variables and 'Chi' square test for qualitative variables.

Ethical clearance: Ethical clearance was obtained from institution and ethical certificate is enclosed.

Results:

Biosocial characteristics of the study population

In the present study, 105 cases of breast carcinoma were evaluated histopathologically and subsequently evaluated for IHC markers ER, PR, HER2/neu and Ki-67. The age of the patients ranged from 29 to 85yrs with mean age of 51.02yrs. Maximum number of cases was seen in the age group of 41-50yrs (30.50%) followed by 31-40yrs (24.80%). In the present study most of the patients were premenopausal (57 cases; 54.0%) and rest were postmenopausal women (48 cases; 46.0%).

Clinical and histopathological profile of breast cancer

Out of the 105 cases of breast cancer studied, right breast was more commonly involved comprising of 62 cases (59.00%). Left breast was involved in 43 cases (41.00%). On gross examination, the tumour size ranged from 1 to 10 cms . 72 cases (68.60%) measured between 2-5 cms followed by 19 cases (18.10%) <2 cms and 14 cases (13.30%) >5 cms. Out of the 105 cases, 96 cases (91.00%) were invasive ductal carcinoma, followed by 4 cases (4%) of mucinous carcinoma. four case papillary and one case of invasive lobular carcinoma were noted. Lymph vascular invasion was noted in 47 cases (44.8%) and absent in 58 cases (55.20%). Majority of the cases (59 cases; 56.20%) did not show metastasis in the lymph nodes. 46 cases (43.8%) that showed metastatic deposit, maximum number of metastatic lymph nodes of a single case was 27. Histological grading showed majority of the cases to be grade II (45 cases). Grade I was seen in 14 cases and Grade III in 43 cases.

Relationship of ER , PR and HER2/neu with biosocial and pathological profile

Positivity for ER, PR and HER2/neu each was more common in 41-50 yrs age group and negativity for the same was more common in same age group. Both ER/PR positivity and negativity were seen mainly in premenopausal women. HER2/neu positive cases were more common in postmenopausal while negative in pre-menopausal women. right breast predominated irrespective of expression of ER and PR. Irrespective of expression of ER, PR and HER2/neu, tumour size of majority of cases were between 2-5 cms. Most of the cases showing positivity or negativity for ER, PR and HER2/neu were of stage IIA. ER positive tumours were mainly of grade II (54.3%) and ER negative were high grade tumours (45.7%). This was statistically significant ($p<0.05$). PR positive and HER2/neu negative tumours were also of lower histological grade. Majority of the cases were positive for lymphovascular invasion and lymph node metastasis irrespective of ER, PR, HER2/neu expression. **[Table No. 1]**

Relationship of Molecular Classification with biosocial and pathological profile

Luminal A cases were mostly in 41-50 , Luminal B in 51-60 years age group (35.70%) and Triple Negative in 41-50 years age group (31.40%). HER2 Positive was mainly seen in younger age group of 31-40 years (27.8%) in whom poor prognosis was observed. Luminal A cases were more common in premenopausal women while Luminal B and HER2 Positive in postmenopausal women. Triple Negative cases were more in premenopausal women. The side of the breast involved in Luminal A, Triple negative and Her-2 positive was right while in Luminal B was left. Majority of cases of all subtype belong to 2-5 cms tumour size which was significant ($P<0.05$). Most of the cases in all subtypes were of stage IIA. Luminal A tumours were mainly of low and intermediate grade (77.3%) while other three molecular subtypes were mostly high grade tumours. This relation between molecular classification and histological grade was statistically significant ($p<0.05$). Majority of the cases were negative for lymph vascular invasion and for lymph node metastasis irrespective of molecular subtype. **[Table No.2]**

