ORIGINAL ARTICLE

FREQUENCY AND CORRELATION OF MOLECULAR SUBTYPES OF BREAST CANCER WITH CLINICOPATHOLOGICAL FEATURES

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Background: Traditional clinicopathological classification of breast cancer has limitations as tumours with similar clinical and histological features behave differently regarding outcome and responsiveness to chemo/immunotherapy. The objectives of the study were to determine the frequency of different molecular subtypes of breast cancer based on immunohistochemical staining and to find the correlation of each subtype with clinicopathological features. **Methods**: Sixty patients with histologically diagnosed invasive ductal carcinoma were enrolled in this cross sectional study. Immunohistological staining of the tumour samples and based on receptor status tumours were classified in four subtypes, Luminal A, Luminal B, *HER2/neu* oncogene amplification subtype and Tripple negative subtype. Clinical features, stage of disease at presentation and histopathological grade of the tumours was also recoded in each subtype. Prevalence of each subtype was calculated and correlation with clinical and pathological features was determined. **Results:** Mean age of the patients was 47.55 years. Protective role of breast feeding was not confirmed in this study as 58 (96.67%) patients breast fed their children. Only two (3.33%) patients gave family history of breast cancer in the study. Thirty three (55%) patients had grade 2 tumours, 26 (43.33%) had grade 3 tumours while only one patient had grade 1 tumour. HER2/neu amplification subtype was the most common molecular subclass in the study, comprising 30% of all the patients. Ten patients (16.67%) in this study belonged to triple negative group. Triple negative disease was found in younger women with mean age of 40–60 years. **Conclusion:** Breast cancer particularly triple negative disease was found in younger age group and patients usually present in advanced stage of their disease.HER2/neu positive breast cancer was the most common subtype in this study.

Keywords: Oestrogen receptors, Progesterone receptors, Molicular Targeted therapy, Gene expression profiling, microarray analysis, Fluorescence, in situ hybridization,

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INTRODUCTION

Breast cancer is heterogeneous disease comprising a number of distinct subtypes with diverse clinical behaviour and outcome.[1] Traditional clinic-pathological classification of breast cancer has limitations as tumours with similar clinical and histological features behave differently regarding outcome and responsiveness to chemo/immunotherapy.[2] Genetic analysis have revealed a large number of molecular and genetic alterations in breast tumours, which can explain the varied and diverse clinical behaviour. The cellular and molecular heterogeneity of breast tumours and the large number of genes potentially involved in controlling cell growth, death, and differentiation emphasize the importance of studying multiple genetic alterations in concert.[3] Systematic investigation of expression patterns of thousands of genes in tumours using complimentary DNA (cDNA) micro arrays, and their correlation to specific features of phenotypic variation, provides the basis for an improved taxonomy of cancer.[3] Based on this analysis, there were characterized different molecular subclasses of breast cancer like luminal, basal, and HER-2.neu, oncogene amplified. Prognosis and response to adjuvant therapy was significantly different in each subtype.[4] Some investigators classified breast cancer into five or even more subtypes on the same gene hierarchical pattern.[3,5] Better understanding of the molecular classes of breast cancer, independent of their prognostic and predictive values, may also lead to new biological insights and eventually to better therapies that are directed toward particular molecular subsets.[5] This is called molecular targeted therapy which has revolutionized the management of this deadly disease.[4]

Molecular classification of the breast cancer on gene analysis, although elegant, is more expensive and not widely available. The immune-histochemical staining of the breast cancer tissue identifies different gene products in the cell and provides a surrogate of gene analysis. This correlates well with the gene analysis and can be called poor man's genetic testing**.** The objectives of the study were to determine the frequency of different molecular subtypes of breast cancer based on immune-histochemical staining and to find the correlation of each subtype with clinic-pathological features**.**

MATERIAL AND METHODS

This cross-sectional study was conducted in Department of General Surgery, Ayub Teaching Hospital, Abbottabad from 1st January, 2010 to 31st December 2010. All female patients with diagnosed invasive carcinoma of breast on the basis of histopathology were

