⊘ OPEN ACCESS

Determining the Expressions of Cytokeratin 5/6 by Immunohistochemistry in Basal Like Triple Negative Breast Carcinoma and Its Correlation with Histomorphological Grade

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Abstract		Original Research Article

***Introduction*:** Breast cancer is the most commonly happening female cancer and the leading cause of cancer deaths worldwide. Breast cancer is a heterogeneous disease and it encompasses a variety of entities with distinct morphological appearances and clinical behaviors. Triple-negative breast cancer (TNBC) represents 12.5– 15% of all breast cancers. Its molecular dissimilarity also brings a significantly different clinical course. It has been observed that basal-like breast cancers exhibit a gene expression profile very similar to that of myoepithelial cells. ***Aim of the study*:** To identify the basal like TNBC cases and correlate them with histomorphological grade. ***Material & 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*:** A total number of 41 cases were selected for the study 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 $\alpha = 0.05$ level. Out of 41 cases 1 (2%) case was grade 1, 24 (59%) cases were grade 2 and 16 (39%) cases were grade 3. According to the grade and the status of the membrane /cytoplasmic expression of CK 5/6 protein, grade I, grade II and grade III tumors show 00(00 %), 14(53%) and 12(46%) expression of CK5/6 respectively. It shows the distribution of cases according to the expression of CK5/6 and their relation with grades .It appears that percentage of CK5/6 expression is high in high grade tumor. ***Conclusion*:** Triple-Negative Breast Cancer' encompasses a heterogeneous group of tumors that possess distinctive pathological and clinical features. CK5/6 expression could serve as biomarker for identifying TNBC patients with poor survival. These patients are not benefited from neoadjuvant chemotherapy.
Keywords: Breast Cancer, Immunohistochemistry, Histomorphological, Expressions, Cytokeratin 5/6.

INTRODUCTION

Breast cancer is the most commonly happening female cancer and the leading cause of cancer deaths worldwide. Breast cancer is a heterogeneous disease

and it encompasses a variety of entities with distinct morphological appearances and clinical behaviors. Triple-negative breast cancer (TNBC) represents 12.5– 15% of all breast cancers. Its molecular dissimilarity

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also brings a significantly different clinical course. The outcome of this cancer is much more aggressive. Very often already at the moment of diagnosis the presence of distant metastases is ascertained. Many risk factors for breast cancer have been defined. The most important are female sex, older age, early age at first menstruation, lack of physical exercise, late age of the last menstrual period, long-term hormone replacement therapy, history of exposure to ionizing radiation, high body mass index (BMI) in young women. In such cases median survival is only one year. Very characteristic of TNBC is also absence of an association between the size of the primary tumor and the presence of metastases in the regional lymph nodes. It was necessary to establish a more detailed classification of lesions belonging to the triple-negative subgroup because molecular studies showed high variability within this type. 75–80% of tumors classified as TNBCs belong to the basal-like breast cancer group. The BLBC subtype is characterized by a particularly high histological grade, high mitotic index and low differentiation. Most commonly these tumors are described as anaplastic and metaplastic lesions. One of the reasons for worse prognosis is the high neovascularization level caused by vascular endothelial growth factor (VEGF) overexpression [1]. Basal-like Triple Negative Breast Carcinoma (TNBC) has increasing attention due to overall poor prognosis even after therapy. A panel of four antibodies (ER, HER1, HER-2, and cytokeratin 5/6) can accurately identify basal-like TNBC. Great importance is also attributed to genetic factors. Among them, carrier status of the BRCA1, BRCA2, TP53, PTEN and CHEK2 genes seems to be significant. The lifetime risk of breast cancer in women with a mutation in the BRCA1 or BRCA2 gene is 60–80%. The basic method used in diagnosis of breast cancer is histopathological assessment. Evaluation of estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor 2 (HER2) expression is a standard diagnostic procedure. Immunohistochemical staining makes it possible to assess and allows to determine patient's prognosis much more properly and establish the most appropriate method of treatment. It has been observed that basal-like breast cancers exhibit a gene expression profile very similar to that of myoepithelial cells. However, it has been concluded that BLBCs show an incomplete phenotype of myoepithelial cells. Most frequently they show CK5/6 and EGFR expression with concomitant lack of SMA, CD10 and p63 expression.