Relationship of Ki-67 index with biosocial and pathological profile

Majority of the cases were of low Ki-67 index (42 cases) followed by 41 cases of high proliferative index. Intermediate Ki-67 index of 16-30% nuclear positivity was seen in 22 cases . Mean age of cases with low proliferative index were higher at 51.52yrs compared to that of high proliferative index which was 47.29yrs. Most of the cases with low proliferative index were in 41-50 and 51-60 years age group and those with high proliferative index in 21-30 years and 41-50 years age group. Tumours in younger age group of 21-30 years showed high Ki-67 index and in elderly age group of >60 years showed low Ki-67 index. High Ki-67 index was more common in premenopausal women. Right breast predominated irrespective of Ki-67 index. Most of the cases were of stage IIA irrespective of the proliferative index assessed by Ki-67. Low Ki-67 tumours were mainly intermediate grade (47.6%) while high Ki-67 tumours were mostly high grade. Majority of the cases were positive for lymph vascular invasion irrespective of Ki-67 index. On the other hand, lymph node metastasis was mostly negative in cases with low proliferative index and positive in high proliferative index cases. **[Table No.3]**

Relationship of Ki-67 with different Marker and Molecular Classification

ER and PR positive cases were mainly of low Ki-67 index while ER and PR negative tumours were of high Ki-67 index. HER2/neu positive and negative cases were mostly of low Ki-67 index. Correlation of ER/PR status with Ki-67 was highly significant ($p<0.01$). All 22 cases of Luminal A subtype were of low Ki-67 index. while Triple Negative and HER2 Positive were of high proliferative index. Mean Ki-67 score of Luminal A was significantly lower at 4.58% while that of Luminal B was slightly higher at 23.33%. Highest mean Ki-67 score were of Triple Negative and HER2 Positive tumours at 35.50% and 35.56% respectively. Molecular classification correlation with Ki-67 was found to be statistically significant ($p<0.05$). **[Table No.4]**

Breast Carcinoma Cases according Immuno-histochemical Expression of Histological Subtypes

Out of the 96 cases of infiltrating ductal carcinoma, majority were ER negative (68.7%), PR negative (69.8%) and HER2/neu negative (68.7%). Most of the IDC cases were of Triple Negative subtype (48.5%) and of high proliferative index (41.7%). out of four mucinous carcinoma cases three were ER, PR positive and all were HER2/neu

negative three were luminal A and one was triple negative. Three have low ki-67 index and one have intermediate index. Out of four papillary carcinoma two were ER, PR positive and three were her2/neu negative two were luminal A and two were triple negative. **[Table No.5]**

Discussion:

