Original Article

Incidence, Epidemiology and Clinico-Pathological Status of Different Molecular Subtypes of Breast Cancer in NICRH, Dhaka

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Abstract

Background: *Molecular subtype determination of breast carcinoma is still an enigma in our perspective. We are far behind the genetic analysis but immunohistochemistry is commonly ensured now a days.* ***Objective:*** *To observe the incidence, epidemiological and clinico-pathological status of different molecular subtypes of breast cancer patients.* ***Materials and method:*** *At first 141 patients were enrolled by purposive sampling. Among them 138 patients were finalized according to the eligibility criteria. A pre-structured, peer reviewed, properly tested, interview and observation based data collection sheet was prepared. Data regarding epidemiological profile, clinical profile and histopathological profile were collected, compiled, edited and analyzed. Mean, frequency, chi-square test were adopted for analysis. Statistics were found significant at <0.05.* ***Results:*** *Mean age of patients was 43.20±9.69 years. Mean BMI was 25.26±13.47. Out of 138 patients, only 4.34% had positive family history, 64.49% and 35.5% had left and right sided breast cancer respectively, 65.2% had tumour size 2-5cm which was followed by 27.53% cases with >5cm sized tumour in maximum diameter. Among the five major molecular subtypes both luminal A and triple negative breast cancer (TNBC) showed high prevalence (27.53%). Association of molecular subtypes with histopathological grading revealed TNBC was the most aggressive among all molecular subtypes. Axillary lymphadenopathy was present in almost all cases.* ***Conclusion:*** *Luminal A and TNBC were positive in most of the cases whereas TNBC showed higher association with advance histopathological grade. Clinical status was almost similar in all subtypes.*

Keywords: *Breast cancer; molecular subtypes; clinico-pathological status.*

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Introduction

Breast cancer is a heterogeneous disease with multiple classification systems.[1] Recently added molecular classification can better explain the breast tumour molecular biology than other classifications.[2,3] It can warn the clinicians regarding the prognosis of breast cancer of a

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particular molecular type that emphasizes on its clinical care. The original molecular classification has been derived from thorough investigations on fresh frozen tissue that is based upon molecular expression of ER, PR, Her-2 and Ki67. Five major subtypes are commonly extracted in this regard that is associated with several molecular alterations and distinct clinical outcome including therapeutic response. These molecular subtypes are luminal A, luminal B, Her-2 enriched, triple negative breast cancer (TNBC) and basal/normal like breast cancer.[3] Besides there may be some others additional subgroups like interferon-enriched,[4] molecular apocrine[5] and so on.

Triple negative breast cancer (TNBC) may be defined as tumours that lack expression of estrogen receptor (ER), progesterone receptor (PR) and Her-2.[6] This subtype may encompass other molecular subtypes of breast cancer. These include claudin like tumours, which are reported to be enriched with cells that have properties exactly similar to those of stem cells and to have features of epithelial to mesenchymal transition, the interferon rich subgroups. This subgroup encompasses tumours with a considerably better prognosis. The normal breast like subgroup may be an artifact i.e. it may comprise samples enriched with a disproportionately high content of stromal and normal cells.[7]

The dreadful statistics of South-East Asia showed that 76,000 women die of breast cancer every year.[8] In Bangladesh, there is no recognized and useful cancer registry at national level except the domestic registry of National Institute of Cancer Research and Hospital (NICRH), Mohakhali, Dhaka. It is estimated that an annual new breast cancer case burden is not less than 30,000.[9]

Simultaneously, we are far behind the usefulness of molecular subtypes in case of prognosis and clinical care of breast cancer patients. National Institute of Cancer Research and Hospital is a well equipped super specialty centre where maximum bulk of breast cancer patients of the country attends to seek one stop medical services. For this reason, this study aimed at to observe the incidence and clinical presentation as well as histopathological features of TNBC patients.

