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Original Research Article

Hormonal Status and Molecular Classification in Breast Carcinoma Cases and Their Correlation with Clinical Parameters

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Abstract:

Background: The diagnosis of breast disease is largely based on triple assessment which includes clinical examination, radiology and tissue biopsy. The presence or absence of cancer in a suspicious clinically or mammographically detected abnormality can only be reliably diagnosed by tissue biopsy. This study evaluated the hormonal status and molecular classification of breast carcinoma cases, and its correlation with clinical parameters.

Materials and Methods: This prospective study was carried out in the department of Pathology, which included all the modified radical mastectomy (MRM)/lumpectomy/biopsy (BCS) specimens which were received in Pathology department during study period, with clinico-radiological suspicion of breast cancer. All the Hematoxylin and Eosin (H&E) stained slides and block were retrieved from the records and reviewed to study the histological features. Histopathological categorization of breast carcinoma was done under CAP protocol. Size of tumour, histological type, and tumour grading under the Nottingham modification of the Bloom–Richardson system were recorded.

Results: Majority of females were above 40 years of age, and 85.4% of them presented with advanced disease (Tumor size >2cm). Maximum cases 71.56% belonged to Nottinghams grade II and III. Association between large tumor size and poor prognosis was statistically significant. Her2 enriched and triple negative breast cancer were most common. TNBC category had higher percentage of grade II and grade III tumors (77.27%).

Conclusion: A large proportion of population were TNBC in our study, with higher grade (Grade II+III). Proportion of patients aged below 50 years with poor survival was higher in TNBCs as compared to Non-TNBC breast cancers.

Keywords: Breast cancer, Molecular classification, Immunohistochemistry.

Introduction

Carcinoma breast is emerging as one of the leading causes of cancer related deaths not only world -wide but also in India. This is supported by the recent ICMR data which states breast cancer contribution to total cancer burden in India accounting for 27.3% outnumbering cancer cervix and ovary which accounts for 14% of the total cases. [1] Although the incidence rate is higher in the west, the disability adjusted life years (DALYS) show highest burden for breast cancer in middle income countries including India where there are increasing incidence rates and a higher proportion with late stage of disease at diagnosis. [2] Even more worrisome in the present scenario is the shift in breast cancer with new victims being younger females, with an unfavourable morphological and hormonal profile. The diagnosis of breast disease is largely based on triple assessment which includes clinical examination, radiology and tissue biopsy. The presence or absence of cancer in a suspicious clinically or mammographically detected abnormality can only be reliably diagnosed by tissue biopsy. Available biopsy techniques include Fine Needle Aspiration biopsy (FNAB), Core Needle

Biopsy (CNB) open and excision biopsy. [3] After the worldwide establishment of anti ER/PR/HER2 neu therapy for carcinoma breast there has been a constant hunt for novel therapeutic options which would address the above-mentioned high grade and hormone non responding group i.e. triple negative breast cancer (TNBC). The present study was designed to evaluate the hormonal status and molecular classification based on immune-histochemical expression of ER/ PR/ Her2nu/ CK5/6 and Ki67 in breast carcinoma cases, and to correlate them with clinical parameters.

Materials and Methods

This prospective study was carried out in the department of Pathology for a period of two year, after obtaining approval from institutional ethical committee. Study included all the modified radical mastectomy (MRM)/lumpectomy/biopsy (BCS) specimens which were received in Pathology department during study period, with clinico-radiological suspicion of breast cancer. Patient characteristics including age, presenting complaint, past medical and family histories were noted from the clinical datasheet. In case of MRM/lumpectomy/BCS, a previous record of neo-adjuvant chemotherapy was taken. Follow up data of the patients was retrieved by telephone calls to the patients and the current status of the patient was noted (Alive, recurrence or Demised with the time of death). Total 207 specimens with suspected breast carcinoma were received during study period, of which 145 cases were included in the study fulfilling the inclusion and exclusion criteria. (Figure 1)