included in the study. Patients not consenting to participate in the study, patients with benign breast tumours and patients with in situ breast cancers were excluded from study. Patients with clinical diagnosis/suspicion of breast cancer were provisionally enrolled for the study. To achieve the histological diagnosis incisional, excisional or trucut needle biopsy was taken. Histopathologically confirmed cases were selected finally for the study after taking their informed consent. The patients' age and menopausal status were recorded as well as history of breast feeding to children born to each married patient. Family history of this disease in first degree relatives was asked and recorded. The disease was staged according to TNM Classification. After definitive surgical procedure resected specimen was submitted to Shaukat Khanum Memorial Hospital and Research Centre Lahore for immuno-histotochemistry to assess the three receptors in tumor cells in addition to detailed histopathology. For oestrogen/progesterone receptors, monoclonal antibody against this receptor was used and scoring was performed according to Allred Guidelines as shown in table-1. Allred scores 0-2 were considered negative while scores 3-8 were reported positive. For HER-2/neu assessment sections of the tumour were stained with a polyclonal antibody against this oncoprotein according to Envision System scoring and reporting criteria as shown in table-2. A test result of 0 to 1+ was considered as negative and 3+ as positive. A Her2/neu result of 2+ were submitted for fluorescent in-situ hybridization (FISH) for Gene amplification. Data was entered and analysed on SPSS-16.

RESULTS

Sixty patients were enrolled in this study. Mean age was 47.55±13.61 with the range of 21–80 years. Out of 60 patients, 21 (35%) were postmenopausal while 39 (65%) were pre-menopausal. Fifty-eight (96.67%) patients had positive history of breast feeding while 2 (3.33%) had no history of breast feeding. Family history for carcinoma breast was positive in 2 (3.33%) patients while it was negative in 58 (96.67%) patients.

Of total 60 patients, one (1.67%) had well differentiated carcinoma, 33 (55%) had moderately differentiated carcinoma while 26 (43.33%) had poorly differentiated carcinoma. Of 60 patients 2 (3.33%) had stage-I disease, 23 (38.33%) had stage-II disease, 27(45%) had stage-III disease and 8 (13.33%) had Stage IV disease. Twenty six patients (43.33%) had grade 3 tumours, 33 (45%) had grade 2 tumours and only one (1.67%) patient was found to have grade 1 tumour at the time of presentation. The final distribution of these patients according to IHC classification is given in table-3. The various clinical parameters of these classes are given in table-4.

Table-1: All red score for ER status

Percentage staining score	Proportion of positive staining cells	Intensity score	Average intensity of positively stained cells
0	none	0	None
1	<1/100	1	Weak
2	1/100 to 1/10	2	intermediate
3	1/10 to 1/3	3	Strong
4	1/3 to 2/3		
5	>2/3		

Allred Score=percent staining score + intensity score. Allred score 0-2=Negative for ER/PR receptors. Allred score 3–8=Positive for ER/PR receptors.

Table-2: Criteria for reposting

Score	Definition
0	No imunostaining
1+	Weak immunostaining less than 30%
2+	Complete immunostaining, either uniform or weak in at least 10% of cells
3+	Uniform intense membranous staining in at least 30% of cells

Criteria: 0-1 scores = Negative for HER2/neu receptors, 3+ scores= Positive for HER2/neu receptors, 2+ scores = Borderline/ weakly positive for HER2/neu receptors

Table-3: Distribution of four molecular subtypes of breast cancer (n=60)

Group	No	Percentage
Luminal A (ER/PR+,Her2-)	17	28.33
Luminal B (ER/PR+,Her2+)	15	25
Her 2 +ive (ER/PR-,Her2+)	18	30
Tripple negative (ER/PR-,Her2-)	10	16.67
Total	60	100

Table-4: Clinical features of different types of carcinoma breast

Parameter		Luminal A	Luminal B	Her2/neu +	Triple Negative
No		17	15	18	10
Mean age		44.06±10.64	47.33±14.39	54.89±14.84	40.60±9.49
Menopausal status	Pre	14 (82.35%)	10 (66.67%)	6 (33.33%)	Nine (90%)
	Post	3 (17.65%)	5 (33.33%)	12 (66.67%)	1 (10%)
Breast feeding	Yes	16 (94.11%)	14 (93.33%)	18 (100%)	10 (100%)
	No	1 (5.89%)	1 (6.67%)	0 (0%)	0 (0%)
Family history	Yes	0 (0%)	2 (13.33%)	0 (0%)	0 (0%)
	No	17 (100%)	13 (86.67%)	18 (100%)	10 (100%)
Grade	I	0 (0%)	0 (0%)	1 (5.55%)	0 (0%)
	II	10 (58.82%)	12 (80%)	9 (50%)	2 (20%)
	III	7 (41.18%)	3 (20%)	8 (44.45%)	8 (80%)
Stage	I	0 (0%)	1 (6.67%)	0 (0%)	1 (10%)
	II	8 (47.06%)	5 (33.33%)	9 (50%)	1 (10%)
	III	6 (35.29%)	9 (60%)	6 (33.33%)	6 (60%)
	IV	3 (14.65%)	0 (0%)	3 (16.67%)	2 (20%)