Materials and method

Patients who were diagnosed with breast cancer and underwent curative surgery in the department of Surgical Oncology at National Institute of Cancer Research & Hospital (NICRH), Mohakhali, Dhaka, Bangladesh, from January 2015 to December 2016 were included in the study. The inclusion criteria were i) with or without axillary lymph node metastasis on pathological examination and ii) available results of immunohistochemistry (IHC) for HRs and HER-2/neu. The exclusion criteria were i) patients who received neoadjuvant chemotherapy and ii) patients who received adjuvant trastuzumab. We evaluated each patient's clinicopathological features, molecular biomarkers, clinical outcome and follow up findings.

The patients were invited to the OPD at three-month intervals during the first year following adjuvant treatment and in six-month intervals during the next year. During each visit, physical examinations were carried out and the patients were asked to undergo blood analyses (complete blood count, routine biochemical tests, and tumour markers), mammography and/or USG of both breasts and axilla, abdominal USG and additional examinations including bone profile and bone scintigraphy, if indicated and all of the patients were monitored for recurrence/metastasis.

Expressions of ER, PR, and HER-2/neu were analyzed in the specimens of breast cancer tissue

of Bangladeshi women after modified radical mastectomy. In this study, ER-, PR- and HER2-negative patients were considered to have triple negative breast cancer, while patients who were positive for any of these markers were defined as "other breast cancers". Breast cancer stage at diagnosis was defined by the American Joint Committee on Cancer (AJCC) Cancer Staging Manual.[10]

The clinical details like age, sex, duration of symptoms, laterality, size of the tumour, axillary lymph node status and imaging findings were recorded for each case. After carrying out the detailed gross examination, all tissues were fixed in 10% buffered formalin. Multiple sections were taken from the tumour and its margins and all the lymph nodes. Histopathological study of the specimen was done by Haematoxylin and Eosin staining and as per standard protocol. Grading of the tumour was done by modified Bloom Richardson grading system. Immunohistochemistry (IHC) for ER, PR and Her-2/neu was performed on representative blocks of paraffin embedded tissue in each case. Sections of 3-4 micron thickness were submitted for IHC staining. Antigen retrieval was done by HIER method using citrate buffer at pH 2.5 for ER/PR and pH 6 for Her-2/neu. The normal epithelial component present in the tissue section served as internal control for ER/PR. IDC-NOS with known Her-2/neu over expression was used as external positive control for Her-2/neu with each lot of staining. The ER+ve cells showed nuclear staining where the percentage of positive cells was counted and the intensity of staining was recorded. For PR also nuclear staining was observed and accordingly scored. Her-2/neu is a membrane stain and Her-2/neu positive cells showed intense membrane staining without cytoplasmic staining.

Following scoring system was used for noting down the results of immunohistochemistry in each case.[11]

Interpretation of IHC in Carcinoma Breast

All red score for ER and PR evaluation in Ca breast.

Proportional score (PS)	Percentage of positive cells	Intensity score	Intensity of positive
0	0	0	None
1	<1	1	Weak
2	1-10	2	Intermediate
3	11-33	3	Strong
4	34-66		
Total score (PS-IS)		**Interpretation**	
0-2		Negative	
3-8		Positive	

Interpretation of Her-2Neu by IHC.

IHC score	Criteria
0 (Negative)	No reactivity/Reactivity in d10% tumour cells
1+ (Negative)	Faint weak reactivity in >10% of tumour cells but only a portion of the membrane is positive
2+ (Equivocal)	Weak to moderate complete membrane reactivity in >10% of tumour
3+ (Positive)	>30% of tumour cell must show circumferential intense and uniform membrane staining. A homogenous (Chicken wire) pattern should be present

The study protocol was approved by the Institutional Review Board (IRB) of NICRH.

Statistical Analysis

The comparisons of clinicopathological variables and patterns of relapse between TN breast cancer and non-TN breast cancer were made using Pearson's χ^2 test or Fisher's exact test as appropriate. Two-sided p values of, < 0.05 were considered statistically significant. A significance level of 0.05 was used for covariate entry. SPSS Version 23 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Table I shows that out of 138 patients maximum 53(38.4%) patients belonged to 41-50 years age group which was subsequently followed by 50(36.2%) patients in 31-40 years age group.