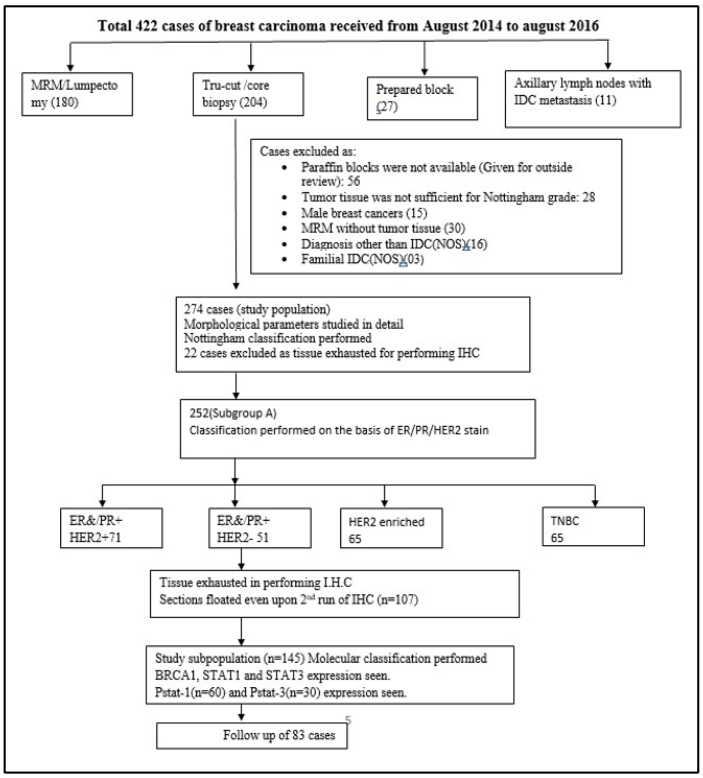
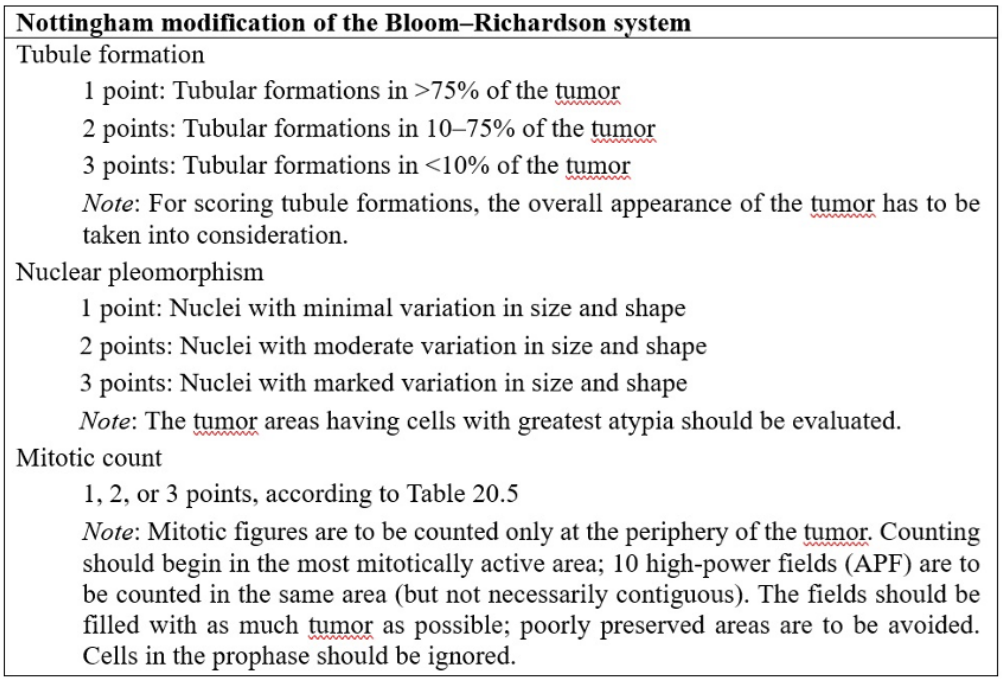


