RESEARCH ARTICLE

Clinicopathological Characteristics of Triple Negative Breast Cancer at a Tertiary Care Hospital in India

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Abstract

Background: Triple-negative breast cancer (TNBC), characterized by the lack of expression of estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2, is typically associated with a poor prognosis. The majority of TNBCs show the expression of basal markers on gene expression profiling and most authors accept TNBC as basal-like (BL) breast cancer. However, a smaller fraction lacks a BL phenotype despite being TNBC. The literature is silent on non-basal-like (NBL) type of TNBC. The present study was aimed at defining behavioral differences between BL and NBL phenotypes. Objectives: i) Identify the TNBCs and categorize them into BL and NBL breast cancer. ii) Examine the behavioral differences between two subtypes. iii) Observe the pattern of treatment failure among TNBCs. Materials and Methods: All TNBC cases during January 2009-December 2010 were retrieved. The subjects fitting the inclusion criteria of study were differentiated into BL and NBL phenotypes using surrogate immunohistochemistry with three basal markers 34βE12, c-Kit and EGFR as per the algorithm defined by Nielsen et al. The detailed data of subjects were collated from clinical records. The comparison of clinicopathological features between two subgroups was done using statistical analyses. The pattern of treatment failure along with its association with prognostic factors was assessed. Results: TNBC constituted 18% of breast cancer cases considered in the study. The BL and NBL subtypes accounted for 81% and 19% respectively of the TNBC group. No statistically significant association was seen between prognostic parameters and two phenotypes. Among patients with treatment failure, 19% were with BL and 15% were with NBL phenotype. The mean disease free survival (DFS) in groups BL and NBL was 30.0 and 37.9 months respectively, while mean overall survival (OS) was 31.93 and 38.5 months respectively. Treatment failure was significantly associated with stage (p=.023) among prognostic factors. Conclusions: Disease stage at presentation is an important prognostic factor influencing the treatment failure and survival among TNBCs. Increasing tumor size is related to lymph node positivity. BL tumors have a more aggressive clinical course than that of NBL as shown by shorter DFS and OS, despite having no statistically significant difference between prognostic parameters. New therapeutic alternatives should be explored for patients with this subtype of breast cancer.

Keywords: Triple-negative breast cancer - basal-like - non-basal-like - prognosis -India

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Introduction

Invasive breast carcinoma is the most common malignant tumor in women worldwide. Although the incidence of breast cancer has increased globally over the last several decades (Hortobagyi et al., 2005; Anderson and Jakesz, 2008; Porter, 2008), the greatest increase has been reported in Asian countries (Green and Raina, 2008). It is expected that in coming decades, Asia would account for majority of new breast cancer patients diagnosed globally. With rising incidence and awareness, breast cancer is the commonest cancer in urban Indian females (Takiar and Vijay, 2010) and the second commonest in the rural Indian women (HBCR, 2001). Over 100,000 new breast cancer patients are estimated to be diagnosed annually in India (Nandakumar et al., 1995; Agarwal et al., 2007). With a rising trend in incidence reported from various registries of National Cancer Registry Programme, presently India has become a country with the largest estimated number of breast cancer deaths worldwide (PBCR, 2001, Nandakumar et al., 2005).

Breast cancer is a heterogeneous disease and it encompasses a variety of entities with distinct morphological appearances and clinical behaviors. In recent years, it has become evident that this diversity is the result of genetic alterations (Badve et al., 2011). Molecular profiling has provided biological evidence for heterogeneity of breast cancer through the identification of intrinsic subtypes. These subtypes consist of two estrogen receptor (ER) positive types (Luminal A and Luminal B),

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and three ER-negative types (human epidermal growth factor receptor-2[HER2] expressing, basal-like and normal breast-like) (Perou et al., 2000; Sorlie et al., 2001). Triple-negative breast cancer (TNBC), characterized by the absence of ER, progesterone receptor (PR) expression and no overexpression of HER2 is typically associated with a poor prognosis, due to the aggressive tumor phenotype. The recurrence pattern of TNBC also differs from other biological subtypes of cancer. In addition, the most characteristic sites of metastases consist of the brain and lungs (Hicks et al., 2006; Reis-Filho et al., 2008; Niwińska et al., 2010). Further, TNBC is only partially responsive to chemotherapy and presents a lack of clinically established targeted therapies. TNBC accounts for 10-17% of all breast carcinomas (Sorlie et al., 2001; Sorlie et al., 2003; Hu et al., 2006; Reis-Filho and Tutt, 2008). Moreover, TNBC consist of two subtypes; basal-like (BL) and non-basal-like (NBL).

