DOI: 10.7860/JCDR/2016/20475.8539

Original Article

Pathology Section

Clinicopathological Study of Triple Negative Breast Cancers

GUNADALA ISHITHA[1], MARIE THERESE MANIPADAM[2], SELVAMANI BACKIANATHAN[3], RAJU TITUS CHACKO[4], DEEPAK THOMAS ABRAHAM[5], PAUL MAZHUVANCHARY JACOB[6]

ABSTRACT

Introduction: Triple Negative Breast Cancers (TNBC) are a subset of breast cancers which are composed of different molecular subtypes. The most common is the basal like subtype, which has an adverse prognosis and limited treatment options.

Aim: This study was undertaken to assess the clinico-pathologic and immunohistochemical subtypes of triple negative breast cancers and assess how each of these subtypes correlate with clinical behaviour and survival outcomes.

Materials and Methods: Fifty-three (22.2%) of 238 cases of primary invasive breast carcinomas diagnosed from January 2010 to June 2011 were found to be negative for immunohistochemical markers- ER, PR and HER2. These fifty three cases were included in the study and were classified into four histological subtypes proposed by Ishikawa et al. Basal markers- CK5/6, EGFR and CK14 were done on these cases and they were further classified immunohistochemically into basal and non basal subtypes. The morphological features, disease free survival and overall survival were evaluated for both basal and non basal subtypes.

Results: Majority (96%) of TNBC cases were classified according to WHO as invasive ductal carcinoma (NOS). Type C Ishikawa histological subtype was found to be the commonest subtype in both basal and non-basal TNBC. Of 53 TNBC cases, basal immunohistochemical markers were performed on 47 cases only because of paucity of tissue. Of these 47 cases, thirty-five (74.4%) were found to be of basal like subtype and all these cases were picked up by a combination of CK5/6 and EGFR.

Conclusion: High grade morphological features, hormonal markers with additional use of basal markers can help identify the basal like subtype of TNBC, thereby predicting breast cancer survival. The combination of CK5/6 and EGFR identified all cases of basal subtype. EGFR in addition also has potential therapeutic implications. The morphological features and survival outcomes were not significantly different between basal and non-basal phenotypes.

Keywords: Basal like, Estrogen, Non basal, Progesterone

INTRODUCTION

Triple Negative Breast Cancers (TNBC) are defined as tumours that are negative for estrogen and progesterone receptors as assessed by Immunohistochemistry (IHC), combined with lack of over-expression of HER2 when tested by IHC or absence of its gene amplification when tested by fluorescence in situ hybridization technique [1]. Hence these cancers do not respond to hormonal therapy and chemotherapy is currently the treatment of choice [2].

TNBC account for 10-20% of all breast cancers worldwide and different molecular subtypes have been identified, the basal like subtype being the most common [3,4]. The basal like subtype is associated with an aggressive clinical behaviour, present usually in younger women with early development of recurrence, distant metastasis and poor survival [5].

Gene expression profiling, the gold standard to identify the molecular subtypes of TNBC is difficult to use in routine clinical practice because of its prohibitive cost and suboptimal results obtained with paraffinized material [6]. There are no specific morphological parameters that can identify these tumours reliably by routine histology. Many studies have therefore used basal immunohistochemical markers as a surrogate to identify the basal like subtype [7]. These markers permit identification of the basal like subtype at an affordable cost with feasibility of use in routine clinical practice and without the need for fresh tissue [6,8,9].

This study was undertaken to assess the frequency and evaluate the clinico-pathological features of TNBC in our institution. We used three immunohistochemical markers as a surrogate for gene expression profiling, to classify TNBC into basal and non-basal subtypes. We studied in detail the morphological features of both basal and non basal like subtypes.

In addition we also attempted to classify TNBC into four prognostically different histological subtypes as described by Ishikawa et al., [10]. The immunohistochemical and histological classifications were also correlated with disease free and overall survival outcomes.

MATERIALS AND METHODS

This study was approved by the institutional review board with waiving off the need for informed consent as fresh frozen paraffin embedded tissues of a retrospective cohort of patients were used for the study. A total of 238 cases of primary invasive breast carcinomas diagnosed between the periods January 2010 to June 2011, in the department of pathology in our institution were screened for 3 IHC markers, ER, PR and HER2. Of these 238 cases, 53 cases were found to be triple negative for ER, PR and HER2 and were included in the study. Cases with insufficient tissue sample required for performing further IHC were excluded.

The clinical details were obtained from the hospital records through the electronic database and patient charts from the medical records. The clinical features analysed included age, sex, size, side, quadrant of tumour involvement, chemotherapy status, clinical staging, metastasis, family history and treatment received.

The histomorphological features were studied in detail. The cases in our study were classified according to Ishikawa subtypes of TNBC [10] as follows: Type A- “atypical” medullary carcinoma, Type B- carcinoma with a central acellular zone, Type C- other invasive ductal carcinomas, Type D- special types.

