FREQUENCY OF DIFFERENT MOLECULAR SUBTYPES OF BREAST CANCER IN PAKISTANI POPULATION: A SINGLE CENTER STUDY

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ABSTRACT

Objective: Breast cancer is a malignant disease caused by an uncontrolled growth of cancerous cells in the breast. It is a heterogeneous disease, consists of several molecular subtypes with different biological behavior, epidemiological risk factors, natural histories, response against local and systemic treatment and also prognosis. The objective of the study was to determine the frequency of different molecular subtypes of breast cancer at our center.

Materials and Methods: A descriptive cross-sectional study was performed on 214 cases at Chughtai Institute of Pathology from 1st September 2018 to 30th April 2019. The clinical parameters like age and provisional diagnosis were recorded. The immunohistochemical (IHC) analysis was performed on serial sections using immune-enzymatic soluble complex method. The antibodies used were Ki67, ER, PR and HER2 polyclonal antibodies from DAKO. Scoring of ER and PR IHC were done according to Allred Score for ER and PR evaluation in breast cancer protocol template of College of American Pathologists (CAP). Similarly, HER2 scoring was also done according to reporting guidelines of HER2 testing by IHC given in breast cancer protocol template of CAP.

Results: There were 55.97±16.40 years of age and 3.91±1.78 cm of the tumor size. 38.8% has luminal A, 30.8% had triple negative, 15.5% had luminal B and 14.9% had HER2 enriched molecular subtypes. According tumor sites, 87 had UOQ site ,51 had central site, 50 had LIQ site, 20 had LOQ site, and 6 had UIQ site. The histologic grades showed that tumors with Grade II morphology were 51.9%, Grade III were 42.9% and Grade I were 5.2%. There were 104 (48.6%) patients with right laterality and 110 (51.4%) patients with left laterality.

Conclusion: Luminal A molecular subtype of breast cancer was found as the most common subtype in this study.

Key Words: Frequency, Molecular subtypes, Breast cancer, Immunohistochemistry.

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INTRODUCTION

Breast cancer is one of the most common cancers in women worldwide [1]. Pakistan has the highest rate of breast cancer among Asian countries [2]. It has variable risk factors including genetics, age at menarche, age at menopause, geographical variation, age at first pregnancy, diet, exposure to radiation and exogenous hormonal therapy [3]. It is expected that identification of genetic and environment factors that contribute to the development of breast cancer will enhance the prevention effects.

Breast cancers can be classified on basis of histological morphology, genetic profiling and molecular analysis. Tumors having similar histological morphology differ in their clinical behavior and outcome. Better methods, therefore, are required to help assess prognosis and determine the most appropriate treatment for patients on an individual basis [4,5].

Various molecular techniques like fluorescence in-situ hybridization (FISH), chromogenic in-situ hybridization (CISH), polymerase chain reaction (PCR) and immunohistochemistry (IHC) have been used to classify breast cancers on molecular basis to assess clinical outcome and management. Studies have identified four molecular subtypes including luminal A, luminal B, HER2 positive and triple negative (basal like) on basis of expression of hormone receptors [estrogen receptor (ER) and progesterone receptor (PR)], protein Ki67 and human epidermal growth factor receptor (HER2) detected via immunohistochemical stains. Prognosis and response to adjuvant therapy is different for each subtype [5].

A local study showed that HER2 positive is the most common molecular subtype (30%) followed by triple negative/basal like (16.67%), luminal A (28.33%) and luminal B (25%) [6]. International study conducted in Brazil indicated luminal A being 55%, luminal B as 11%, HER2 positive as 13% and triple

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negative/basal like as 18%. Similar study conducted in Indonesia stated that frequency of luminal A as 38.1%, luminal B as 16.7%, HER2 positive as 20.2% and triple negative/basal like as 25%. Another study in China showed frequency of luminal A as 23%, luminal B as 54.3%, HER2 positive as 12.6% and triple negative/basal like as 10.1% [7-9].

Luminal A breast cancers have favorable prognosis as compared to luminal B breast cancers. HER2 positive breast tumors are generally intermediate to high grade tumors, with an aggressive course. The basal-like gene signature is the tripe-negative type with worst prognosis [10-12]. Molecular classification allows us to direct different molecular targeted therapies which have revolutionized the outcome of breast cancer.

Rationale: Breast cancer is heterogeneous disease comprising a number of distinct subtypes with diverse clinical behavior and outcome. International studies conducted in Indonesia, Brazil and China show luminal A and luminal B subtypes as most frequent [7-9]. Local data is limited. A study conducted in Abbottabad showed HER2 positive as the commonest subtype. This implicates the breast cancer in Pakistan is different from the rest of the world in terms of this receptor status [6].