Use of immunohistochemistry has now become an integral part of a complete and comprehensive histopathology report of breast carcinoma. In terms of prognosis and prediction of response to therapy, in addition to histological grade, tumour subtype and lymph node status, assessment of triple markers – ER, PR and HER2/neu, and proliferative activity based on Ki-67 score have become essential requirements for the oncologist. Recent attention has been directed at molecular classification of breast cancer based on triple markers which has prognostic and therapeutic implications. The present study includes 105 breast carcinoma cases which were classified into molecular subtypes based on the expression of ER, PR and HER2/neu and correlated with Ki-67 score. Relationship of expression of these markers with clinic pathological parameters was studied. This study highlights the importance of classification based on IHC and proliferative index based on Ki-67 to assess prognosis and therapeutic response. In the present study most of the cases were in 41-50yrs age group with a mean age of 51.02yrs. This correlated with study by Su et al and Dang et al. [8, 9] Leong et al noticed that in Asia, breast cancer incidence peaks among women in forties while in Western countries, it peaks in sixties due to multiple factors like environmental factors, socioeconomic status, inadequate implementation of screening programmes and lack of appropriate care. [3] Majority of the cases were involving Right breast (59.0%) and found in premenopausal women (55.93%), which was in concordance with most of the studies in India and abroad. [10,11,12,13] Majority of tumours were 2-5cms (68.60%) in size like in studies by Sofi et al and Verma et al. [13,14] and histological grade II (42.90 %) which correlated with studies by Nair et al and Kakarala et al. [12,15] Most common histological subtype was infiltrating ductal carcinoma and lymph node metastasis was absent in most cases. Similar findings were observed in almost all studies. [15, 16,17,18] In this study, majority of the cases were ER, PR and HER2/neu negative. ER and PR were negative in two other studies by Indian authors; [12, 18] whereas in most international studies hormonal receptors were usually positive. [6, 11, 16, 17, 19] This shows a different trend in Indian population where patients usually present with high tumour grade and advance stage with lymph node metastasis this suggesting a possibility of ethnic variation related to difference in gene expression. In terms of age group, ER/PR positivity was more common among elderly (>60yrs) compared to younger women (<30yrs) which correlated with study by Sofi et al. [13] Nishimiura et al and Inwald et al also found higher Ki-67 index in women of younger age group than elderly as seen in present study. [17, 19] In the present study, most of the cases belonged to Triple Negative subtype (40.00%) which correlated with study by Dang et al. [9] Luminal A was the most common molecular subtype in all other Indian and international studies. [5, 8, 14, 16, 19, 21] regional variation can be attributed to the increased incidence of triple negative cases. Subtypes with worse prognosis like Triple Negative and HER2 Positive were more common amongst younger age group while those with better prognosis, Luminal tumours were more common among elderly age group. Similar observation was made by Su et al, Nair et al and Dang et al. [8, 9, 12] ER expression in the present study was compared with study by Sofi et al. [13] ER negative cases were more common in premenopausal like in Dang et al while Sofi et al found more in postmenopausal women. [9,13] ER negative tumours were of higher grade in both the studies. Size of majority of cases was 2-5 cm. However lymph node metastasis was almost half in the present study unlike that by Sofi et al. [53] Although most studies also showed consistency with our study. [14, 15, 16, 17] PR negative tumours were common in premenopausal women and of higher grade which correlated with Sofi et al. [13] Tumour size was 2-5 cms in both studies while lymph node metastasis was more common in study by Sofi et al. HER2/neu expression was compared with Ivkovic – Kapicl et al. [6] Mean age was slightly more in HER2/neu negative cases in both the studies. HER2/neu positive cases in study by Ivkovic – Kapicl et al were of larger size, higher grade and showed lymph node metastasis, while in the present study tumours were mainly of same size range(2-5 cms) and lymph node metastasis were mostly absent as in HER2/neu negative cases. [6] However, HER2/neu positive tumours were of higher grade as in study by Ivkovic – Kapicl et al and majority of the cases with lymph node metastasis were HER2/neu negative in both the studies. [6] Molecular classification correlated with study by Onitilo et al. [20] Triple Negative and HER2 Positive tumours were seen in younger age, of larger size, higher grade and metastasis to lymph node compared to luminal tumours in both studies. Ki-67 expression was compared with study by Nishimura et al. [22] Age did not correlate, but high Ki-67 index was associated with higher grade and lymph node metastasis in both studies. Yang et al observed similar age and tumour size variation with Ki-67 as the present study. [11] ER positive, PR positive and HER2/neu negative tumours were of low proliferative index in both studies. Similar observation was made in most of the studies. [5,6,9,11,17] Molecular classification correlated with Ki-67 in both the studies, wherein Luminal tumours were of low proliferative index and Triple Negative and HER2 Positive tumours were of high proliferative index. This was in concordance with studies by Verma et al, Dang et al and Aleskandarany et al. [9, 14, 23] Mean Ki-67 score of various parameters like menopausal status, tumour size, histological grade, lymph node metastasis and immuno-histochemical expression of ER, PR and HER2/neu in the present study correlated well with Ki-67 score in study by Inwald et al. [17] Under molecular classification, Luminal A cases had the lowest mean Ki67 score while Triple Negative and HER2 Positive had the highest, which was in agreement with study by Nishimura et al. [22] Cheang et al suggested that the most appropriate Ki-67 index cut-off point to distinguish Luminal B from Luminal A tumours was 13.25%. [24] In the present study too, mean Ki-67 score of Luminal B tumours was above the cut-off at 31.50% while that of Luminal A was 7.77%.