DISCUSSION

Important and notable trend of the disease towards younger age has been observed in current study. About half of the patients were between 30 to 50 years of age and 8 patients were below 30 years. The mean age was 47.55±13.6 years. This is in contrast to the commonly reported incidence with mean age of 60 years.[6] Surveillance Epidemiology and End Results (SEER) from national cancer institute of United States in its 2003-2007 statistics have reported only 1.9% of total breast cancer patients were below 34 years and 32% between 35–54 years of age .The mean age was also 61 years.[7] The disease occurring at younger age in current study can be explained in terms of racial and social differences. Higher average life span and commonly used hormonal replacement therapy in United States account for higher mean age for this disease there, as compared to my study. Black and Asian women also get the disease at an earlier age than the white.[8,9] Higher prevalence of BRCA1&BRCA2 mutations in Pakistani population might be a factor for higher proportion of younger patients in my study.[9] The same finding was translated in the menopausal status of the patients. Sixty five percent of my patients were premenopausal. This fact has been reported by other Pakistani workers aswell.[10]

Regarding tumour grades, moderately differentiated carcinoma was the most common, including 33 patients out of 60. Only one patient had well differentiated tumour, while 26 had poorly differentiated disease. The major preponderance of the patients was in stage 3 on their first assessment, comprising of 45% of total patient population in the study. This might be the reflection of the higher no. of patients with less differentiated tumours in the study. Only two patients had stage one disease at the time of first presentation. Eight patients were already in stage four when entered in the study. This is a very unfortunate situation and reflects the ignorance, poverty and malpractice in our society. The traditional "purda" in our conservative society is also a contributing factor in this regard. The role of unethical, unscientific methods of treatment by quacks, *Hakeems* and homeopaths cannot be disregarded. Late presentation with advanced disease in our country has been reported by many Pakistani workers.[10] This study used IHC classification and stratified all breast cancer cases into four subtypes. HER2/neu amplification subtype was the most common molecular subclass in the study, comprising 30% of all patients. This figure is higher than those reported in other studies.[11] However, some studies have reported the same prevalence of this subtype of breast cancer in South Asian women.[12–14] This means breast cancer in India and Pakistan is different from the rest of the world in terms of this receptor status. This is one explanation of poor outcome from breast cancer in these countries as these tumours tend to be aggressive with high proliferation index.

Luminal A was the next common subtype in this study, comprising of 17 (28.33%) cases. Luminal B subtype comprised of 15 (25%) cases. Combined together both constituted 32 (53.33%) patients. This finding is in contrast to many international studies reporting much higher proportion of ER/PR positive cancers.[12,15] Again Pakistani women are unfortunate. Madhuri Kakarala and her colleagues from University of Michigin 2010 has reviewed the SEER Data from 1988 to 2006 and calculated that frequency of ER negative breast cancer subtype is much higher in Indian and Pakistani women than their American and European counterparts. The authors have revealed that 30.6% of breast cancers in Indian and Pakistani women were ER negative in contrast to Caucasian women in whom this percentage was 21.8%.[16] Higher proportion of oestrogen receptor negative breast cancers in our subcontinent is another reason of aggressive disease and is further indication to promote screening and public awareness programs to catch the disease at an earlier stage. Ten patients (16.67%) in this study belonged to triple negative group. All three receptors were negative in these patients. Onitilio and colleagues (2009) have reported 13.4% of their analysis of 1134 invasive breast cancer patients.[15] Most of other studies also report the same frequency of this variant of breast cancer.[17,18] This study revealed some interesting findings regarding mean age for different subtypes of breast cancer. Triple negative disease was found in younger women with mean age of 40.60, while the mean age for HER2/neu positive group was 54.89 years. Mean age for Luminal A&B were 47.33 and 44.06 respectively. Regarding triple negative disease mean age in this study was in accordance to the other studies but the patients with HER2/neu+ in this study were much older than reported by most of other workers.[17,19,20] This difference might be due to different genetic make of Pakistani women as other authors from this part of the world have also reported HER2/neu positive breast cancer in older patients.[10,21,22]

CONCLUSION

In conclusion carcinoma of breast is a common clinical problem in our community. Patients usually present late due to various reasons. Triple negative and HER2/neu positive are more common subtypes. There is an urgent need for breast cancer screening, health education and public awareness programs to catch the disease in initial stage when it is curable. Molecular studies and immunohistochemical staining facilities are not available in public sector setup and high cost of such facilities in private setup is beyond the reach of poor patients of our country. Arrangements of such facilities

at government institutions are recommended. Breast cancer societies have been developed in most parts of the world particularly in west to raise the public awareness and help the patients. Need for such societies is more intense in countries like Pakistan where ignorance, poverty and quackery are endemic.

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