

A comparative Study of Clinicopathological Characteristics and Expression of Basal Markers (CK5/6 & EGFR) in Triple Negative and Non Triple Negative Breast Carcinomas in Kashmir Valley

Subuh Parvez Khan, Syed Besina Yasin and Fiza Parvez Khan

Department of Pathology, Sher e Kashmir Institute of Medical Sciences, Soura ,Srinagar, J&K, India

ABSTRACT

Background: The aim of this study was to compare the clinicopathological characteristics of Triple Negative Breast Carcinomas with Non Triple Negative Breast Carcinomas (NTNBC). Evaluation of expression of Epidermal Growth Factor Receptor (EGFR), CK5/6 in TNBC and their comparison with NTNBC was done.

Methods: 25 TNBC and 35 NTNBC were selected. The clinicopathological parameters of these two groups were compared. Each group was further immunostained for basal markers(CK5/6 and EGFR). The expression of markers in these two groups was studied and compared with each other.

Results: The mean age of TNBC and NTNBC were 47 and 49 years respectively. The majority (48%) of cases from TNBC as well as NTNBC (45%)were in size range of 2-5 cm(T2 stage). IDC-NOS was predominant histological type seen in 92% of TNBC and 100% NTNBC.TNBCs had a significantly higher tumor grade than NTNBC at presentation. LVI was seen in 40% TNBC cases and 42% NTNBC cases. Majority (52%) of TNBC cases were node negative while majority (37.14%) of NTNBC cases belonged to N1 stage. IIA was the most common stage in 36% TNBC cases . In NTNBC, majority of the cases (34%) belonged to Stage IIIA.Expression of basal markers was significantly associated with triple negative breast cancers.

Conclusion: TNBCs had a significantly higher tumor grade than NTNBCs at presentation. Expression of basal markers was significantly associated with TNBCs. Since EGFR was significantly associated with triple negative phenotype, TNBC could potentially benefit from EGFR targeted therapeutic strategies.

Keywords: Cytokeratin, EGFR, Triple Negative Breast Neoplasms.

Introduction

Breast cancer is the second most common cancer in the world and, by far, the most frequent cancer among women¹. Breast cancer is a heterogeneous disease and it encompasses a variety of entities with distinct morphological appearances and clinical behaviours. In recent years it has been evident that this diversity is the result of genetic alterations.²

Currently, the most widely used classification system of breast cancer combines histo-morphological information (such as histological subtype and grading) as well as TNM staging information.^{3,4} A new approach to classify breast tumours using molecular characteristics was first described by Sorlie et al.⁵

Triple-negative breast carcinomas lack the expression of estrogen receptor, progesterone receptor and Her2. Although patients with TNBC tend to have a poor prognosis, only chemotherapy is expected to be effective because no therapeutic targets have yet been established. Histological types of TNBCs mainly comprise of highgrade invasive ductal carcinoma, no special type [solidtubular carcinoma (or atypical medullary carcinoma), invasive ductal carcinoma with a large central acellular zone], typical medullary carcinoma, and metaplastic carcinomas.

They can be classified into two subtypes: basal and nonbasal phenotype⁶. Basal type was defined as CK5-/6-positive and/or EGFR-positive, and nonbasal type was defined as having no expression of these two markers. Although the triple-negative phenotype has been considered as sufficient to identify the 'basal-like' tumours, increasing evidence has shown that the terms 'basal-like' and 'triple-negative' are not synonymous.² The triple-negative group of breast cancer is not a homogeneous disease entity. However, a substantial fraction of these tumours belongs to the basallike tumour type, which does form a homogeneous group.

The basal-like group comprises 8% to 20% of all breast cancers.^{7,5,8-15} The majority of these tumors are ductal of no special type, but occasionally are tubular mixed,¹⁴metaplastic,¹⁶ or medullary cancers.¹⁷They have common features including younger patient age, high

histologic tumor grade, marked cellular pleomorphism, high nuclear-cytoplasmic ratio, lack of tubule formation, high mitotic index, frequent apoptotic cells, scant stromal content, a pushing border of invasion, central geographic or comedo-type necrosis. Basal-like cancer is associated with an aggressive clinical history, development of locoregional and distant metastasis (particularly in the first 5 years,¹² shorter survival, and a relatively high mortality rate.^{13,14}

Materials and Methods

The study was conducted in the Department of Pathology of Sher-e-Kashmir Institute of Medical Sciences (SKIMS), a tertiary care referral centre of Kashmir valley for a period of 7 years from December 2008 to November 2015. This study is approved by institutional review board. All the patients diagnosed with primary breast cancer enrolled in our hospital during this duration were analysed. 60 cases of invasive breast cancer were selected whose complete clinical profile and the paraffin block was available. Based on the hormonal status, the 60 cases were divided into 25 Triple Negative Breast Carcinomas(TNBC) and 35 Non Triple Negative Breast Carcinomas (NTNBC). The clinicopathological parameters of these two groups were compared.

