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Clinical Outcome of Cytokeratin 5/6 by Immunohistochemistry in Basal like Triple Negative (ER, PR and HER2 negative) Breast Carcinoma

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ABSTRACT

Background: Breast cancer is a heterogeneous disease, which can be classified into biologically, morphologically and clinically meaningful entities. Prognostic indicators based on currently available clinical and histopathologic variables such as tumor size, tumor grade, lymph node status and hormone receptor status already exist and are used to predict a patient's clinical outcome in certain situations. Classification of breast cancer based on the microarray method is difficult to put into widespread clinical practice due to very high costs. Aim of the study: To evaluate the clinical outcome of cytokeratin 5/6 by immunohistochemistry in basal like triple negative (ER, PR and HER2 negative) breast carcinoma. **Methods:** This cross sectional study was conducted in the department of BSMMU from January 2016 to December 2017. A total of 41 cases previously diagnosed as TNBC by histological and immunohistochemical examinations were selected for the study. During collecting specimen, all relevant information were recorded systemically in a prescribed proforma. Histopathogical type of tumor was done according to WHO classification of breast tumor, 2012 and grading was done followings Nottingham modification of the Bloom -Richardson Grading System, and recorded. Immunohistochemistry of ER, PR and HER-2/neu expression of all cases were performed using Dako Autostainer Plus at the immunohistochemistry laboratory, of department of Pathology, BSMMU. Statistical analyses of the results were obtained by window based computer software devised with Statistical Packages for Social Sciences (SPSS-20). Prior to the commencement of this study the thesis protocol was submitted to the Institutional Review Board (IRB) of BSMMU, Dhaka for approval and was approved. **Results:** Out of these 41 cases; most of the cases belong to a range of 31 to 40 years. Minimum age was 26 years, maximum age was 70 years. The mean age was 42. There is no association between age and CK 5/6 immunoreactivity. It is not statistically significant at α = 0.05 level. Cases 1 (2. %) case was grade 1, 24 (59%) cases were grade 2 and 16 (39%) cases were grade 3. It appears that maximum cases belong to grade-II. Number of CK 5/6 positive case was '0'(zero) and negative was I (one) in grade-1, number of positive cases were 14 and negative were 10 in grade-II and number of positive cases were 12 and negative cases were 4 in grade-III. It appears that grade-I tumor is negative and only the high grade cases shows positivity. 11 (27 %) cases are highly positive to CK 5/6, 15 (36 %) cases are low positive to CK 5/6 and 15 (37 %) cases are negative to CK 5/6. **Conclusion:** A significant overlap was observed between the triple-negative breast cancers and basal like breast carcinoma, the "triple negativity" should not be used as a surrogate marker for the basal-like breast cancers. By adding, CK 5/6 and/ or EGFR as the positive markers to the triple negative phenotype, a significantly worse outcome group can be identified among the triple-negative cases.

Keywords: Clinical Outcome, Breast Carcinoma, Immunohistochemistry, Heterogeneous.

INTRODUCTION

Breast cancer is a heterogeneous disease, which can be classified into biologically, morphologically and clinically meaningful entities. Approximately 12 to 17% are triple-negative breast cancers lacking expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2).[1] In particular, triple-negative breast cancers are clinically problematic as there is no approved targeted systemic therapy for these lesions; in some series, patients with triple negative breast cancers have an increased risk of recurrence between the first and third years after diagnosis and an increased mortality in the first 5 years after treatment. Currently, chemotherapy is the only systemic therapy available for triple-negative breast cancers. Triple-negative breast cancer patients with extensive clinical information was reviewed histopathologically and subjected to a panel of immunohistochemical biomarkers characterizing 'Core Basal' lesions. Breast cancer is a heterogeneous disease composed of growing number of recognized biological subtypes. Prognostic indicators based on currently available clinical and histopathologic variables such as tumor size, tumor grade, lymph node status and hormone receptor status already exist and are used to predict a patient's