Most common histologic subtype, infiltrating ductal carcinoma was found to be of Triple Negative molecular subtype in most cases. Though most studies showed Luminal A to be most common molecular subtype amongst IDC cases, Triple Negative was the second most common. [8, 20, 21, 25] Among lobular carcinoma cases in majority of studies, Luminal A was the most common subtype. However in the present study, the single case of lobular carcinoma was Triple Negative which was observed in a small percentage of cases in these studies. [8, 20, 21, 25] Single case was not representative of the whole population leading to discrepancy. Out of four cases of mucinous carcinoma three were of luminal A subtype which correlated with Yang et al, Onitilo et al , Su et al and Engstrom et al . [8, 20, 21, 25] 50% of papillary carcinoma case was of Triple Negative type like in study by Su et al and Engstrom et al. [8, 21] Molecular classification correlation with age, histological grade and Ki67 was statistically significant ($p<0.05$). ER/PR also correlated with histological grade and Ki67 ($p<0.01$). Statistically significant correlation was also found for these parameters in other Indian studies; [5, 9, 14] and few international ones. [7, 22, 20] This implies that higher grade and high proliferative index tumours were usually ER/PR negative and of Triple Negative or HER2/neu molecular subtype.

Conclusion:

Breast cancer is a global disease with rising incidence in Indian women. As advances have been made in breast cancer diagnosis and treatment, more attention has been directed to markers of increased risk for developing the disease, which are hormone receptors, HER2/neu expression and proliferative index using Ki-67. Molecular classification based on triple markers into Luminal A, Luminal B, Triple Negative and HER2 positive, is considered a better predictive factor for prognosis and treatment than routine histopathology. In the present study of 105 breast carcinoma cases, most of the cases were of Triple Negative subtype. Younger patients had a higher frequency of Triple Negative and HER2 positive tumours while older patients had a higher rate of Luminal tumours. Assessment of proliferative activity using Ki-67 revealed low proliferative index in most cases. However higher Ki-67 index was associated with younger age, higher grade tumours, lymph node metastasis, negative ER/PR and, Triple Negative and HER2 positive subtypes. There was significant statistical correlation between age, molecular classification, ER/PR status and Ki-67. Correlation of histological grade with molecular classification and hormonal status was also statistically significant. A greater understanding of the molecular classification of tumours based on triple markers helps in development of targeted therapies that lead to increased efficacy, decreased toxicities, and better selection of patients who will benefit from treatment. Ki-67 being an independent prognostic factor should be evaluated routinely in breast carcinoma cases. It also plays a role in making treatment decisions and to monitor response to treatment.