The terms TNBC and basal type often are used inter-changeably as there is an overlap in the biological and clinical characteristics of these tumors (Yamamoto et al., 2009). While basal-like breast cancer (BLBC) was originally a term used for a molecular subtype, the term TNBC applies to tumors which are negative for expression of ER, PR and HER2 on immunohistochemistry (IHC). Some authors have claimed that the basal type is composed almost entirely of TNBC and therefore, the TNBC phenotype could reliably be used as a surrogate for the basal type (De Ruijter et al., 2011). However, Rouzier et al. (2011) revealed that ER and HER2 expression were seen in 5% and 14%, respectively of basal type those had been diagnosed by gene expression profiling. Therefore, significant heterogeneity exists within the group of patients diagnosed with TNBC. Although it is currently accepted that gene expression profiling is the Gold standard for identification of BLBC (Rakha et al., 2008), additional efforts have been made to characterize the BL tumors with standard IHC. While gene expression profiling is a sophisticated and expensive method, IHC methods are cheaper, easy to reproduce and can be easily undertaken in routine diagnostic laboratories. Since then, many studies have attempted to translate the gene expression profiling results into more user-friendly methods of determining protein expression by IHC.

The IHC definition of these tumors encompasses tumors which show immunopositivity for one or more of the basal cytokeratins (CK5/6, CK14, CK17), either alone or in combination with other basal markers (EGFR, c-Kit, P-cadherin, nestin, osteonectin, vimentin, and laminin) (Rakha and Reis, 2009). Basal cytokeratins (CKs) represent a large number of high molecular weight (HMW) CKs mainly seen in the basal cell layers of stratified epithelium. Rakha and Ellis (2009) recommended 4 basal markers, namely CK5/6, CK14, CK17 and EGFR of which at least 2 should be positive to be termed as BLBC. Another panel was proposed by Nielsen et al. (2004) where BLBCs are defined as those lacking both ER and HER2 expression and expressing CK5/6 and EGFR.

This panel has shown the specificity of 100% and sensitivity of 76% for the identification of BLBCs. In this study, the expression of three basal markers viz. 34βE12, c-Kit and EGFR was used to characterize TNBC patients into BL and NBL subtypes. Furthermore, the clinicopathological features and follow-up data were examined to determine the difference between outcomes of two subgroups pattern of failure among the patients of TNBC.

Materials and Methods

Case Selection

The study was approved by the Institutional Review Board of Rajiv Gandhi Cancer Institute and Research Centre (RGCI and RC), Delhi. The subjects with TNBC were identified from the records of Department of Pathology, RGCI and RC. The patient selection criteria included the cases which had, early/locally advanced TNBC, adequate material (slides, blocks and clinical records), visited and received treatment in the Institution during January, 2009-December, 2010. A total of 67 subjects befitted the criteria of patient selection. The detailed data regarding patients' clinical history, tumor characteristics, therapy, tumor recurrence etc were collated from their clinical records as per the proforma of study. The follow-up had been maintained by reviewing clinical charts and contacting patients through telephone.

Immunohistochemistry

The immunostaining procedures were performed using formalin-fixed, paraffin-embedded tissue sections. The sections were immunohistochemically stained for ER, PR, HER2, HMWCKs, c-Kit and EGFR according to the protocols provided by the manufacturer (Table 1). For labeling, polymer based strategy was used. ER and PR results were screened manually and interpreted according to H scoring as positive only when more than 10% of tumor cells showed positive nuclear staining. HER2 immunohistochemical analysis was performed using Hercep Test Kit according to the manufacturer's instructions and results were interpreted manually. The tumors which immunohistochemically scored 3+, or 2+ and were FISH-positive, were regarded as HER2-positive.

IHC for 34βE12 was performed first as a work up for detecting expression of the basal markers, and presence of HMWCKs (CK1, 5, 10 and 14) was determined. The cytoplasmic staining pattern was considered as positive staining (Figure 2). After getting positive result for 34βE12, the IHC for c-Kit (CD117) was performed.