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clinical outcome in certain situations. Classification of breast cancer based on the microarray method is difficult to put into widespread clinical practice due to very high costs. For this reason, the classification is primarily based on immunohistochemical techniques in order to assess the expression of the following markers: ER, PgR, HER2, CK5/6 and EGFR. TNBC is high risk due to its rapid drug resistance and recurrence, metastasis, and lack of targeted therapy. Progress in TNBC therapy remains an important challenge. Currently, standard chemotherapy (anthracyclines and taxanes) is still the main strategy, but no molecularly targeted therapeutic agents are being used for TNBC in clinical practice. Treatments that target molecules, such as JAK2, TROP2, AR, EGFR, FGFR2, VEGF, PARP, and mTOR, are still at the stage of investigation. Thus, it is essential to discover new treatment targets for TNBC. With the emergence of a large volume of cancer genomic data, the exploration of genomic profiles for TNBC could improve our understanding of this disease and identify promising targets for TNBC therapy. To develop new targeted therapeutic and immunotherapeutic drugs, we need to explore the heterogeneity in TNBC, effectively stratify TNBC into different subtypes, and identify predictive biomarkers for selective therapy of TNBC based on oncogenomics data. Basal-like breast cancer demonstrates typical metastatic sites. It often tends to spread to the brain and lungs but very rarely to the bones and liver. Those lesions with higher CK 5/6 and EGFR expression demonstrate significantly more often central nervous system and lung recurrence.The triple negative phenotype of breast cancer is a lower disease-free survival, a higher predisposition to form visceral metastases and poorer outcomes as compared to the other subtypes of breast cancer.

MATERIALS AND METHODS

This cross sectional study was conducted in the department of BSMMU from January 2016 to December 2017. A total of 41 cases previously diagnosed as TNBC by histological and immunohistochemical examinations were selected according to following inclusion and exclusion criteria for the study. During collecting specimen, all relevant information were recorded systemically in a prescribed proforma. Aim of the study was to evaluate the clinical outcome of cytokeratin 5/6 by immunohistochemistry in basal like triple negative (ER, PR and HER2 negative) breast carcinoma. Histopathogical type of tumor was done according to WHO classification of breast tumor, 2012 and grading was done followings Nottingham modification of the Bloom -Richardson Grading System, and recorded. Immunohistochemistry of ER, PR and HER-2/neu expression of all cases were performed using Dako Autostainer Plus at the immunohistochemistry laboratory, of department of Pathology, BSMMU. Scoring were done by multiplying the percentage of CK 5/6 expression cells with the intensity of its positivity. Tumors were classified based on positivity and negativity of the stain. No staining observed or membrane staining is observed in less than 10% of tumor cells. A faint/barely perceptible membrane staining is detected in more than 10% of tumor cells. A weak to moderate complete or incomplete membrane staining is seen in > 10% of tumor cells (weakly positive). A strong complete membrane staining is seen in > 30% of tumor cells (strongly positive). Scoring were done by multiplying the percentage of CK 5/6 expression cells with the intensity of its positivity. Then the scores of percentage and staining intensity were multiplied to produce a weighted score for each tumor. A score of less than four (2-3) was considered as low expression and more than the four was considered as high expression. Statistical analyses of the results were obtained by window based computer software devised with Statistical Packages for Social Sciences (SPSS-20). Prior to the commencement of this study the thesis protocol was submitted to the Institutional Review Board (IRB) of BSMMU, Dhaka for approval and was approved.

Inclusion Criteria

- Breast tumor samples were taken from paraffin blocks diagnosed as triple-negative invasive breast carcinoma by histopathology and immunohistochemistry.

Exclusion Criteria

- Inadequate tissue present in paraffin blocks.
- Equivocal results.
- Recurrence breast carcinoma cases.
- Cases treated with neoadjuvant chemotherapy or radiotherapy prior to surgery.