Density of lymphocytic infiltration was graded as mild, moderate and marked according to Thike et al., [11]. Tumours with up to one-third of lymphocytic infiltration were graded as mild, one-third to two-thirds as moderate and more than two thirds was graded as marked. Amount of cytoplasm was assessed depending on the Nucleo-Cytoplasmic (N/C) ratio [12].

Three IHC markers, CK5/6, CK14 and EGFR [Table/Fig-1] were used to categorize these tumours into basal and non-basal subtypes. More than 1% tumour cells staining for any one of these markers was considered as positive [13].

Overall and disease free survival of TNBC and of basal and non-basal subtypes was performed using the Kaplan Meier survival analysis [14].

RESULTS

Fifty three (22.2%) cases were found to be negative for ER, PR and HER2. The microscopic features were analysed in 52 of 53 cases as in 1 case the paraffin embedded block was received as a consult case and returned to the patient after diagnosis.

Type of specimen: Of 53 cases of TNBC, 35 cases (66%) were of modified radical mastectomy specimens, 4 cases (7.5%) were of lumpectomy, 1 case (2%) was a wedge biopsy, 7 cases (13.2%) had only a trucut biopsy and the remaining 6 cases (11.3%) were received as consultation from other hospitals.

Clinical features [Table/Fig-2]: The mean age (with standard deviation) of TNBC patients in our present study was 46±12 years. The youngest patient with TNBC was 23-year-old and the oldest patient was 79-year-old. The average size of tumour was 4.3 cm and 53% of cases presented with stage III and IV disease. There was no statistically significant difference in the laterality of breast involvement.

WHO subtype: Ninety-six percent cases were classified as invasive ductal carcinoma, Not Otherwise Specified (NOS).

Histological grading: Modified Bloom-Richardson histological grading [15] was done only in 49 cases because mitotic activity could not be assessed in 3 cases. None of the cases was found to be of grade 1 [Table/Fig-2].

Borders: Majority of TNBC cases, 37.8% had a predominantly pushing border, 31.1% had both infiltrating and pushing borders and 31.1% had infiltrating border.

Tumour infiltrating lymphocytes: Ninety-eight percent cases of TNBC had lymphocytic infiltrate out of which 54% had mild infiltrate, 33% had moderate infiltrate and 11% had dense infiltrate.

Cytological features of TNBC: Ninety-eight percent cases had polygonal cells, 2% had spindle shaped cells, 72% had hyperchromatic nuclei, 57% had prominent nucleoli, 87% had moderate amounts of cytoplasm and 98% of cases had high N/C ratio.

Necrosis and central acellular sclerosis: Necrosis was present in 4% cases. Only 1 case showed central geographic necrosis. Central acellular sclerosis without necrosis was seen only in 12% of cases.

Metaplasia in TNBC: Three out of 52 cases (6%) of TNBC had shown focal squamous differentiation. Of these 3 cases, 1 case also showed syncytiotrophoblasts with bizarre giant cell formation.

Ductal Carcinoma In Situ (DCIS) in TNBC: Seventeen out of 52 cases (33.3%) of TNBC had an associated DCIS component. Of these 17 patients with DCIS, 8 (47%) cases were found to be triple negative of which 3 cases were found to be basal like both in DCIS and invasive component.

Lymphovascular invasion: It was seen in 69% cases.

Ishikawa subtype: TNBC were classified according to the Ishikawa subtypes. Invasive ductal carcinoma NOS (Type C -67%)

Antibody	Clone	Dilution	Source	Pre-treatment
EGFR	E30	1:50	Dako M7239	Pressure cooking
CK5/6	D5/16B4	1:100	Dako M7237	Pressure cooking
CK14	LL002	1:20	Novocastra NCL- LL002/Biogenex	Pressure cooking

[Table/Fig-1]: Antibody clones used for Immunohistochemical markers.

Parameters(N=number of cases)	Percentage (%)
Age(N=52)	46±12 years
Size(N=33)	4.3±2.56cm
Staging(N=47)	
I	9
II	38
III	38
IV	15
WHO subtype(N=52)	
Invasive ductal carcinoma	96
Other subtypes	4
Histologic grading(N=49)	
Grade II	43
Grade III	57
Tumour borders (N=45)	
Pushing	38
Pushing and infiltrative	31
Infiltrative	31
Tumour infiltrating lymphocytes(N=52)	
Absent	2
Mild	54
Moderate	33
Marked	11
Shape of cells(N=52)	
Polygonal	98
Spindle	2
Type of chromatin(N=52)	
Hyperchromatic	37
Vesicular	28
Hyperchromatic and vesicular	35
Nucleoli(N=52)	
Inconspicuous	8
Present, but not prominent	35
Prominent	57
Amount of cytoplasm(N=52)	
Moderate	87
Abundant	13
Nucleo-cytoplasmic ratio(N=52)	
Low	2
High	98
Amount of intratumoural stroma(N=52)	
Scant	44
Mild	25
Moderate	27
Abundant	4
Necrosis and central acellular sclerosis (N=52)	
Necrosis	
Absent	96
Present	4
Central geographic necrosis(N=52)	98
Absent	
Present	
Central acellular sclerosis without necrosis (N=52)	2
Absent	88
Present	12
Squamous metaplasia(N=52)	
Absent	94
Present	6
Other Metaplasia(N=1)	2
Ductal carcinoma in situ(N=52)	
Absent	67
Present	33
Lymphovascular invasion(N=52)	
Absent	31
Present	69
Ishikawa subtypes	
Type A(Atypical medullary carcinoma)	15
Type B (Carcinoma with central acellular zone)	14
Type C (other invasive ductal carcinoma)	67
Type D (Special types)	4