The presence of cytoplasmic staining along with

Table 1. Specifications of Antibodies Used

Marker	Clone	Manufacturer	Dilution
ER	SP1	Dako	1:50
PR	SP2	Dako	1:50
HER2	Polyclonal	Dako (HERCEP test Kit)	RTU
High molecular weight cytokeratins			
	34βE12	Dako	1:50
c-Kit/CD 117	Polyclonal	Dako	1:400
EGFR	EP774Y	Biocare	RTU

*ER, estrogen receptor; PR, progesterone receptor; EGFR, epidermal growth factor receptor

membranous staining was taken as positive staining (Figure 2B). When the tumor showed positive staining for 34βE12 and negative staining for c-Kit, the IHC for EGFR was performed. The presence of cytoplasmic staining together with membranous staining was considered as positive staining for EGFR (Figure 2C). Thus, TNBC cases with 34βE12+, c-Kit+, EGFR- or 34βE12+, c-Kit-, EGFR+ were defined as BLBC (Figure 1) and rest as non-basal-like breast cancer (NBLBC). In other words, the tumor was considered BL if it expressed 34βE12 and one of the basal markers i.e. c-Kit or EGFR. A positive control, prepared from tissue known to contain the antigen under study was taken on individual slides to determine if the staining system was working properly, positive and negative staining specific and whether the correct procedure was followed.

Statistical analysis

The qualitative data were presented in frequencies and percentages and quantitative data were presented by mean (standard deviation [SD]) or median (interquartile range [IQR]). The subjects with missing information were excluded from the analysis. Student's't' or Mann-Whitney U test was applied for quantitative variables and for categorical variables, chi-square and Fisher's exact tests were used for statistical significance. Univariate and multivariate logistic regression analyses were carried out to identify the prognostic factors associated with BLBC with respect to NBLBC. Overall survival (OS) and disease-free survival (DFS) were measured from date of diagnosis until death/date of last contact and date of diagnosis to relapse/progression of disease/date

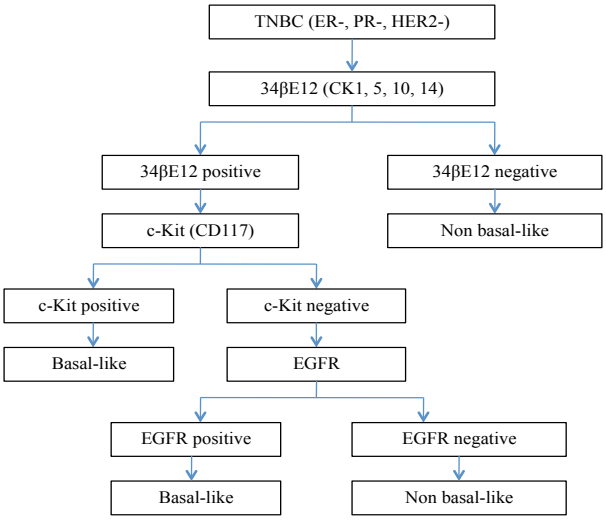

Figure 1. Algorithm for Defining Basal-Like Breast Cancer (adopted from Nielsen et al)

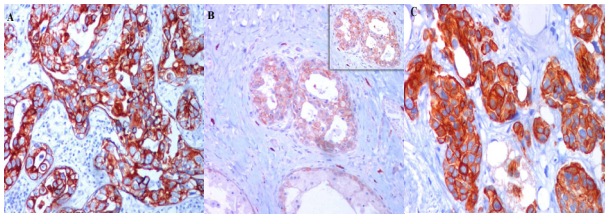

Figure 2. Positive Immunohistochemical Staining for A) 34βE12; B) c-Kit; C) EGFR; 200 X