Table No. 1: Relationship of ER , PR and HER2/neu with different parameters

Age in years-range (mean)	Clinico- pathological parameters					
	ER		PR		HER2/neu	
	Positive (35)	Negative (70)	Positive (34)	Negative (71)	Positive (31)	Negative (74)
20-30	1	1	1	1	1	1
	2.9	1.4	2.9	1.4	3.2%	1.4%
31-40	6	20	6	20	7	19
	17.1	28.6	17.6	28.2%	22.6%	25.7%
41-50	11	21	10	22	6	26
	31.4%	30.0%	29.4%	31.0%	19.4%	35.1%
51-60	9	13	9	13	10	12
	25.7%	18.6%	26.5%	18.3%	32.3%	16.2%
61-70	5	11	5	11	4	12
	14.3%	15.7%	14.7%	15.5%	12.9%	16.2%
71-80	2	3	2	3	2	3
	5.7%	4.3%	5.9%	4.2%	6.5%	4.1%
>80	1	1	1	1	1	1
	2.9%	1.4%	2.9%	1.4%	3.2%	1.4%
Menopausal status						
Pre	18	39	17	40	13	44
	51.4%	55.7%	50.0%	56.3%	41.9%	59.5%
Post	17	31	17	31	18	30
	48.6%	44.3%	50.0%	43.7%	58.1%	40.5%
Side affected						
Right	21	41	20	42	16	46
	60.00%	58.6%	58.8%	59.2%	51.6%	62.2%
Left	14	29	14	29	15	28
	40.00%	41.4%	41.2%	40.8%	48.4%	37.8%
Tumour size (cms)						
<2	5	14	5	14	5	14
	14.3%	20.0%	14.7%	19.7%	16.1%	18.9%
2-5	24	48	23	49	18	54
	68.6%	68.6%	67.6%	69.0%	58.1%	73.0%
>5	6	8	6	8	8	6
	17.1%	11.4%	17.6%	11.3%	25.8%	8.1%
TNM stage						
0	0	0	0	0	0	0
	0	0	0	0	0	0
I.	7	8	6	9	2	13
	20%	11.4%	17.6%	12.7%	6.5%	17.6%
IIA	18	32	18	32	13	37
	51.5%	45.7%	52.9%	45.1%	41.9%	62.1%
IIB	3	10	3	10	3	10
	8.6%	14.3%	8.8%	14.1%	9.7%	13.5%
IIIA	6	14	6	14	8	12
	17.1%	20.0%	17.6%	19.7%	25.8%	16.2%
IIIB	0	0	0	0	0	0
	0	0	0	0	0	0
IIIC	1	6	1	6	5	2
	2.9%	8.6%	2.9%	8.5%	16.1%	2.7%
IV	0	0	0	0	0	0
	0	0	0	0	0	0
Histological grade						
I	5	12	4	13	4	13
	14.3%	17.1%	11.8%	18.3%	12.9%	17.6%
II	19	26	19	26	13	32
	54.3%	37.1%	55.9%	36.6%	41.9%	43.2%
III	11	32	11	32	14	29
	31.4%	45.7%	32.4%	45.1%	45.2%	39.2%
Lymphovascular invasion						
Positive	15	32	15	32	12	33
	42.9%	45.7%	41.2%	46.5%	38.7%	47.3%
Negative	20	38	19	39	19	39
	57.1%	54.3%	55.9%	54.9%	61..3%	52..7%
Lymph node metastasis						
Positive	11	35	11	35	15	31
	31.4%	50.0%	32.4%	49.3%	48.4%	41.9%
Negative	24	35	23	36	16	43
	68.8%	50.0%	67.6%	50.7%	51.6%	58.1%

Table No. 2: Relationship of Molecular Classification with different parameters

Clinico-pathologic Parameter	Luminal A (n= 22)	Luminal B (n=14)	Triple Negative (n= 51)	HER2 Positive (n=18)
Age in yrs	50.48	54.21	49.13	50.54
21-30	0	1	1	0
	0.0%	7.1%	2.0%	0.0%
31-40	4	2	15	5
	18.2%	14.3%	29.4%	27.8%
41-50	9	3	16	4
	40.9%	21.4%	31.4%	22.2%
51-60	4	5	8	5
	18.2%	35.7%	15.7%	27.8%
61-70	4	1	8	3
	18.2%	7.1%	15.7%	16.7%
71-80	1	1	2	1
	4.5%	7.1%	3.9%	5.6%
>80	0	1	1	0
	0.0%	7.1%	2.0%	0.0%
Menopausal status				
Pre	13	5	31	8
	59.1%	37.5%	60.8%	44.4%
Post	9	9	20	10
	40.9%	64.3%	39.2%	55.6%
Side affected				
Right	15	6	31	10
	68.2%	42.9%	60.8%	55.6%
Left	7	8	20	8
	31.8%	57.1%	39.2%	44.4%
Tumour size				
<2	4	1	10	4
	18.2%	7.1%	19.6%	22.2%
2-5	16	9	37	10
	72.7%	64.3%	72.5%	55.6%
>5	2	4	4	4
	9.1%	28.6%	7.8%	22.2%
TNM Stage				
0	0	0	0	0
	0.0%	0.0%	0.0%	0.0%
I	6	2	6	1
	27.3%	14.3%	11.8%	5.6%
IIA	12	6	25	7
	54.5%	42.9%	49.0%	38.9%
IIB	3	0	7	3
	13.6%	0.0%	13.7%	16.7%
IIIA	1	5	11	3
	4.5%	35.7%	21.6%	16.7%
IIIB	0	0	0	0
	0.0%	0.0%	0.0%	0.0%
IIIC	0	1	2	4
	0.00%	7.1%	3.9%	22.2%
IV	0	0	0	0
	0.0%	0.0%	0.0%	0.0%
Grade				
I	3	2	10	2
	13.6%	14.3%	19.6%	11.1%
II	14	5	18	8
	63.6%	35.7%	35.3%	44.4%
III	5	7	23	8
	22.7%	50.0%	45.1%	44.4%
Lymphovascular invasion				
Positive	9	7	25	6
	40.9%	50.0%	49.0%	33.3%
Negative	13	7	26	12
	59.1%	50.0%	51.0%	66.7%
Lymph node Metastasis				
Positive	5	6	26	9
	22.7%	42.9%	51.0%	50.0%
Negative	17	8	25	9
	77.3%	57.1%	49.0%	50.0%