RESULTS

A total of 41 cases previously diagnosed as TNBC by histological and immunohistochemical examinations were selected and reviewed. [Table 1] shows the age distribution of the patients. Out of these 41 cases; most of the cases belong to a range of 31 to 40 years. Minimum age was 26 years, maximum age was 70 years. The mean age was 42. This table shows that, there is no association between age and CK 5/6 immunoreactivity. It is not statistically significant at $\alpha = 0.05$ level [Table 2]. [Figure 1] shows the distribution of tumors according to grade. Out of 41 cases 1 (2. %) case was grade 1, 24 (59%) cases were grade 2 and 16 (39%) cases were grade 3. It appears that maximum cases belong to grade-II. [Table 3] shows the distribution of CK5/6 positive and negative status in

each grade. Total number of cases was 41. Among them, 1 case was grade-1, 24 cases were grade –II and 16 cases were grade-III. Number of CK 5/6 positive case was '0'(zero) and negative was I (one) in grade-1, number of positive cases were 14 and negative were 10 in grade-II and number of positive cases were 12 and negative cases were 4 in grade-III. It appears that grade-I tumor is negative and only the high grade cases shows positivity. [Figure 2] shows the distribution of low positive, high positive and negative expression status of CK 5/6 in TNBC. In this study, among the 41 cases, 11 (27 %) cases are highly positive to CK 5/6, 15 (36 %) cases are low positive to CK 5/6 and 15 (37 %) cases are negative to CK 5/6.

Table 1: Age distribution of TNBC cases

Age (in years)	Number of patient	Percentage
20-30	5	12 %
31-40	14	34 %
41-50	11	26 %
51-60	9	21 %
61-70	2	4 %

Table 2: Association between CK5/6 immunoreactivity and age distribution of TNBC cases

Clinicopathological parameter		No of cases	CK 5/6 immunoreactivity of total 41 cases		P value
			Positive	Negative	
Age (years)	≥ 40	23 (56%)	12 (29%)	11(26%)	3.34 (critical value) P > 0.05
	< 40	18 (43%)	14(34%)	4(10%)	

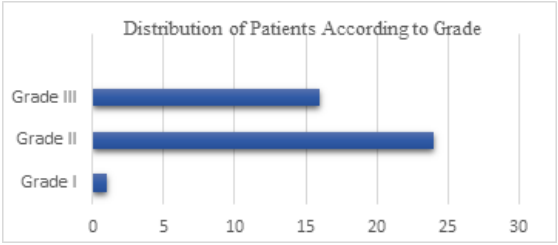

Figure 1: Barr chart showing the distribution of patients according to tumor grade.

Table III: Distribution of CK 5/6 positive and negative status in each grade.

CK5/6 Grade	Positive CK5/6	Negative CK5/6
Grade I- (1)	0	1
Grade II- (24)	14	10
Grade III- (16)	12	4

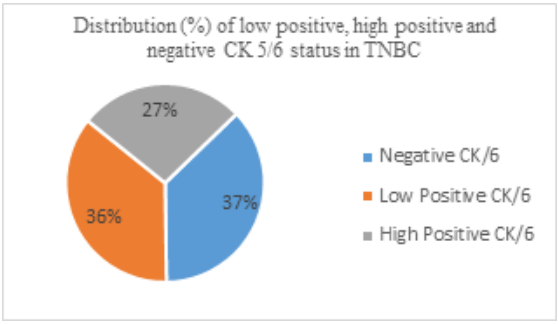

Figure 2: Pie chart showing the distribution of CK 5/6 status.