Table I: Age Distribution (N=138)

Age group (in years)	Frequency (%)
21 – 30	15 (10.9%)
31 – 40	50 (36.2%)
41 – 50	53 (38.4%)
51 – 60	15 (10.9%)
61 – 70	4 (2.9%)
≥71	1 (0.7%)

Table II shows the overall epidemiological status of the participating patients of carcinoma breast. Here it was proclaimed that mean age of the patients was 43.20±9.69 years (range: 23-72 years) and BMI was 25.26±13.47 kg/m^2, 84(60.9%) patients out of 138 were from normal BMI (18.5-25); 95(68.8%) out of 138 patients were from middle socioeconomic status; 44(31.9%) out of 138 patients achieved up to primary education and 128(92.8%) patients were housewives. Among these 138 patients 71(51.44%) and 67(18.55%) were from urban and rural area of residence respectively.

Table II: Epidemiological characteristics (N=138)

Variables	Mean±SD/ Frequency (%)
Age (in years)	43.20±9.69
BMI±SD (in kg/m^2)	25.26±13.47
BMI category	
<18.5 n(%)	3 (2.2%)
18.5 – 25	84 (60.9%)
25.1 – 30	50 (36.2%)
>30	1 (0.7%)
Socioeconomic status	
Poor class (<10000 BDT/month)	42 (30.4%)
Middle class (10000-25000 BDT/month)	95 (68.8%)
Rich class (>25000 BDT/month)	1 (0.7%)
Education status	
Primary	44 (31.9%)
SSC	52 (37.7%)
HSC	37 (26.8%)
Graduate &/over	5 (3.6%)
Occupational status	
Housewife	128 (92.8%)
Service holder	8 (5.8%)
Business	1 (0.7%)
Others	1 (0.7%)
Residence	
Urban	71 (51.44%)
Rural	67 (48.55%)

Table III shows the incidence of different molecular subtypes of carcinoma breast where it was proclaimed that luminal A and TNBC subtypes were the highest in number; 38(27.53%) each and subsequently 32(23.18%), 26(18.84%) and 4(2.89%) patients were determined as luminal B, Her-2 enriched and other molecular subtypes.

Table III: Incidence of molecular subtypes (N=138)

Molecular subtypes	Frequency (%)
Luminal A	38 (27.53%)
Luminal B	32 (23.18%)
TNBC	38 (27.53%)
Her-2 enriched	26 (18.84%)
Others	4 (2.89%)

Table IV shows that out of 138 patients 6(4.34%) had positive family history of carcinoma breast, 128(92.8%) had history of taking OCP, 73(52.9%) patients had breast tumour in upper and outer quadrant, 89(64.49%) patients had carcinoma of left breast and 92(65.2%) had tumour size 2-5cm (T2) in maximum diameter.

Table IV: Clinical presentation of breast tumour (N=138)

Clinical presentation of breast tumour	Frequency (%)
Family History	
Present	6 (4.34%)
Absent	132 (95.65%)
History of taking OCP	
Present	128 (92.8%)
Absent	10 (7.2%)
Anatomical site of lump	
Upper & inner	15 (10.9%)
Lower & inner	5 (3.6%)
Lower & outer	29 (21%)
Upper & outer	73 (52.9%)
Central	16 (11.6%)
Laterality of breast cancer	
Right	49 (35.5%)
Left	89 (64.49%)
Tumour size	
T1 (<2cm)	8 (5.7%)
T2 (2-5cm)	92 (65.2%)
T3 (>5cm)	38 (27.53%)

Table V shows that out of 138 patients, 131(94.28%) had axillary lymphadenopathy whereas 77(55.79%) and 54(39.13%) had single and multiple lymph nodes respectively. Out of these 138 patients 96(69.56%) lymph node(s) were mobile and 35(25.36%) were fixed or matted. On the contrary, 2(1.45%) out of 138 patients had contra lateral lymph nodes; all of these were mobile.

