ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL ANALYSIS OF BREAST CANCER SUBTYPES AND THEIR CORRELATION   WITH Ki 67 INDEX

Shagufta Naeem, Shabana Naz, Anila Riyaz, Fouzia Jehangir, Naeema Afzal
Department of Pathology, Ayub Medical College, Abbottabad-Pakistan

Background: Breast cancer is the second most common cancer in females including Pakistan. Nowadays immunohistochemistry has revolutionized management of breast cancer. In this study, we determined the frequency of various subtypes of breast cancer on the basis of immunohistochemical staining and their correlation with ki 67 index. **Methods:** Fifty consecutive diagnosed breast cancers cases received during the period February 2014 to February 2016, at Ayub Medical College, Pathology Department were included in this cross-sectional study. Immunohistochemistry for Oestrogen receptor (ER), Progesterone receptor (PgR), Her2neu and ki 67 index were applied and the results were recorded. **Results:** Out of 50 cases, 34 (68%) were positive for ER and PgR, 6 (12%) were positive for her 2 neu while 6 (12%) were triple positive and 4 (8%) were triple negative. **Conclusion:** Low risk Luminal A was the most frequently encountered breast cancer category.
Keywords: ER, PgR, Her 2 neu; Triple positive breast cancer; Triple negative breast cancer.

INTRODUCTION

Breast cancer is the most common cancer in women worldwide,[1] and only second to lung cancer[2]. It is a heterogeneous group of diseases with various biological subtypes, different natural history and clinicopathological features.[3] The introduction of gene profiling has led to a novel way of classifying breast cancers leading to more targeted approach to management, thus affecting the prognosis.[4]

The main aim of gene expression profiling is to guide the oncologist to plan specific management for each patient.[4,5] Breast cancer patients having hormone receptor positive tumour with negative node can be treated with tamoxifen alone, as 85% of these patients would do well without the addition of chemotherapeutic agents.[6] On the other hand Her 2 positive tumours generally show relative resistance to tamoxifen. In metastatic breast cancer, targeted therapy like tranzumab is used to improve the survival, response rate and time to progression when used alone or along with chemotherapy.[7]

The purpose of this study is to determine the frequency of various subtypes of breast cancer and their further categorization into low risk and high risk.

MATERIAL AND METHODS

This cross-sectional study was carried out at Pathology Department of Ayub Medical College, Abbottabad from February 2014 to February 2016. Fifty consecutive cases of breast cancer were selected. Patients' data including age, gender and relevant clinical history were noted.

After reviewing Haematoxylin and Eosin stained slides, tissue blocks suitable for immunohistochemical studies were selected. These blocks were sectioned at 3µm thickness, deparaffinized in xylene and rehydrated with decreasing concentration of ethanol. Heat induced epitope retrieval in Tris/EDTA buffer at pH 9.0 buffer was used for ready to use primary antibodies ER, PgR, Her 2 neu and ki 67 (clone by Dako Corporation). Immunohistochemical (IHC) analyses of Oestrogen receptor (ER), Progesterone Receptor (PgR), Her 2 neu and ki 67 index were performed on formalin fixed, paraffin-embedded cancer tissue blocks.

For Oestrogen and Progesterone receptors Allred scoring method is used. In this method, the proportion of nuclei (on a scale from 0–5) and intensity (from 0–3) of staining are summed to give an overall score. Her 2neu positivity was considered on the basis of strong complete membrane staining in more than 10% of the tumour cells. Ki 67 index of <14% was considered low risk while >14% were high risk.   The results of immunohistochemistry were recorded.

Data was analysed by using SPSS version 19. Frequencies and percentages were recorded for categorical variables while mean and standard deviation were calculated for the quantitative variables.

RESULTS

A total of 50 cases of breast cancer were included in the study. All patients included in the study were females. The ages of the patients ranged between 30

and 80 years with average age of 48.9±10 years. Majority of the patients presented in the 4th decade of life. Youngest patient was 30 years of age and was pregnant.

Distribution of various breast cancer subtypes is shown in table-1 indicating Luminal A as the commonest molecular subtype whereas no case of normal like subtype was identified. Frequency of ki67 index in various molecular subtypes is shown in table-2.