Table 2. Clinicopahological Features of Triple-negative Breast Cancer

Characteristic		Frequency (%) (N=67)
Average age (years) (Mean±SD)		48±11.47
Menstrual history	Pre menopausal	28 (41.8)
	Peri menopausal	5 (7.5)
	Post menopausal	34 (50.7)
Laterality	Right	35 (52.2)
	Left	31 (46.3)
	Bilateral	1 (1.5)
Tumor size (cm)	0.1-2	4 (6.0)
	2.1-5	47 (70.1)
	>5	16 (23.9)
LN status (cN)	Negative	30 (44.8)
	Positive	37 (55.2)
Stage	I	2 (3.0)
	II	47 (70.1)
	III	18 (26.9)
Surgery	MRM	54 (80.6)
	BCS	11 (16.4)
	Not done	2 (3.0)
Axilla clearance	Yes	65 (97.0)
	NA	2 (3.0)
Tumor subtype	Ductal carcinoma	61 (91.0)
	Medullary carcinoma	2 (3.0)
	Metaplastic carcinoma	2 (3.0)
	Lobular carcinoma	1 (1.5)
	Apocrine carcinoma	1 (1.5)
Histological grade	I	1 (1.5)
	II	19 (28.4)
	III	47 (70.1)
LN status (pN)	Positive	29 (43.3)
	Negative	36 (53.7)
	NA	2 (3.0)
Extracapsular extension	Present	14 (20.9)
	Absent	14 (20.9)
	NA	38 (56.7)
	Unknown	1 (1.5)
Lymph-vascular invasion	Present	27 (40.3)
	Absent	40 (59.7)
34βE12	Positive	54 (80.6)
	Negative	13 (19.4)
c-Kit	Positive	35 (52.2)
	Negative	19 (28.4)
	NA	13 (19.4)
EGFR	Positive	19 (28.4)
	NA	48 (71.6)
Phenotype	Basal	54 (80.6)
	Non basal	13 (19.4)
Chemotherapy	Yes	56 (83.6)
	No	11 (16.4)
Radiotherapy	Yes	30 (44.8)
	No	37 (55.2)
Failure	No	55 (82.1)
	Yes	12 (17.9)
Disease status at last follow-up	Disease free	52 (77.6)
	Alive with disease	5 (7.5)
	Expired	10 (14.9)
Vital status	Alive	57 (85.1)
	Dead	10 (14.9)
Cause of death	Disease	9 (13.4)
	Other than disease	1 (1.5)
	NA	57 (85.1)

BCS, breast conservative surgery; Ca, carcinoma; LN, lymph node; MRM, modified radical mastectomy; NA, not applicable; SD, standard deviation

of last contact respectively. Survival curves were drawn by the Kaplan-Meier method and differences assessed by the stratified log-rank test. The statistical analyses were performed using SPSS version 20.0 (IBM Corp, Armonk, NY). The results were considered as statistically significant if the p value was <0.05.

Results

A total of 67 cases of TNBC were included in this retrospective study. Table 2 summarizes the background data including clinicopathological, immunohistochemical features, failure of therapy and survival etc. The mean and median ages at diagnosis were 48.3 and 49.0 (range 25-72) years respectively depicting the majority at post-menopausal state (51%; 34/67). The patients predominantly reported at clinical stage II (70%; 47/67). 97% (65/67) of all patients underwent surgery (modified radical mastectomy/breast conserving surgery) along with axillary lymph node clearance. A total of 87% (58/67) patients received chemotherapy (CT) as neo-adjuvant, adjuvant or palliative CT; however, 45% (30/67) of the cohort received radiotherapy. The histopathological evaluation revealed a large proportion of patients with poorly differentiated high grade tumors (70%; 47/67) and infiltrating duct carcinoma (91%; 61/67) as primary histology morphology. The lymph node metastases were noted in 45% (29/65) cases with extra capsular extension in 48% (14/29) among the operated patients. The presence of lymph-vascular invasion was found in 40% (27/67) cases.

The frequency of basal marker expression was assessed; the BL and NBL subtypes accounted as 81% (54/67) and 19% (19/67) respectively of the entire group in study. The median follow-up time was 33 (IQR 28-44) months. Among all patients, 18% (12/67) had treatment failure in the form of progressive disease (during treatment of primary tumor), local recurrence and distant metastasis. Table 4 outlines the site(s) of failure and clinical course of patients. Among patients with treatment failure, 19% (10/54) were with BL and 15% (2/13) were with NBL phenotype. The most common sites of first failure were local, lung and lymphnodes, however brain metastasis was observed as the most common site following first recurrence/failure. The second failure was observed in BLBC patients only. In the entire group, most of patients