Triple negative category included those cases which were negative for ER, PR and Her 2. Non triple negative category included those cases which were ER/PR positive, Her 2 negative or ER/PR positive, Her 2 positive or ER/PR negative, Her 2 positive. Each group was further immunostained for CK5/6 and EGFR(Biocare antibodies,India). The expression of markers in these two groups was studied and compared with each other.

Formalin fixed and paraffin embedded section were cut and placed on glass slides coated with 0.5% poly L lysine. Sections were kept in oven for half an hour at temp 55-60°C-section were deparaffinised by placing in xylene for 5 min. Slides were then dehydrated by transferring to absolute alcohol, 90% alcohol, and then to 70% alcohol followed by rehydrating the sections in running water for 10 min. Endogeneous peroxidase activity was blocked by placing slides in a mixture of methanol and hydrogen peroxide (9:1) for 20 minutes. For antigen retreival, either

heat treatment was given by transferring sections to citric acid buffer pH 6 (preheated) in an oven(for ER,PR,Her 2,CK 5/6)or enzyme treatment was given by using enzyme pronase(for EGFR). After cooling sections, sections were rinsed in distilled water and transferred to citric acid buffer for 5 min. Primary antibodies were added to the sections and left for overnight. In case of EGFR, primary antibody was kept for 30 minutes. Secondary antibody tagged with HRP was left for half an hour. Sections were then washed by citric acid buffer for 5 min. Few drops of 3,3 diaminobenzidine (DAB) were added to the sections for 5 minutes. Sections were washed and then counterstained with Haematoxylin. Slides were washed in water, dried and mounted in DPX. Positive and negative controls were used in all of the cases. Wherever there was a discrepancy, fresh slides were prepared and IHC was repeated with both positive and negative controls.

ER and PR positivity was assessed using Allred Score system. Her 2 neu IHC score was calculated combining the intensity of stain and percentage of cells stained.¹⁸ Score of 0 and 1 were taken as negative, 2 as equivocal and 3 as positive. For CK5/6 and EGFR scoring, any weak or strong cytoplasmic and/or membranous invasive carcinoma cell staining was taken as positive.¹⁹

A statistical analysis was implemented by using the SPSS 16.0 version software. The Chi square test was conducted to assess the relationship between the immunohistochemical markers and other variables. The Fischer exact test was used when the expected cell counts were less than 5. A p-value less than 0.05 was considered significant.

Results and Observations:

The study was conducted on a total of 60 cases of breast carcinomas which included 25 cases of triple negative breast carcinomas (41.7%) and 35 cases of non triple negative breast carcinomas (58.3%). All the patients were female (100%). Triple negative breast cancers had a significantly higher tumor grade than non triple negative cancers at presentation. Expression of basal markers was significantly associated with triple negative breast cancers. Comparision of TNBCs and NTNBCs is summarized in Table 1.

Table1: comparision of clinicopathological and immunohistochemical characteristics between TNBC and NTNBC group.

CHARACTERISTIC	TNBC (25 cases)	NTNBC (35 cases)	P value
≤60	20	24	>0.05
>60	5	10	
Symptoms			
Discharge	1	4	>0.05
Lump	24	31	

CHARACTERISTIC	TNBC (25 cases)	NTNBC (35 cases)	P value
T1	10	13	>0.05
T2	12	16	
T3	1	6	
T4	2	-	
IDC NOS	23	35	>0.05
IDC APO	1	-	
Medullary	1	-	
1	-	5	<0.05
2	19	28	
3	6	2	
present	10	15	>0.05
absent	15	20	
NO	13	12	>0.05
N1	5	13	
N2	5	10	
N3	2	-	
IA	7	9	>0.05
IIA	9	7	
IIB	2	7	
IIIA	4	12	
IIIB	1	-	
IIIC	2	-	
positive	10	3	<0.05
negative	15	32	
positive	11	6	<0.05
negative	14	29	



Fig. 1: Photomicrograph showing high power view of strong membranous immunoreactivity for CK 5/6 in breast carcinoma.(40X).



Fig. 2: Photomicrograph showing cytoplasmic and membranous immunoreactivity for EGFR in breast carcinoma.(40X).

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Discussion

Total number of cases in our study was 60.TNBC comprised 41.66% of total cases i.e. 25 cases and NTNBC comprised 58.33% of cases i.e. 35 cases.

Age Distribution: The mean age of TNBC and NTNBC were 47 and 49 years respectively. Various studies show a significant relationship of TNBC with younger age. These include studies of Thike AA et al ²⁰, Pillai SK et al²¹ and Rao C et al²².