Table-1: Distribution of breast cancer according to the gene profiling (n=50)

Molecular subtype	Number (Percent)
Luminal A	34 (68)
Luminal B	6 (12)
Her 2 positives	6 (12)
Basal-like	4 (6)
Normal-like	0 (0)

Table-2: Frequency of ki67 index in various molecular subtypes

Molecular subtype	Low risk (<14%)	High risk >14%)
Luminal A	27 (79.47%)	7 (20.5)
Luminal B	2 (33%)	4 (67)
Her 2 Positive	0 (0%)	6 (100)
Basal-like	0 (0%)	4 (100)
Normal –like	0 (0%)	0 (0)

DISCUSSION

Breast cancer is most common cancer in females[2,8] and among these invasive ductal carcinoma is the commonest type[8].The diagnosis is primarily made histologically, however ancillary testing supports diagnosis, classification, prognosis, and prediction of response to therapy. With DNA microarray, it has been divided into 5 major subtypes: luminal A, luminal B, Her 2neu positive, basal-like (triple negative) and normal breast-like group.[9]

The mean age in this study was 48.9 years which is comparable to other studies carried in Pakistan. [10]However the average age in European countries is 61 years.[11]

Hormone receptors are expressed by 60-70% of breast cancer. However, in our setting 80% of the tumours are hormone receptor positive, the difference may be due to small sample size.[12] The luminal A type cancers are consistently oestrogen receptor positive and have favourable prognosis as these patients respond well with hormone therapy with or without chemotherapy. However further stratification using ki 67 indexes will identify the high risk category for appropriate management.[12,13] In our setting 34 (68%) cases were Luminal A subtype which is different from the study carried out by Wang J, Sang D et al which may be due to racial difference or small sample size.[13] Ki 67 index was low in majority of Luminal A subtype, this in concordance with study carried out by Inic Z and Zegarac M et

al.[14] This categorization is important as it will determine prognosis, treatment protocol and recurrence. It can play an important role in follow-up of the patients.

Luminal B type tumours are positive for all three receptors with high ki 67 index[15]and are prognostically not as good as Luminal A. Luminal B involves the lymph nodes more frequently than Luminal A which adversely affects the prognosis.[14] In our study we had 12% cases of Luminal B subtype which is in concordance with cohort study carried by SU Y, Zheng Y, et al.[16]

HER-2/neu positive cases accounts for 20% of total cases which is same as study carried out by Witton CJ and Reeves JR[17] and all of them showed a high ki 67 index[18]. Her 2 neu positive breast cancers are poorly differentiated invasive ductal carcinoma with nuclear grade III, brisk mitosis and no tubule formation. Clinically, they present as rapidly growing masses with poor prognosis. Treatment protocol of these tumours is different.

The triple negative cases account only 12%, which is in consonance with a study conducted by Rakha EA, Ellis IO.[16,19] Two of these cases were associated with pregnancy while two patients had strong family history of endometrial carcinoma in first degree relatives. Basal like breast cancer (BLBC) is considered most common in African-Americans, especially young and premenopausal women. These tumours are more common in women with early age of menarche, increased parity, and first full-term pregnancy before age of 26 years. Other factors include lack of breast feeding and lactation suppressants.[20,21]

CONCLUSION

Majority of cases 68% were positive for ER and PgR so they belong to luminal A subtype and among these 79.47% belong to low risk category, while majority of Luminal B, all of Her 2neu positive and triple negative showed a high ki 67 index.

Recommendations: Further studies for comparison of grading and p53 expression needs to be performed in our setting to further improve management and survival of patients of this region.

AUTHORS' CONTRIBUTION

SN, SN, AR: Write-up, literature search and discussion. FJ: Data collection and compilation, NA: write-up and proof read the article.

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Address for Correspondence:
Dr. Shagufta Naeem, Assistant Professor Pathology Department, Ayub Medical Abbottabad-Pakistan
Cell: +92 334 895 2197
Email: drshaguftanaeem@yahoo.com