Table 3. Associations between BLBC and Prognostic Factors compared with NBLBC

Variable (N)		Phenotype (N=67)		Univariate analysis		Multivariate analysis	
		BLBC n(%)	NBLBC n(%)	Chi-square	OR (95%CI)	AOR (95%CI)	p value
Age (n=67)	≤50	28 (51.9)	8 (61.5)	0.40	1 (Ref)	1 (Ref)	0.750
	>50	26 (48.1)	5 (38.5)		0.67 (0.20-2.32)	0.66 (0.15-2.98)	
Tumor size (n=67)	0.1-2	3 (5.6)	1 (7.7)	0.57	1 (Ref)	1 (Ref)	0.570
	2.1-5	39 (72.2)	8 (61.5)		1.63 (0.15-17.7)	1.64 (0.12-22.2)	
	>5	12 (22.2)	4 (30.8)		1.00 (0.08-12.6)	0.08 (0.002-3.37)	
Stage (n=65)	2	36 (69.2)	11 (84.6)	1.23	1 (Ref)	1 (Ref)	0.325
	3	16 (30.8)	2 (15.4)		2.32 (0.46-11.7)	18.78 (0.79-446)	
Tumor subtype (n=67)	Ductal Ca	50 (92.6)	12 (92.3)	0.01	1 (Ref)	1 (Ref)	0.998
	Others	4 (7.4)	1 (7.7)		0.96 (0.10-9.39)	0.99 (0.10-9.93)	
Grade (n=66)	2	15 (28.3)	4 (30.8)	0.03	1 (Ref)	1 (Ref)	0.996
	3	38 (71.7)	9 (69.2)		1.06 (0.28-3.93)	1.43 (0.31-6.50)	
LN status (pN) (n=65)	Positive	23 (44.2)	6 (46.2)	0.02	1 (Ref)	1 (Ref)	0.998
	Negative	29 (55.8)	7 (53.8)		0.93 (0.27-3.13)	0.45 (0.10-2.10)	
Lymphovascular invasion (n=67)	Positive	23 (42.6)	4 (30.8)	0.61	1 (Ref)	1 (Ref)	0.538
	Negative	31 (57.4)	9 (69.2)		1.67 (0.46-6.10)	1.76 (0.38-8.20)	

*LN, lymph node

Table 4. Site Distribution of Disease Failure Including Clinical Course

S No	Phenotype	Disease-free survival (months)	Site(s) of failure	Treatment after failure	Site(s) of PD	Treatment at progression	Time relapse to death (months)	Overall survival (months)
1	Non Basal	29	Bone & lung	CT	NA		NA	37
2	Basal	37	Bone & LNs	CT	NA		NA	39
3	Basal	5	Lung	CT	NA		8	14
4	Basal	3	Local	RT	Brain	RT followed by CT	5	9
5	Basal	9	Liver, lung & LNs	RT followed by CT	Brain	RT	11	20
6	Basal	13	Liver & brain	CT	Local	No treatment received	16	29
7	Basal	15	Local & skin	CT	NA		7	22
8	Basal	22	Lung	No treatment received	NA		3	25
9	Non Basal	50	Local	No treatment received	NA		NA	50
10	Basal	8	Liver & LN	CT	NA		6	15
11	Basal	31	Local	Surgery	NA		NA	32
12	Basal	10	Local & LNs	CT	NA		NA	29