MATERIAL AND METHODS

After approval from ethical committee, a descriptive cross-sectional study was performed at Department of Histopathology, Chughtai Institute of Pathology, Lahore in eight months (1st September 2018 to 30th April 2019). A total of 214 cases (Confidence interval: 95% and margin of error: 5%) [6] were retrieved from archives of Chughtai Institute of Pathology, using non-probability consecutive sampling technique. All mastectomies, lumpectomies and core needle biopsy specimens with diagnosis of epithelial malignancy in females between age 20-80 years were included. Blocks from outside laboratories for review and immunohistochemistry were also included. Poorly preserved and autolysed specimens (specimen received in weak formalin) and tumors with HER2 2+ score by immunohistochemistry were excluded.

The histological preparation was performed by classical method for inclusion in paraffin followed by hematoxylin-eosin staining. The immunohisto-chemical analysis was performed on serial sections using immune-enzymatic soluble complex method. The antibodies used were Ki67, ER, PR and HER2 polyclonal antibodies from DAKO.

Scoring of ER and PR IHC were done according to Allred Score for ER and PR evaluation in breast cancer protocol template of College of American Pathologists (CAP). Similarly, HER2 scoring was also done according to reporting protocol of HER2 testing by IHC given in breast cancer protocol template of CAP.13 Ki-67 proliferation index was also recorded in the hotspots. Data was entered and analyzed using SPSS version 20. The mean and standard deviation was calculated for quantitative variables including age and tumor size. Qualitative variables including molecular subtypes, tumor site, histological grade, pathologic stage and laterality were presented in the form of frequencies and percentages. Effect modifier like age was controlled through stratification. Post-stratification Chi-square test was applied by taking P value of 0.05 as significant.

RESULTS

The study was carried out over a period of eight months. Two hundred and fourteen patients of breast cancers were included.

There were 48 patients (22.4%) in age group 20-40 years, 78 patients (36.4%) in age group 41-60 years and 88 patients (41.2%) in age group 61-80 years. The mean ± SD age was 55.97±16.40 years (Table-1). According to tumor size, there were 116 patients (54.3%) between 1.0-3.5 cm, 85 patients (39.7%) between 3.6-7.0 cm and 13 patients (6%) between 7.1-10.0 cm with mean and standard deviation of 3.91±1.78 cm (Table-2). When the patients were distributed according to the molecular subtypes, 83 patients (38.8%) were classified as luminal A, 66 patients (30.8%) as triple negative, 33 patients (15.5%) as luminal B and 32 patients (14.9%) as HER2 enriched molecular subtypes (Table-3). According to tumor site, there were 51 cases (23.8%) with tumor in central quadrant, 50 cases (23.4%) in LIQ site, 20 cases (9.3%) in LOQ site, 6 cases (2.8%) in UIQ site and 87 patients (40.7%) in UOQ site (Table-4).

Table-5 shows the histologic grade with 11 patients (5.2%) having grade I, 111 patients (51.9%) with grade II and 92 patients (42.9%) with grade III (Figure-I; A).

There were 21 patients (9.8%) with pathologic stage PT1, 144 patients (63.3%) with pathologic stage PT2 and 49 patients (22.9%) with pathologic stage PT3 (Table-6). According to laterality, there were 104 (48.6%) patients having right laterality and 110 (51.4%) patients with left laterality (Table-7). Frequency of ER, PR and Her2 are presented in form of tables. (Table 9-11). The positivity of these immunohistochemical stains is shown in Figure-I; B, C and D.

When the molecular subtypes were stratified according to age, statistically significant difference

(P<0.05) was found between age and molecular subtype.

Table-1: Frequency and percentage of age (n = 214).

Age (years)	No.	%
20 – 40	48	22.4
41 – 60	78	36.4
61 – 80	88	41.2
Mean ± SD	55.97±16.40	

Key: SD = Standard deviation

Table-2: Frequency and percentage of tumor size (n = 214).

Tumor size (cm)	No.	%
1.0 – 3.5	116	54.3
3.6 – 7.0	85	39.7
7.1 – 10.0	13	6.0
Mean±SD	3.91±1.78	

Key: SD = Standard deviation

Table-3: Frequency and percentage of molecular subtypes (n = 214).

Molecular subtypes	No.	%
HER2 enriched	32	14.9
Luminal A	83	38.8
Luminal B	33	15.5
Triple negative	66	30.8

Table-4: Frequency and percentage of tumor site (n = 214).

Tumor site	No.	%
Central	51	23.8
LIQ	50	23.4
LOQ	20	9.3
UIQ	6	2.8
UOQ	87	40.7

Table-5: Frequency and percentage of histologic grade (n = 214).

Histologic grade	No.	%
I	11	5.2
II	111	51.9
III	92	42.9

Table-6: Frequency and percentage of pathological stage (n = 214).