Figure 1: Study Flowchart

All the Hematoxylin and Eosin (H&E) stained slides and block were retrieved from the records and reviewed to study the histological features.3-4 micrometer thick sections were taken from each block for Immunohistochemical staining for markers like Estrogen receptor (ER), Progesterone receptor (PR), Her2 neu receptor (HER2), Cytokeratin CK5/6, and Ki67. Both positive and negative tissue control and reagent control were performed with the immunohistochemistry panel. Histopathological categorization of breast carcinoma was done under CAP protocol which included the diagnostic information such as - specimen identification procedure, laterality, lymph node sampling, site and size of the tumour, histological type, and tumour grading under the Nottingham modification of the Bloom–Richardson system (Figure 2).



Nottingham modification of the Bloom–Richardson system

Tubule formation

1 point: Tubular formations in >75% of the tumor

2 points: Tubular formations in 10–75% of the tumor

3 points: Tubular formations in <10% of the tumor

Note: For scoring tubule formations, the overall appearance of the tumor has to be taken into consideration.

Nuclear pleomorphism

1 point: Nuclei with minimal variation in size and shape

2 points: Nuclei with moderate variation in size and shape

3 points: Nuclei with marked variation in size and shape

Note: The tumor areas having cells with greatest atypia should be evaluated.

Mitotic count

1, 2, or 3 points, according to Table 20.5

Note: Mitotic figures are to be counted only at the periphery of the tumor. Counting should begin in the most mitotically active area; 10 high-power fields (APF) are to be counted in the same area (but not necessarily contiguous). The fields should be filled with as much tumor as possible; poorly preserved areas are to be avoided. Cells in the prophase should be ignored.

Figure 2: Nottingham modification of the Bloom–Richardson system

Statistical Analysis

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. Chi square test, ANOVA, and Student t test were used. p-value of <0.05 was considered significant.

Results

The mean age of study patients was 48.40±11.35 years, with age ranging from 27 to 90 years. Most common age group was 41-50 years (46.67%), followed by 31-40 years (21.67%). Left breast (50.677%) was more commonly affected than Right breast (49.33%). Tumour tissue specimens of only 252 cases were sufficient for IHC and Nottingham's classification could be performed only on these cases. Out of 252 cases, Nottingham Grade I was observed in 68 patients, Grade II was observed in 162 cases, and Grade III was observed in 22 cases. Hormonal status of 20.24% cases was ER/PR+ HER-, of 28.17% cases was ER/PR+ HER+, of 25.79% cases was HER2 enriched and rest of the 25.79% was TNBC. (Table 1)

Table 1: Patient Characteristics

Parameters	Number	Percentage
Age (years) (n=300)		
<30	15	5.00
31-40	65	21.67
41-50	140	46.67
51-60	60	20.00
>60	20	6.67
Laterality (n=300)		
Left	152	50.67
Right	148	49.33
Nottingham's Grade (n=252)		
Grade I (Score 3-5)	68	26.98
Grade II (Score 6-7)	162	64.29
Grade III (Score 8-9)	22	8.73
Hormone Profile of Study Population (n=252)		
ER/PR+ HER-	51	20.24
ER/PR+ HER+	71	28.17
HER2 enriched	65	25.79
TNBC	65	25.79

Tumour size of only 102 specimens could be detected. Size of largest dimension of tumour in majority of the cases was >5 cm (78.43%). (Table 2)

Table 2: Size of Largest dimension of Tumour (n=102)

Size	Number	Percentage
<2 cm	6	5.88
2-5 cm	16	15.69
>5 cm	80	78.43
Total	102	100.00

Outcome was available for 83 cases. Approximately three-quarter of the cases were alive (75.90%). Only 1 case had reported recurrence. Duration of follow-up ranged from 5-26 months, median duration was 10 months, and mean duration was 10.38±7.19 months. (Figure 3)