Table No. 3: Relationship of Ki-67with different parameters

Clinico-pathologic Parameter	Ki-67		
	<15% (n=42)	16-30% (n=22)	>30% (n=41)
21-30	1	0	1
	2.4%	0.0%	2.4%
31-40	8	2	16
	19.0%	9.1%	39.0%
41-50	14	8	10
	33.3%	36.4%	24.4%
51-60	9	5	8
	21.4%	22.7%	19.5%
61-70	8	3	5
	19.0%	13.6%	12.2%
71-80	1	4	0
	2.4%	18.2%	0.0%
>80	1	0	1
	2.4%	0.0%	2.4%
Menopausal status			
Pre	22	19	26
	52.4%	40.9%	63.4%
Post	20	13	15
	47.6%	59.1%	36.6%
Side affected			
Right	25	13	24
	59.5%	59.1%	58.5%
Left	17	9	17
	40.5%	40.9%	41.5%
Size (cms)	0-2	2-5	>5
<2	7	5	7
	16.7%	22.7%	17.1%
2-5	27	17	28
	64.3%	77.3%	68.3%
>5	8	0	6
	19.0%	0.0%	14.6%
TNM Stage			
0	0	0	0
	0.0%	0.0%	0.0%
I	10	2	3
	23.8%	9.0%	7.3%
IIA	18	14	18
	42.9%	63.6%	43.9%
IIB	4	2	7
	9.5%	9.1%	17.1%
IIIA	10	2	8
	23.8%	9.1%	19.5%
IIIB	0	0	0
	0.0%	0.0%	0.0%
IIIC	0	2	5
	0.0%	9.1%	12.2%
IV	0	0	0
	0.0%	0.0%	0.0%
Grade			
I	9	5	3
	21.4%	22.7%	7.3%
II	20	9	16
	47.6%	40.9%	39.0%
III	13	8	22
	31.0%	36.4%	53.7%
Lymphovascular invasion			
Positive	19	10	18
	45.2%	45.5%	43.9%
Negative	23	12	23
	54.8%	54.5%	56.1%
Lymph node Metastasis			
Positive	14	9	23
	33.3%	40.9%	56.1%
Negative	28	13	18
	66.7%	59.1%	43.9%

Table No. 4: Relationship of Ki-67 with different Markers and Molecular Classification