Pathological stage	No.	%
PT1	21	9.8
PT2	144	63.3
PT3	49	22.9

Table-7: Frequency and percentage of laterality (n = 214).

Laterality	No.	%
Right	104	48.6
Left	110	51.4

Table-8: Stratification of molecular subtypes according to age (n = 214).

Age (years)	HER2 enriched	Luminal A	Luminal B	Triple negative
20 – 40	8	22	9	9
41 – 60	10	27	18	33
61 – 80	14	34	6	24

$\chi 2 = 12.24$; df. = 6; P = 0.057

Table-9: Frequency and percentage of estrogen receptor (n = 214).

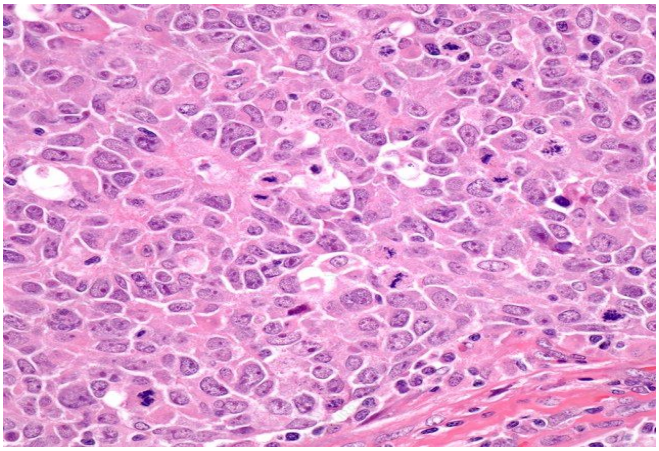
Estrogen Receptor	No.	%
Negative	100	46.7
Positive	114	53.3

Table-10: Frequency and percentage of progesterone receptor (n = 214).

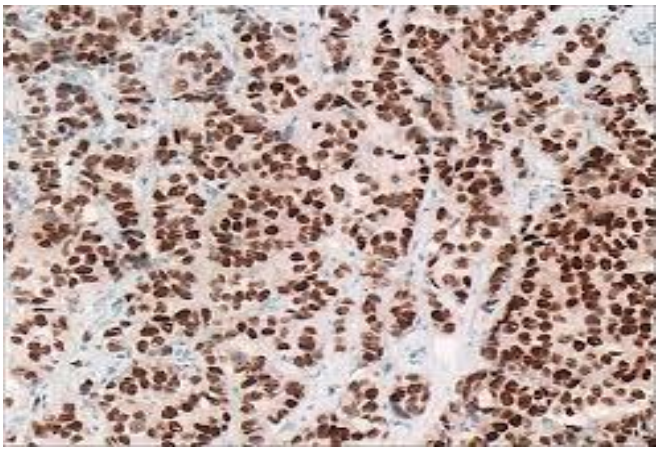
Progesterone Receptor	No.	%
Negative	96	44.9
Positive	118	55.1

Table-11: Frequency and percentage of HER2 (n = 214).

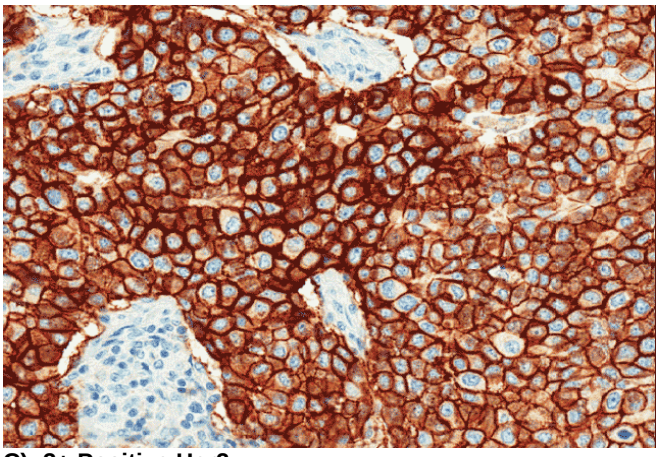
HER2	No.	%
0	84	39.3
1+	59	27.6
2+	5	2.3
3+	66	30.8



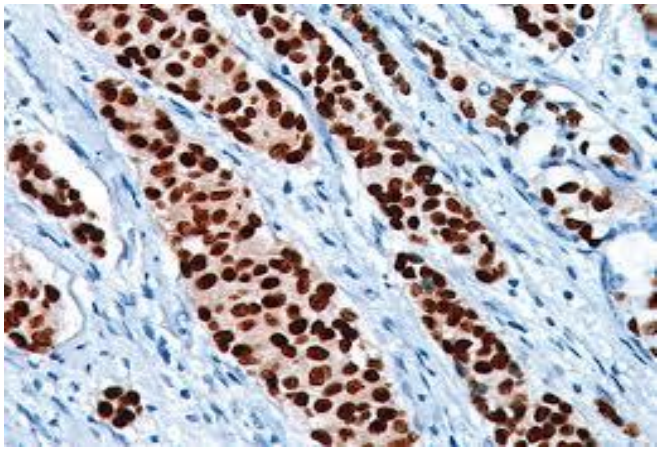
A): Invasive Ductal Carcinoma, GIII



B): Positive ER



C): 3+ Positive Her2



D): Positive PR

Figure-1: Histopathology and immunohistochemical interpretation.