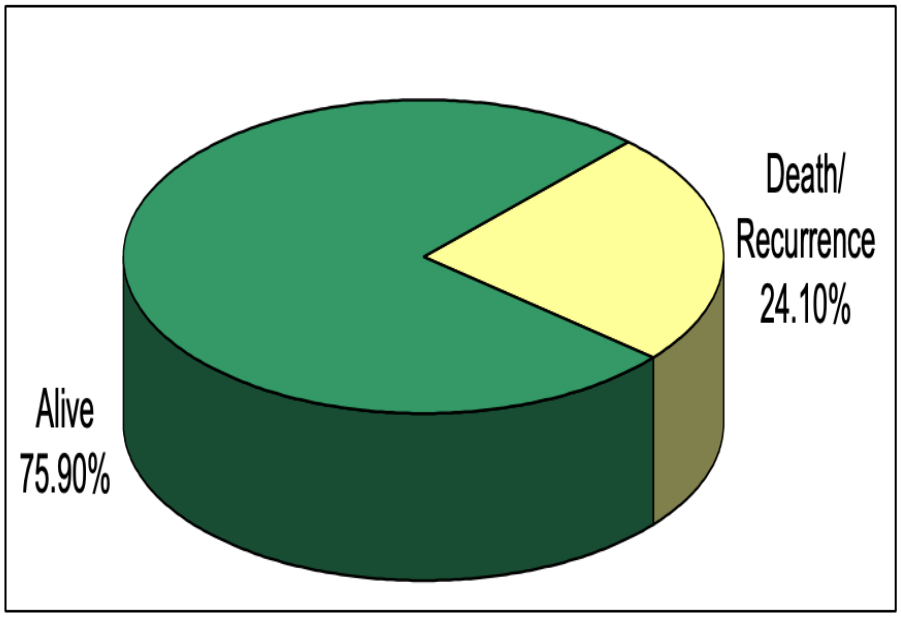


Figure 3: Outcome in Study Patients

Proportion of alive cases was higher in Grade I (81.82%) as compared to Grade II+III (73.77%) cases; however no statistically significant difference was observed (p=0.517). Mean tumour size in cases of death/recurrence (280.38±285.05 cc) was found to be statistically significantly higher (p<0.001) than that of Alive cases (55.73±103.50 cc). (Table 3)

Table 3: Association of Outcome with Nottingham's Grade and Tumor size

Association of Outcome with Nottingham's Grade (n=83)					
Outcome	**Number**	**Grade I (n=22)**		**Grade II+III (n=61)**	
		No.	**%**	**No.**	**%**
Alive	63	18	81.82	45	73.77
Death/Recurrence	20	4	18.18	16	26.23
Association of Outcome with Tumor size (n=62)					
	Number	**Min.**	**Max.**	**Mean**	**SD**
Alive	45	1	512	55.73	103.50
Death/Recurrence	17	9	864	280.38	285.05
Total	62	1	864	117.33	198.11

Ki67 is a marker of proliferation. An increase in Ki67 protein levels was observed with increase in Mitosis score and Nottingham grade. Difference in Ki67 protein levels in cases with different mitosis score was found to be statistically significant (p<0.001), however, it was not significant with Nottingham grade (p=0.078). Ki67 levels in cases with Grade I was minimum followed by Grade II and maximum in cases with Grade III. (Table 4) Ki67 levels of 2 cases was inconclusive.