*CT, chemotherapy; LN, lymph node; NA, not applicable; RT, radiotherapy

Table 5. Associations between Treatment Failure and Prognostic Factors

Variable (N)	Failure		Chi-suqare	p value
	No n(%)	Yes n(%)		
Age (years)				
≤50	27 (49.1)	9 (75.0)	2.66	0.123
>50	28 (50.9)	3 (25.0)		
Tumor size (cm) (n=63)				
2.1-5	41 (80.4)	6 (50.0)	4.74	0.071
>5	10 (19.6)	6 (50.0)		
Stage (n=65)				
2	42 (79.2)	5 (41.7)	6.90	0.023*
3	11 (20.8)	7 (58.3)		
Tumor subtype (n=67)				
Ductal Ca	50 (90.9)	11 (91.7)	0.02	0.997
Others	5 (9.1)	1 (8.3)		
Grade (n=66)				
2	17 (31.5)	2 (16.7)	1.05	0.484
3	37 (68.5)	10 (83.3)		
LN status (pN) (n=65)				
Positive	21 (39.6)	8 (66.7)	2.90	0.114
Negative	32 (60.4)	4 (33.3)		
Extracapsular extension (n=28)				
Positive	9 (45.0)	5 (62.5)	0.70	0.678
Negative	11 (55.0)	3 (37.5)		
Lymphovascular invasion (n=67)				
Positive	20 (36.4)	7 (58.3)	1.98	0.280
Negative	35 (63.6)	5 (41.7)		
Phenotype (n=67)				
No	44 (80.0)	10 (83.3)	0.07	0.997
Yes	11 (20.0)	2 (16.7)		
Vitas status (n=67)				
Alive	52 (94.5)	5 (41.7)	21.70	.000**
Dead	3 (5.5)	7 (58.3)		

*Ca, carcinoma; LN, lymph node; *p<0.05; **p<0.001

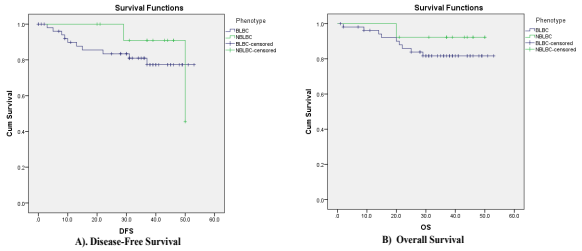

Figure 3. Kaplan-Meier Curves for (A) Disease-free Survival and (B) Overall Survival for All Patients

were found to be alive and disease free (78%, 52/67) and 7% (5/67) were alive with disease, while 15% (10/67) had expired at last contact. Among dead patients, disease was the main cause of death (90%, 9/10) except for 1 patient who was free of disease and died of kidney failure.

The analysis between failure of cancer therapy and well established prognostic parameters viz, age, tumor size, stage, tumor subtype, tumor grade, lymphnode status, extracapsular extension, lymph-vascular invasion and phenotype etc showed that failure was significantly associated only with stage (p=0.023) among prognostic factors and vital status (p<0.001) (Table 5). Multivariate regression analysis was done to see the correlation between BLBC and prognostic factors in comparison with NBLBC. No statistically significant association was seen between prognostic parameters and the two subtypes (Table 3). A significant correlation between size of tumor and the incidence of lymph node positivity was observed (p=0.037).

Survival Analysis: At average follow-up of 33.21 months, the subset BL showed relatively shorter DFS as well OS than that of NBL (Figure 3). The mean DFS in group BLBC and NBLBC was 29.98 and 37.92 months respectively, while mean OS was 31.93 and 38.54 months respectively. No statistical significant differences in DFS and OS were detected between two subtypes.

Discussion

TNBC contributes a large proportion of breast cancer deaths despite its small proportion among all breast cancers. Our study was designed to see the difference between the clinical outcomes of BL and NBL subgroups of TNBC along with the pattern of failure in same cohort. In our study, TNBC comprised 18% of the total breast cancer cases which is comparable to that of a study showing the prevalence of TNBC as 19.9% in Indian population (Patil et al., 2011). In contrast to this, other studies showed different frequencies of triple negativity as 25% (Ambroise et al., 2011) and 11.8% (Sharma et al., 2013) in Indian data. The median age at diagnosis was 49 years which showed similarity to (Thike et al., 2010; Rao et al., 2013) and variation from other studies (Dent et al., 2007; Suresh et al., 2013). Clinically stage II was the commonest stage at presentation in accordance to the previous findings (Niwińska et al., 2010; Rao et al., 2013; Suresh et al., 2013) followed by III and I. This reflects the awareness among population presenting to a private tertiary cancer care centre located in a metropolis. The bias towards MRM (54/65) was highly significant in our study. Despite of CT planned as per treatment protocol, a number of patients (9/67) did not choose to have CT at our centre.