DISCUSSION

Breast cancer is becoming a major health issue in many countries including Pakistan. About 1 million women per year are being diagnosed with this cancer [2]. In Asia, Pakistan has highest breast cancer incidence and it is increasing day by day as it escapes early detection due to lack of awareness regarding disease presentation and screening programs. In Pakistan, one in every eighth women experiences breast cancer. Studies revealed that every year 40,000 women die of breast cancer in Pakistan [13].

Breast cancer is a heterogeneous disease with variable clinical, pathological and molecular features. The histological classification system was introduced which divided breast cancer into numerous morphological subtypes. However, this subtyping did not help in deciding a definitive treatment categorization. This heterogeneous nature of the disease has significant implications both for physicians and their patients increasingly as targeted therapy is given according to the specific molecular subtype. So, later on further subtyping on basis of gene expression, types came into existence with distinct treatment strategies and prognosis. Two of them are derived from ER- tumors (basal like and Her-2 positive) and two from ER+ subtypes (luminal A and B). Still, there are certain subtypes which do not express either of the hormone receptors or Her-2 and are called triple negative subtype [26].

The study population in our study ranged from 20-80 years with a mean age of 55.97 years. Bennis et al [14] reported mean age of 45 years in a similar study of 366 cases in Morocco. Akbar et al6 found mean age of 47.55 years in a study of 60 cases in Pakistan. The mean age of our study population was higher as compared with study population of Bennis et al [14] and Akbar et al [6]. However, Zhu et al [15] found a mean age of 51 years in a similar study of 3198 cases in China.

The mean tumor size in our study was 3.91 cm and 94% tumors were of more than 2 cm size in their largest dimension. Kumar et al [16] from India also found similar results. They reported mean tumor size of 3.4 cm with 85.8% of their cases having tumor size more than 2 cm. However, Zhu et al [15] reported mean tumor size of 2.1 cm in his study. The higher mean tumor size in our study and in India is most probably due to late presentation during the progression of the disease owing the existing low socio-economic status in the subcontinent. Another important cause may be the lack of self-awareness and mammographic screening programs.

Luminal A was the commonest subtype found in this study, comprising 83 (38.8%) cases, followed by 66 patients (30.8%) as triple negative, 32 patients (14.9%) as HER2 enriched, and 33 patients (15.5%) as luminal B molecular subtypes This finding is in common to many international studies reporting much higher proportion of ER/PR positive cancers [17,18]. Although most common subtype in our study was Luminal A, a significant number (45.7%) were ER/PR negative (Triple negative and Her-2 enriched group combined). A study conducted in University of Michigan by Madhuri Kakarala reviewed the SEER Data from 1988 to 2006 and showed higher frequency of ER negative breast cancer subtype in Indian and Pakistani women. 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% [19]. Higher proportion of estrogen receptor negative breast cancers in our subcontinent is another reason of aggressive disease and is further indication to promote screening and public awareness programmes to catch the disease at an earlier stage. 66 patients (30.8%) in this study belonged to triple negative group. Onitilio and colleagues have reported frequency of triple negative breast cancer as 13.4%, of their analysis of 1134 invasive breast cancer patients [20]. Most of other international studies also report the same frequency of this variant of breast cancer [21,22]. Regarding triple negative disease mean age in this study was in accordance to the other studies but the patients with Her2 in this study were much older than reported by most of the other international studies [23]. The finding was not significant. However, this difference might be due to different genetic makeup of Pakistani women as other authors from this part of the world

have also reported Her2 positive breast cancer in older patients, similar to our study [24, 25].

CONCLUSION

In conclusion, carcinoma of breast is a common clinical problem in our community. Patients usually present late due to various reasons. Luminal A molecular subtype is the commonest subtype in this study. There is an urgent need for breast cancer screening, health education and public awareness programmes to catch the disease in initial stage when it is curable. Molecular studies and immunohisto-chemical 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 treat the patients. Need for such societies is more intense in countries like Pakistan where ignorance, poverty and quackery are common.

AUTHORS CONTRIBUTION

Fatima Khalid: Concept, design, result interpretation and content writing

Ghazi Zafar: Design, analysis, result interpretation, critical revision

Sameen Afzal & Anila Chughtai: Critical revision

Beenish Fatima: Result interpretation

Akhtar Sohail Chughtai: Financial support and final critical revision

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