Table 4: Association of Ki67 with Mitosis Score and Nottingham Grade

Parameters	No. of cases	Min.	Max.	Mean	SD
Mitosis score					
Score 1	58	1	80	14.90	17.67
Score 2	63	1	80	16.95	16.08
Score 3	22	1	90	35.23	28.00
Nottingham Grade					
Grade I	43	1	80	17.12	18.58
Grade II	79	1	90	17.49	20.73
Grade III	21	1	80	28.05	19.07
Total	143	1	90	18.93	20.10

Association of molecular class with Nottinngham grade and outcome was not found to be statistically significant (p>0.05). (Table 5)

Table 5: Association of Molecular Classification with Nottinngham grade and Outcome

Molecular Classification						
Nottingham's Grade		**L/A**	**L/B**	**Her2**	**TNNBL**	**Basal**
Grade I (n=45)	Number	12	12	15	5	7
	%	38.7	38.7	40.5	16.6	14.2
Grade II (n=100)	Number	19	28	22	25	6
	%	61.2	70	59.4	83.3	85.7
Total		31	40	37	30	7
Outcome						
Alive (n=63)	Number	12	24	13	13	1
	%	75	92.3	68.4	68.4	33.3
Death/Recurrence (n=20)	Number	4	2	6	6	2
	%	25	7.6	31.2	31.2	66.6
Total		16	26	19	19	3

Out of 145 cases, 37 (25.52%) were TNBC and rest 108 were non-TNBC. Though proportion of TNBC cases was higher in age group ≤40 years (26.83%) as compared to >40 years (25.00%), but this difference was not statistically significant (p>0.05). Proportion of TNBC cases was found to be statistically significantly higher (p=0.024) in Nottingham Grade II+II (31.00%) as compared to Grade I (13.33%). Though not statistically significant, poorer outcome was observed in TNBCs [death/recurrence (36.4%)] as compared to outcome of non TNBC cases (19.6%). (Table 6)

Table 6: Comparison of study parameters between TNBC and Non-TNBC groups

Parameters		**TNBC (n=37)**	**Non-TNBC (n=108)**	**Total**	**p-value**
		N (%)	**N (%)**		
Age (In years)	25-40	11 (29.73%)	30 (27.78%)	41(28.27%)	0.82
	41-50	15 (40.54%)	43 (39.81)	58 (40%)	
	51-60	8 (21.62%)	24 (22.22)	32 (22.06%)	
	>60	3 (8.11%)	11 (10.19)	14 (9.65%)	
	Total	37 (100%)	108 (!00%)	145 (100%)	
Nottingham Grade	Grade I	6 (13.33%)	39 (86.67%)	45 (100%)	0.02
	Grade II + Grade III	31 (31%)	69 (69%)	100 (100%)	
	Total	37 (25.52%)	108 (74.48%)	145 (100%)	
Outcome (n=83)	Alive	14 (63.6%)	49 (80.3%)	63 (75.90%)	0.11
	Death/ Recurrence	8 (36.4%)	12 (19.6%)	20 (24.09%)	

Discussion

The large majority of breast cancers are detected during the reproductive years. The incidence curve starts rising at puberty, increases steeply up to menopausal age, and levels off afterwards. [4] In our study population, maximum number of breast carcinoma patients were perimenopausal with a median age of 45 years and mean age of 47.98±10.55 years. (Table1) Our findings were in agreement with the study by Pandey ST et al [5], who observed that most common age group of breast carcinoma patients was 41-50 yrs (37.2%), with a mean age of 47.76 years. 24.9% of our study cases belonged to younger age group (<35 years). This is in concordance with the previous studies which quote that the proportion of young patients varies from about 10% in developed to up to 25% in developing Asian countries including India, which carry a poorer prognosis. [6] In our study, left breast was involved in 50.41% of cases and right breast in 49.59% of case. (Table1) Several previous studies have documented that breast carcinoma is slightly more frequent in left breast than in right. [7] As far as Nottinghams grade was concerned, maximum of our cases belonged to grade II (65.14%) followed by Grade I (28.44%) and a small subset (6.42%) belonged to grade III. (Table1) These findings are concordant with observation of a study from Punjab where grade II was maximum (57.1%).[8] However, total of grade II and grade III cases found by their study was 85%, while in our study it was 71.56%. On further analysis, we found that mitoses was a major determinant of high grade in differentiating Grade II and Grade III.