A majority of TNBC are high grade invasive ductal carcinomas of no special type and a few were medullary Ca, metaplastic Ca and apocrine Ca suggesting that TNBC may occur in all histological subtypes of breast malignancies with probable association with pathogenesis, progression and prognosis (Reis-Filho et al., 2008; Thike et al., 2010; Kutomi et al., 2012). A fairly large proportion (81%) of cases fell into the subtype BL comparable with other authors (Bertucci et al., 2008; Rakha et al., 2009; Badve et al., 2011; Niwińska et al., 2011). However Rakha et al. (2007) have reported about 50% of patients with BL subtype. In the present study, no statistically significant association could be seen between prognostic parameters considered here and the two subtypes in agreement with some studies (Niwińska et al., 2010; Choccalingam et al., 2012). Conversely several studies have shown that the basal type is associated with tumor size and nuclear grade (Nishimura and Arima, 2008; Sasa et al., 2008; Iwase et al., 2010). Our limited study population may be the reason behind non-association.

Studies have shown that BL subtype has been associated with poor clinical outcomes (Sorlie et al., 2003; Sotiriou et al., 2003; Nielsen et al., 2004). The differences in survival between patients with BLBC and NBLBC have been evaluated in some studies, but the

results were not unambiguous. Albeit not significant, the DFS of basal subtype was shorter than that of NBL subtype in our study. Similar observations were made in other studies (Rakha et al., 2009; Niwińska et al., 2011; Choccalingam et al., 2012). We did not observe statistically significant differences between BL and NBL subgroups with respect to treatment failure. Our results revealed more frequent local relapses and metastases to lung and LNs whereas some studies show brain and lungs to be the most characteristics sites of metastases among TNBC (Hicks et al., 2006; Reis-Filho et al., 2008; Niwińska et al., 2010). Our results also showed that BLBC are different from NBLBC in pattern of treatment failure. Of all 12 patients who had treatment failure, 10 belonged to group BL and only 2 cases were with NBL type. The cases those experienced treatment failure twice were also from group BLBC only. All 9 patients those had disease related mortality, were under group BLBC. In our cohort, BLBC had a high propensity of local relapses together with metastatses to lymphnodes, brain and liver compared to NBLBC. In the study by Niwińska et al. (2011), a very homogenous group of TNBC patients with brain metastasis was assessed which showed that lungs and brain were the most relevant sites of distant metastases, similar to findings of Rakha et al. (2009). Though not many studies have been undertaken in India on this subject, performance of IHC for basal markers as in the present study would contribute to unraveling the mystery of TNBC in the Indian setting further.

More recently, the subtyping of three large clinical trials (MA.5 [Levine et al., 2005], GEICAM/9906 [Martín et al., 2008] and MA.12 [Bramwell et al., 2010]) using the PAM50 qRT-PCR based assay showed that approximately 30% of TNBC identified by central pathology review do not fall under the BL subtype category (Cheang et al., 2012). Lately, Lehmann et al. (2012) and Masuda et al. (2013) have reported that TNBC can be classified into 7 subtypes (6 defined subtypes and an unstable group) by gene expression microarray. The subtypes were characterized as BL 1, BL 2, immunomodulatory, mesenchymal, mesenchymal stem-like, luminal androgen receptor and unstable. These studies show that significant biological heterogeneity exists within the group of patients detected with TNBC.

The current study has some limitations. Our limited study population could not show the diversity in clinical outcome between the two subgroups of TNBC. Another drawback is the short duration of follow-up as the longer follow-up may make clearer differences in OS and DFS between two subgroups.

In conclusion, disease stage at presentation is an important prognostic factor influencing the treatment failure and survival among TNBC. The increasing tumor size is related to lymph node positivity. We show that identification of basal markers (HMWCKs, c-Kit and EGFR) positivity within this group of TNBC could identify a subgroup of tumors, BLBC. It has been shown that BLBC consistently overexpress HER1 or EGFR (Nielsen et al., 2004; Lakhani et al., 2005; Masuda et al., 2005), EGFR inhibitors may have a role in the treatment of this tumor subtype. The basal markers are not consistently used in the standard histological diagnosis of breast cancer. As existing prognostic markers do not identify this group, patients with BL and NBL tumors are currently treated similarly. The BL tumors have an aggressive clinical course than that of NBL as shown by shorter DFS and OS, despite having no statistically significant difference between the prognostic parameters of two subtypes. New therapeutic alternatives should be investigated for patients with this subtype of breast cancer.

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