Table V: Clinical features of axillary lymph nodes (N=138)

Clinical features of lymph nodes	Frequency (%)
Axillary lymphadenopathy	
Present	131 (94.28%)
Absent	7 (5.07%)
Number of palpable lymph node	
Single	77 (55.79%)
Multiple	54 (39.13%)
No palpable lymph node	7 (5.07%)
Mobility of lymph nodes	
Mobile	96 (69.56%)
Fixed or matted	35 (25.36%)
Contra lateral axillary lymph nodes	
Present	2 (1.45%)
Absent	136 (98.55%)

Table VI shows that out of 138 patient 2(1.45%) showed the features of distant metastasis where 1(0.72%) each showed brain metastasis and presence of fixed left supraclavicular lymph nodes. Rest 136(98.55%) patients showed no features of distant metastasis.

Table VI: Pattern of distant metastasis (N=138)

Distant metastasis	Frequency (%)
Present	
Brain metastasis	1 (0.72%)
Left supraclavicular	1 (0.72%)
Absent	136 (98.55%)

Table VII shows correlation between molecular subtypes of carcinoma breast with their grades where maximum subtypes featured grade II disease. Among 120 grade-II diseases 34(28.83%), 30(25%), 27(22.5%), 25(20.83%), 4(3.33%) belonged to luminal A, luminal B, TNBC, Her-2 enriched and other molecular subtypes respectively. Among the rest of the cases, 16(11.59%) and 2(1.45%) cases were grade-III and I respectively. Among these 16 patients with grade-III, 11(68.75%) were TNBC whereas luminal A was the highest in both grade-I (100%) and II (28.33%). These statistics showed significant correlations between the variables (p=0.034; p=0.012). Besides, in most of the cases our patients manifested axillary lymphadenopathy; 97.36%, 87.5%, 94.73%, 100% each are observed in luminal A, luminal B, TNBC, Her-2 enriched and others respectively (p=0.219).

Table VII: Correlation between molecular subtyping of carcinoma breast and their grades (N=138)

Grade	Molecular sub typing category (N=138)				
	Luminal-A (n=38)	Luminal-B (n=32)	TNBC (n=38)	Her-2 enriched (n=26)	Others (n=4)
Grade-I	2 (5.26%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Grade-II	34 (89.47%)*	30 (93.75%)	27 (71.05%)	25 (96.15%)	4 (100%)
Grade-III	2 (5.26%)	2 (6.25%)	11 (28.94%)**	1 (3.84%)	0 (0%)
Axillary lymph nodes* **					
Present	37 (97.36%)	28 (87.5%)	36 (94.73%)	26 (100%)	4 (100%)
Absent	1 (2.63%)	4 (12.5%)	2 (5.26%)	0 (0%)	0 (0%)

Pearson's chi square significance is *0.034,0.012 and ***0.219**

Discussion

Breast cancer has been recognized as a multifaceted disease which is composed of distinct biological subtypes with diverse natural history. It may be presented as a varied spectrum of clinico-pathological and molecular features. These features have distinct therapeutic and prognostic uses.[12] Our study confirmed those features of breast cancer once again.

We have collected data from 138 patients who fulfilled all the eligibility criteria. Majority cases (38.4%) belonged to 41-50 years which was subsequently followed by 36.2% cases of 31-40 years age group. The mean age of the participants was 43.20±9.69 years. Our findings were almost nearer to the report of Tiwari et al.[13] where they claimed the mean age of their respondents was 47.76±11.08 years. They found 37.1% patients in 41-50 years age group which was 38.4% in our counterpart.

Geographic variation in breast cancer incidence can be attributed to racial and genetic differences, cultural differences, as well as environmental

exposures that vary worldwide.[14] It is not only the headache for developed world but also a threatening terror for the least developed world at present. Especially urban females are mostly affected in developing countries due to westernization of lifestyle.[15] Age at marriage, reduced breast feeding and increased junk food consumption may be the attributable factors for developing breast cancer in relatively early age than before.[16]

This study observed 64.49% breast cancer in left side which was supported by the report of Ambroise et al.[17] who reported 59.2% in this regard. This study observed that the commonest anatomical site of breast cancer was upper and outer quadrant (52.9%) which was in accordance with Naeem et al.[18] Painless lump (87.5%) was the highly frequent clinical presentation followed by nipple discharge in 8.31% and these results were supported by the findings of Sofi et al.[19] where they claimed that 85.3% and 7.3% of their cases had painless lump and nipple discharge respectively.