Symptoms: Most common clinical presentation in our study was left sided lump . Left sided breast lump was the most common symptom in other studies as of Lakshmaiah KC et al²³ and Suresh P et al.²⁴

Primary Tumor: The majority (48%) of cases from TNBC were in size range of 2-5 cm (T2 stage) while as least number (8%) of cases belonged to T4 stage . Majority of cases (45%) from NTNBC were also in the T2 stage. No statistical significance was seen between primary tumor and tumor group. Our results are comparable to those of Yuan N et al²⁵ and Pillai SK et al.²¹

Histological Type: Invasive Ductal carcinomas-NOS(IDC-NOS) was the predominant type of tumor in TNBC comprising 23 out of 25 cases(92%).There was one case of IDC with apocrine differentiation and one case of medullary carcinoma. All the 35 (100%) cases of NTNBC were of IDC NOS type. There was no statistical correlation between histological type and tumor group. Gaopande V L et al²⁶ and Qui J et al²⁷ also found no statistically significant relation between histological type and tumor group.

Tumor Grade: 19 out of 25 cases of TNBC were of Grade 2, hence the most common grade constituting 76% of cases. Grade 3 was seen in 6 cases(24%). In NTNBC group, Grade 2 was seen in 28 cases accounting for 80% while it was followed by grade 1 seen in 5 cases(14.28%). Statistically significant association was found between tumor grade and tumor group with a p value of 0.037. Statistically significant association between tumor grade and tumor group was seen by other studies including. Li C Y et al. Li C Y et al²⁸ conducted a study in Tianjin Medical University cancer Institute and Hospital, China in which they found 42.20% of TNBC cases belonged to Grade 2, and 34.47% cases belonged to Grade 3 versus 45.66% of NTNBC belonged to Grade 2 and 32.59% cases of NTNBC belonged to Grade 3. This association of tumor group with tumor grade was statistically significant. Some other studies which showed significant association between tumor group and tumor grade include those of Nabi MG et al²⁹, Gaopande V L et al ²⁶ and Albergaria A et al.³⁰

Lymphovascular Invasion: The presence of Lymphovascular invasion (LVI) is an independent poor

prognostic factor.In our study,10 out of 25 (40%) TNBC cases and 15 out of 35(42%) of NTNBC cases had presence of LVI. No statistical significance was found between presence of LVI and tumor group. Our study is comparable to Tawfiq O et al ³¹ and Hashmi AA et al³² They also did not find any significant association of LVI with tumor group.

Nodal Stage: 52% i.e. 13 out of 25 of TNBC cases were node negative .In NTNBC group, 37.14% i.e. 13 out of 35 cases belonged to N1 stage.No association was seen between nodal metastasis and tumor group. No statistical association was seen by Nabi MG²⁹ ,Pillai SK²¹.

Tumor Stage: Tumor Stage is an important prognostic parameter. In our study IIA was the most common stage in TNBC seen in 9 out of 25 cases accounting for 36% .In NTNBC, majority of the cases i.e. 12 out of 35 cases(34%) belonged to Stage IIIA.No statistical correlation was seen between the tumor group and tumor stage. No association was also seen by Li C Y et al²⁸ and Yuan N et al²⁵

CK5/6 AND EGFR Expression: CK 5/6 was expressed in 10 (40%)cases from TNBC group(Figure 1)while as it was expressed in only 3(8%) cases from NTNBC group. The expression of CK5/6 was significantly higher in the TNBC group than the NTNBC group with a p value of 0.009. EGFR expression was present in 11 (44%) of TNBC cases(Figure 2) while as it was present in only 6(17%) of NTNBC cases. EGFR expression was significantly higher in TNBC group as compared to NTNBC group with a p value of 0.040.

Our study found a significant correlation of basal markers with the negative hormone status. Our results are similar to many other studies carried out in reputable institution across the globe. Pintens S et al³³ carried out a similar study in Belgium. They found basal markers expression in 88% of TNBC. On comparision with NTNBC, they found high association of basal markers with the TNBC than NTNBC. Similar significant correlation between basal like tumor and negative hormone status was seen by Pillai SK et al²¹ Choccalingam C et al³², Rakha EA et al³⁵, Abulkhair O et al³⁶ and Rao C et al²².

Hence, in our study there was a significant overlap between triple negative carcinomas and basal like carcinomas. Oncologists may incorporate the use of targeted therapy in basal marker positive cancers as is currently being done in hormone receptor positive cancer. This may benefit a subgroup of patient population after carefully selecting them for this treatment.

Conclusion

In conclusion, triple negative breast cancers had a significantly higher tumor grade than non triple negative

cancers at presentation. Expression of basal markers was significantly associated with triple negative breast cancers. Since EGFR was significantly associated with triple negative phenotype, TNBC could potentially benefit from EGFR targeted therapeutic strategies.

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*Corresponding author: Dr. Subuh Parvez Khan, Sher e Kashmir Institute of Medical Sciences, Soura, Srinagar. 190011, INDIA Phone: +91 9469343742 Email: khansubuh@gmail.com

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