METHODOLOGY AND MATERIALS

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 identify the basal like TNBC cases and correlate them with histomorphological grade. 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 number of 41 cases were selected for the study 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 (Table-1). 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). 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). 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 (Table-4). According to the grade and the status of the membrane /cytoplasmic expression of CK 5/6 protein, grade I, grade II and grade III tumors show 00(00 %), 14(53%) and 12(46%) expression of CK5/6 respectively. After the statistical analysis by SPSS 20 Spearman rank correlation coefficient was 0.99. T value

was 43.84 with a 39 degree of freedom, (df=n-2), P value is < 0.05. It shows the distribution of cases according to the expression of CK5/6 and their relation with grades .It appears that percentage of CK5/6 expression is high in high grade tumor (Table-5).

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 case.

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

Table-3: Distribution of patients according to tumor grade.

Grade	Case	Percentage
Grade-I	1	2%
Grade-II	24	59%
Grade-III	16	39%

Table-4: 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

Table-5: Correlation between tumor grade with low and high expression of CK 5/6.

Tumor grade	Low expression of CK 5/6	High expression of CK 5/6
Grade 1	00 (00%)	00 (00%)
Grade 2	10 (38%)	4 (15%)
Grade 3	5 (19 %)	7 (27 %)

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 groups. 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, Winter [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. There are a number of studies showing the relationship between the histologic grade and hormone negativity in breast carcinomas [7, 8]. Carey *et al.*, [8] examined hormone receptor negative tumors and found that 26% of cases were triple negative and that these tumors were mainly of high histological grade. Dabbs *et al.*, [9] reviewed morphologically stained 16 triple negative breast carcinomas and reported that all tumors were of high grade according to the Nottingham score. Similar to them all of our triple negative cases were high grade cases constituted the majority of them (75%). TN carcinomas are highly proliferative breast tumors and could be identified by basal cytokeratin expression. Rakha *et al.*, [7] examined a series of 1944 patients and reported positive immunohistochemical expression of CK5/6 and/or CK14 in 157 (55.7%) TN cases. Similarly Toyoma *et al.*, [10] examined all their TN breast 52 carcinomas and found 31% positive for EGFR, 52% positive for CK5/6 and 55% positive for CK14. 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. According to the grade and the status of the membrane /cytoplasmic expression of CK 5/6 protein, grade I, grade II and grade III showed 00 (00 %), 14(53%) and 12(46%) of low and high expression of CK5/6 respectively. After the statistical analysis by SPSS 20 Spearman rank correlation coefficient was 0.99, t value was 43.84. With a 39 degree of freedom (df = n-2), the P value is < 0.05. It appears that percentage of CK5/6 expression is higher in high grade of basal like TNBC, which is statistcally significant. This result is consistent with other study results [11, 10, 7] *et al*., Another study, performed by Abdelrahman *et al*., [12] 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 Nigam [11] evaluated 36 cases of TNBC and observed that 61.11% were CK5/6-positive. Siziopikou *et al*., [13] investigated CK5/6 and EGFR expressions in 271 patients (48; 18% of them were TN). Of these cases 32 (67%) were CK5/6 positive and 22 (69%) EGFR positive. In another study Collins *et al*., [14] searched the frequency of EGFR and basal cytokeratin expressions in TN breast cancers with or without BRCA-1 mutations and found high rates of positivity, pointing out the association of basal-like phenotype with basal cytokeratins and/or EGFR expression. We recommend doing both CK5/6 and EGFR staining to evaluate TN cases to find better results.

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 AND RECOMMENDATIONS

Triple-Negative Breast Cancer' encompasses a heterogeneous group of tumors that possess distinctive pathological and clinical features. 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. CK5/6 expression could serve as biomarker for identifying TNBC patients with poor survival. These patients are not benefited from neoadjuvant chemotherapy.

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