Parameters	Ki-67			Mean Ki-67 Score	Total n=105	χ^2	p value
	<15% (n=42)	16-30% (n=22)	>30% (n=41)				
Marker							
ER Positive	26 (61.9%)	3 (13.6%)	6 (14.6%)	15.48	35	25.7	<0.05
ER Negative	16 (38.1%)	19 (86.4%)	35 (85.4%)	33.88	70		
PR Positive	25 (59.5%)	3 (13.6%)	6 (14.6%)	15.87	34	25.5	<0.05
PR Negative	17 (40.5%)	19 (86.4%)	35 (85.4%)	33.43	71		
HER2/neu Positive	7 (16.7%)	6 (27.3%)	18 (43.9%)	34.38	31	7.4	<0.05
Molecular Classification							
Luminal A	22 (100.0%)	0 (0.0%)	0 (0.0%)	7.77	22	45.5	<0.05
Luminal B	4 (28.5%)	3 (21.4%)	7 (50.0%)	31.50	14		
Triple Negative	13 (25.4%)	16 (31.3%)	22 (43.1%)	31.50	51		
HER2 Positive	3 (16.6%)	3 (16.6%)	12 (66.6%)	38.61	18		
HER2/neu Negative	35 (83.3%)	16 (72.7%)	23 (56.1%)	24.97	74		

Table No. 5: Distribution of Breast Carcinoma Cases according Immunohistochemical Expression of Histological Subtypes

	IDC (n=96)	Mucinous (n=4)	Papillary (n=4)	Lobular (n=1)
ER Positive	30 (31.3%)	3 (75.0%)	2 (50.0%)	0 (0.0%)
ER Negative	66 (68.7%)	1 (25.0%)	2 (50.0%)	1 (100.0%)
PR Positive	29 (30.2%)	3 (75.0%)	2 (50.0%)	0 (0.0%)
PR Negative	67 (69.8%)	1 (25.0%)	2 (50.0%)	1 (100.0%)
HER2/neu Positive	30 (31.3%)	0 (0.0%)	1 (25.0%)	0 (0.0%)
HER2/neu Negative	66 (68.7%)	4 (100.0%)	3 (75.0%)	1 (100.0%)
Molecular subtype				
Luminal A	18 (18.6%)	3 (75.0%)	2 (50.0%)	0 (0.0%)
Luminal B	14 (14.4%)	0 (0.0%)	0 (0.0%)	(0.0%)
Triple –ve	47 (48.5%)	1 (25.0%)	2 (50.0%)	1 (100.0%)
HER2 +ve	18 (18.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ki-67				
<15%	36 (37.5%)	3 (75.0%)	2 (50.0%)	1 (100%)
15-30%	20 (20.8%)	1 (25.0%)	1 (25.0%)	0 (0.0%)
>30%	40 (41.7%)	0 (0.0%)	1 (25.0%)	0 (0.0%)

References:

1. Rosai J. Breast. In: Rosai J. Rosai and Ackerman's Surgical Pathology. 10th edition. St. Louis: Mosby; 2011: 1659-1770.
2. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. 2013[cited 2014 September 11]; Available from: http://globocan.iarc.fr
3. Leong SPL, Shen Z, Liu T, Agarwal G, Tajima T, Paik N, et al. Is breast cancer the same disease in Asian and Western countries?. World J Surg. 2010; 34: 2308–2324.
4. Lester SC. The Breast. In: Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia: Saunders; 2010: 1065-97.
5. Singhai R, Patil VW, Patil AV. Status of HER-2/neu receptors and Ki-67 in breast cancer of Indian women. Int J App Basic Med Res. 2011; 1(1): 15-19.
6. Ivkovic-Kapicl T, Knezevic-Usaj S, Djilas-Ivanovic D, Panjkovic M. Correlation of HER-2/neu protein overexpression with other prognostic and predictive factors in invasive ductal breast cancer. Anticancer Research. 2007; 21: 673-678.
7. Ensani F, Hajsadeghi N, Amoozegar-Hashemi F and Haddad P. Evaluation of the effects of biological prognostic and predictive factors on survival of breast cancer patients. Acta Medica Iranica. 2007; 45(2): 95-100.
8. Su Y, Zheng Y, Zheng W, Gu K, Chen Z, Li G et al. Distinct distribution and prognostic significance of molecular subtypes of breast cancer in Chinese women: a population-based cohort study. BMC Cancer. 2011; 11:292-302.
9. Dang M, Mysorekar V. Correlation of the expression of estrogen receptor, progesterone receptor, HER2/neu and Ki-67 with clinical features and tumour histopathology in breast carcinoma. RGUHS dissertation. 2012.
10. Naeem M, Khan N, Aman Z, Nasir A, Samad A, Khattak A. Pattern of breast cancer: experience at lady reading hospital, Peshawar. J Ayub Med Coll Abbottabad. 2008;20(4): 22-25.
11. Yang X, Wang F, Chen C, Peng C, Zhang J, Li Y. High Ki-67 Expression is a Poor Prognostic Indicator of 5-Year Survival in Patients with Invasive Breast Cancer. Asian Pacific J Cancer Prev.2011; 12: 3101-3104.
12. Nair GL, Tewari V. Prognostication and correlation between histopathology and IHC of breast cancer. RGUHS Dissertation. 2011.
13. Sofi GN, Sofi JN, Nadeem R, Shiekh RY, Khan FA, Sofi AA et al. Estrogen Receptor and Progesterone Receptor Status in Breast Cancer in Relation to Age, Histological Grade, Size of Lesion and Lymph Node Involvement. Asian Pacific J Cancer Prev.2012; 13 (10): 5047-5052.