DISCUSSION

Out of the total 41cases 26 (63.41%) cases showed positive Cytokeratin staining. Number of Cytokeratin positive cases was highest in 31-40 age group followed by 41-50 age group. It is lowest in extreme of age. No association was observed between age of the patient and Cytokeratin 5/6 expression in our study. However, Rehim et al,[2] showed an inverse correlation of Cytokeratin 5/6 with the patient's age. Another study results showed, a large number of patients with the positive basal marker expression were belong to the age group of 41 to 50 years,[3] which is consistent with present study. In present study, among the 41 cases all cases were diagnosed as invasive ductal carcinoma (NST). Other study also found same type of results. In a recent study Atik et al,[4] found that majority of cases were invasive ductal carcinoma (44.4%) but the spectrum was wide despite the small number of patients included in their work. Similar to these study Nofech-Mozes et al,[5] and Williams et al,[6] described the vast majority of ductal histology (92% and 91%, respectivelly) in their series. In present study, Out of 41 cases 1 (2. %) cases were grade 1, 24 (59%) cases were grade 2 and 16 (39%) were grade 3. Number of CK 5/6 positive case was '0'(zero) and negative was I (one) in grade-1, number of positive cases were 14 and negative were 10 in grade-II and number of positive cases were 12 and negative cases were 4 in grade-III. It appears that grade-I tumor is negative and only the high grade cases shows positivity. CK5/6 is one of the commonest cytokeratin's expected in basal like breast cancers. Previous studies showed percentages of CK5/6 positivity at 61% to 62% Sood et al.[7] However, the staining of the basal cytokeratin 5/6 is challenging to be detected by immunohistochemistry because of focal and weak reactivity. Also, the use of a single basal marker as cytokeratin 5/6 might miss about half of basal-like tumors. Therefore, Nielson et al,[8] described that it is better to define the basal-like breast cancers by detecting triple negative breast carcinoma with positive CK5/6 and/or EGFR. However due to lack of resource and 53 unavailability we did not do any EGFR staining. So some basal type cases might have been missed. Another study, performed by Abdelrahman et al,[9] result showed that, morphologically aggressive phenotype of TNBC were grade II-III infiltrative ductal carcinomas. CK5/6 and EGFR expressions were found in 57.1% and 71.4% of the cases respectively. However, the combined expression of

both CK5/6 and EGFR was observed in 48.6% of cases. However, Sood and Nigam7 evaluated 36 cases of TNBC and observed that 61.11% were CK5/6-positive. There are a number of studies showing the relationship between the histologic grade and hormone negativity in breast carcinomas (Rakha et al, Carey et al).[10,11] Carey et al,[11] examined hormone receptor negative tumors and found that 26% of cases were Triple negative and that these tumors were mainly of high histological grade. Similarly Toyoma et al,[12] examined all their TN breast 52 carcinomas and found 31% positive for EGFR, 52% positive for CK5/6 and 55% positive for CK14. Prognostic indicators based on currently available clinical and histopathologic variables such as tumor size, tumor grade, lymph node status and hormone receptor status already exist and are used to predict a patient's clinical outcome in certain situations (Olivotto et al).[13] The triple – negative phenotype of breast cancer has been reported to have different incidences amongst different ethnic groups, with a lower diseasefree survival, a higher predisposition to form visceral metastases and poorer outcomes as compared to the other subtypes of breast cancer (Tan et al).[14] However, it has been found that patients with a basal-like triple negative phenotype had a significantly poorer response to the chemotherapy, so alternative therapeutic strategies for these patients become more desired (Nogi et al).[15]

Limitations of the Study

Study period was of short duration. Small sample size was also a limitation of the present study. So, the results of the study may not reflect the exact picture of the whole country.

CONCLUSION

Breast cancer is a heterogeneous disease composed of growing number of recognized biological subtypes.CK5/6 is one of the commonest cytokeratins expected in basallike breast cancers. Although, a significant overlap was observed between the triple-negative breast cancers and basal like breast carcinoma, the "triple negativity" should not be used as a surrogate marker for the basal-like breast cancers. By adding, CK 5/6 and/ or EGFR as the positive markers to the triple negative phenotype, a significantly worse outcome group can be identified among the triple-negative cases.

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