Only 8.3 % of all cases had tumor size <2cm, while remaining cases (91.7%) were more than 2cm in size. (Table 2) This strongly supports the fact that in developing countries like India, breast cancer patients are diagnosed at a relatively late stage. This can be attributed to illiteracy, lack of awareness, lack of breast cancer screening programmes, poor economic infrastructure and low priority in public health schemes. [6] Approximately 75.90% of our patients were alive, and only one case had reported recurrence. (Figure 3) In our study, association between clinical outcome and tumour size was statistically significant ($p<0.001$). (Table 3) The mean size of tumour in females who succumbed to disease was maximum, followed by those with recurrence, and the mean tumor size was least in alive patients in our study. According to a study by Narod S et al [9], tumour size was a strong predictor of 15-year survival in both node-positive and node-negative cancer subgroups. Increasing Nottingham's grade is associated with poor prognostic profile. All cases belonging to Nottinghams grade1 were alive and none showed recurrence or death. In grade 2 patients 72.73 % were alive, 9.09% showed recurrence and 18.18% demised. Nottinghams Grade III showed worst outcome amongst all, i.e., 50% were alive whereas 25% showed recurrence and another 25% succumbed to the disease.

Various techniques have been developed to quantify proliferation rates, including, mitotic-count estimates, Ki67/MIB1 labelling, cyclin A index, measurement of DNA synthesis, and flow cytometry.[10-12] According to a study conducted by Weidner N et al[13] a strong correlation was seen between Ki67 antigen expression & mitotic figure index (MFI). In our study, increased mitoses correlated with high Ki67, though the correlation was statistically weak ($p>0.05$). (Table 4) In 4 of our cases, mitotic score was high (>23 mitoses/10 hpf), while their ki67 value ranged was low (1-5). Since, this was an unexpected finding as a high mitotic index implements high Ki67, we repeated the Ki67, but the results were same. An increasing trend in Ki67 levels with increase in Nottingham Grade was observed in our study. This was in concordance with the expected results as Grade 3 being high grade tumors have increasingly proliferative capacity and high mitotic count than Grade 2 and Grade 1 tumors. [5] Mean Ki67 labelling index of patients who succumbed to the disease was higher than recurrence and alive group. [5]

According to the western literature, most common subtype amongst molecular classification of breast cancer is Luminal A comprising approximately (50%) of total cases. [7] Few of the studies also support the above findings. However, we found contrasting results. In our study, maximum percentage of cases were of TNBC and Her2 enriched, followed by Luminal B and Luminal A. (Table 5) Similar findings have been reported from other parts of India. A study from north eastern Indian population stated that TNBC accounts for a significant portion of breast cancers in India (31.9%) based on IHC markers. [14] Also, Akhtar M et al [15] in Maharashtra found that TNBC forms a large proportion (43.7%) of carcinoma breast patients in a central Indian scenario. This may be contributed to different genotypic compositions of the study population. TNBC are hormone therapy deprived group with limited treatment options. Increasing percentage of this subgroup in our study population indicates a thorough study of this subgroup. Further analysis showed that 77.27% of TNBC belonged to grade II and grade III as compared to 57.5% of Non-TNBC cases. (Table 6) Our results are in concordance with various studies which have stated that TNBC are aggressive set of breast cancer with higher histologic grade, larger tumor size, and more often are lymph-node positive. [16]

Conclusion

This study concluded that breast carcinoma was more frequent in females between 41-60 years, with high clinical stage and grade. Both left and right side

were equally involved in our study population. A large population were TNBC in our setup, and higher grade (Grade II+III) was significantly observed in TNBCs. Proportion of patients aged below 50 years with poor survival was higher in TNBCs as compared to Non-TNBC breast cancers. A relationship between poor prognosis and high Ki67 was seen, thus reinforcing the fact that highly proliferative tumours are associated with poor prognosis. Higher mitosis score was associated with higher Ki-67, which implies that both mitosis score and Ki-67 can be used as indicators of tumor proliferation.

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