Majority cases of this study revealed tumour size as 2-5cm (65.2%) which was subsequently followed by >5cm sized tumour (27.53%). Patel et al.[20] in their study presented the similar findings.

Infiltrating duct cell carcinoma was the sole morphological category where grade-II was the highest (86.95%) which was followed by grade-I (20.45%) tumour. This study found no lobular and inflammatory breast cancer. Hussain et al. noticed the similar findings.[21] All invasive epithelial tumours were graded here according to the Modified Blood Richardsm Grading.[22]

Recently, a refined assessment of hormone receptors in breast carcinoma has become mandatory to choose therapeutic agents according to the recommendations and guideline for adjuvant systemic therapy of early breast cancer proposed by the international consensus panel during the St. Gallen conference in 2005.[23] The guidelines proposed 3 disease responsiveness groups. Firstly, endocrine responsive; secondly endocrine response uncertain and finally, endocrine nonresponsive. Immunohistochemistry is the method of choice to detect and quantify estrogen receptor (ER) and progesterone receptor (PR). Immunohistochemistry is preferred because of its relative simplicity, low cost, speed of performance, application to small samples, precise identification of reactive elements, simple method of fixation and storage, ability to be applied to archival material and better ability to predict response to adjuvant endocrine therapy owing to validation studies of ER and PR.[24,25]

Breast cancer mortality was evident as decreased in case of positive ER/PR status which is associated with various demographic factors and clinical tumour characteristics.[26] Likewise, lower recurrence is evident following breast conservation surgery.[27]

This study showed the incidence of luminal A, luminal B, TNBC, Her-2 enriched and others as 27.53%, 23.18%, 27.53%, 18.84% and 2.89% respectively. There were 4 global molecular subtypes out of 8 possible subtypes commonly used by the researches.[27]

The independent prognostic and predictive role of PR expression irrespective of ER expression has become a topic of great dispute as demonstrated by the report from the ATAC (Arimidex, Tamoxifen, Alone or in Combination) adjuvant trial. It is a large global trial comparing the efficacy of tamoxifen with that of aromatase inhibitor anastrazole. This trial revealed that the patients with ER+/PR+ tumour had a lower recurrence rate than those with ER+/PR- tumours.[28] The same study also observes that the patients with ER+/PR- tumours respond nearly as well as anastrazole as those with ER+/PR+ tumours suggests that the ER signaling pathway is functional in many ER+/PR- tumours, consistent with the popular fact that the PR gene is regulated by the estrogen pathway.[28]

As it is yet to be very common to use microarray analysis in our perspective, we have utilized the

immunohistochemistry based molecular subtyping. There is substantial variation in ER results both in intralaboratory and interlaboratory perspective because of fixation, antigen retrieval and staining methods may vary from laboratory to laboratory.[29] Likewise, substantial discrepancy among Her-2 results from same specimen in different laboratories has also been reported.[30]

In this study, luminal A and TNBC are the most prevalent molecular subtypes that claimed 27.53% of total sample size each. Almost similar findings regarding luminal A was observed in the reports by Hao et al. and interestingly, higher figure were observed in previous Western and Indian Studies.[31]

Fan et al. reported the normal like molecular subtype as the least common.[32] Their report can be matched in our aspect as 2.89% cases were identified as other variety where normal like is also a category.

In this study, 28.94% cases of TNBC manifested grade-III histopathological status whereas 5.26% each was observed in case of luminal A and luminal B. Among 120 grade-II cases, 34(28.33%) were luminal A which was mostly prevalent. TNBC is usually associated with more aggressive histopathological features. Our findings confirmed the statement again.

The study revealed significant differences among the association of different molecular subtypes and histopathological grading ($p=<0.05$).

Conclusion

Clinicopathologically it was assumed that TNBC was aggressive than any other variety from the point of view of grade-III. On the contrary, luminal A was a little more aggressive in grade-II. Axillary lymphadenopathy was almost common in all the varieties. So, it may be concluded here that TNBC is more aggressive though maximum cases in our centre present in advanced stage of any subtype.

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