14. Verma S, Bal A, Joshi K, Arora S and Singh G. Immunohistochemical characterization of molecular subtypes of invasive breast cancer: a study from North India. Acta Pathologica, Microbiologica Et Immunologica Scandinavica. 2012 ;120(12):1008-19.
15. Kakarala M, Rozek L, Cote M, Liyanage S and Brenner DE. RBreastrt icancer histology and receptor status characterization in Asian Indian and Pakistani women in the U.S. - a SEER analysis. BMC Cancer. 2010; 10:191-198.
16. Yamamoto-Ibusuki M, Yamamoto Y, Yamamoto S, Fujiwara S, Fu P, Honda Y et al. Comparison of prognostic values between combined immunohistochemical score of estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, Ki-67 and the corresponding gene expression score in breast cancer. Modern Pathology. 2013; 26: 79–86.
17. Inwald EC, Klinkhammer-Schalke M, Hofstädter F, Zeman F, Koller M, Gerstenhauer M, Ortmann O. Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat. 2013; 139: 539–552.
18. Kuraparthy S, Reddy KM, Yadagiri LA, Yutla M, Venkata PB, Kadainti SVS and Reddy RPV. Epidemiology and patterns of care for invasive breast carcinoma at a community hospital in Southern India. World Journal of Surgical Oncology. 2007; 5:56-62.
19. Nishimura R, Osako T, Okumura Y, Hayashi M, Toyozumi Y and Arima N. Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer. Experimental and Therapeutic Medicine. 2010; 1: 747-754.
20. Onitilo AA, Engel JM, Greenlee RT and Mukesh BN. Breast Cancer Subtypes based on ER/PR and Her2 Expression: Comparison of Clinicopathologic Features and Survival. Clinical Medicine & Research. 2009; 7(1/2): 4-13.
21. Engstrøm MJ, Opdahl S, Hagen AI, Romundstad PR, Akslen LA, Haugen OA et al. Molecular subtypes, histopathological grade and survival in a historic cohort of breast cancer patients. Breast Cancer Res Treat. 2013; 140:463–473.
22. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol. 2010;28:2784–2795.
23. Aleskandarany MA, Green AR, Benhasouna AA, Barros FF, Neal K, Reis-Filho JS et al. Prognostic value of proliferation assay in the luminal, HER2-positive, and triple-negative biologic classes of breast cancer. Breast Cancer Research. 2012; 14:R3-R13.
24. Cheang MCU, Chia SK, Voduc D, Gao D, Leung S, Snider J et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009; 101: 736-750.
25. Yang XR, Sherman ME, Rimm DL, Lissowska J, Brinton LA, Peplonska B et al. Differences in Risk Factors for Breast Cancer Molecular Subtypes in a Population-Based Study. Cancer Epidemiol Biomarkers Prev. 2007;16:439-443.