[Table/Fig-2]: Clinicopathological features of triple negative breast carcinomas.

was found to be the commonest subtype, followed by "atypical medullary carcinoma" (type A -15%), carcinoma with central acellular zone (type B-14%) and special type (type D -4%).

Immunohistochemical findings: Three markers, CK14, CK5/6 and EGFR were used on 47 cases to identify the basal like subtype in TNBC. 35 cases (74.4%) were classified as basal like subtype based on positivity for any one basal marker. A total of 24 (51%) were found to be positive for CK5/6, 17 (36%) were positive for CK14 and 29 (62%) were positive for EGFR [Table/Fig-3].

EGFR as a single marker picked up the maximum number of cases of basal like subtype of TNBC. The combination of CK5/6 and EGFR picked up all cases of basal like subtype (100%) as compared to the combinations of CK5/6 and CK14 (80%) and CK14 and EGFR (91%) [Table/Fig-4].

In our study, the only clinical parameter which showed statistical significance was the size of tumours, which was found to be larger in the basal type (p-value: 0.023). There was no significant difference in the microscopic features between basal and non-basal TNBC, except for the amount of cytoplasm (p-value: 0.049) [Table/Fig-5]. In our study, follow up was done for a period of 1.5 years up to 3 years. The median disease free survival for TNBC was 27 months and the median overall survival was 33 months [Table/Fig-6]. There was no significant difference in survival outcomes between basal and non- basal TNBC [Table/Fig-7].

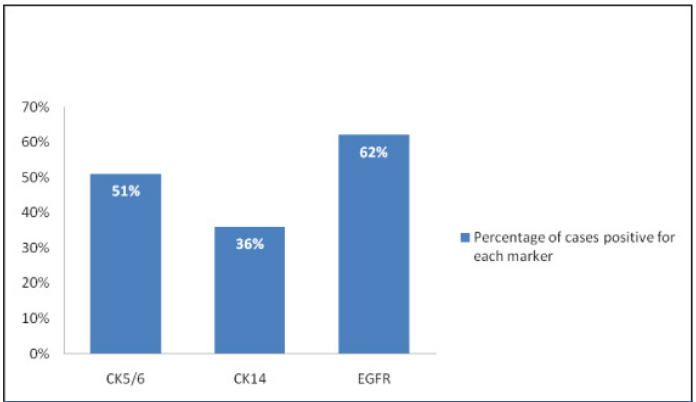
Treatment and follow up: Treatment given is depicted in [Table/Fig-8]. Anthracycline and taxane based chemotherapy was given to all 49 patients. 22 patients received radiotherapy.

A total of 12 patients presented with metastases, which included 5 of basal and 5 non-basal types and the remaining 2 cases did not have basal markers done due to paucity of tissue. 11 patients relapsed with metastases, of which 7 were of basal type and 3 of non-basal type. Only 1 case did not have basal markers done. The sites for metastases are shown in [Table/Fig-9]. There was no evidence of residual disease in 23 patients, 2 patients had died and 11 patients were lost to follow-up.

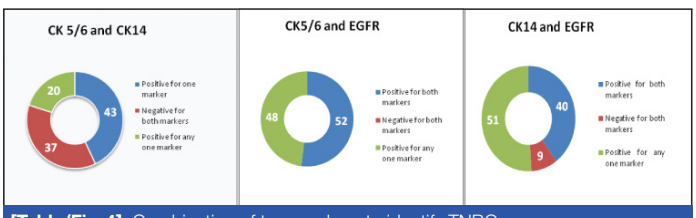
DISCUSSION

Only few Indian studies on TNBC including the basal like subtype have been published. There is a need to identify these tumours because of its aggressive behaviour, bad prognostic and treatment outcomes as shown in literature [16–23].

The frequency of TNBC in our institution constituted 22.2%. Studies from different parts of the world have shown a varying frequency of 10-20%. Studies from India have shown a slightly higher range, 11.8% to 31.9% [16–24]. The incidence is lower in studies from other parts of Asia, including Korea (14.7%), China (12.6%) and Japan (8.4%) [25-27]. TNBC are more frequent in African Americans (26.5%) and in African women (34%) when compared to Non-African Americans (16.0%) [28].



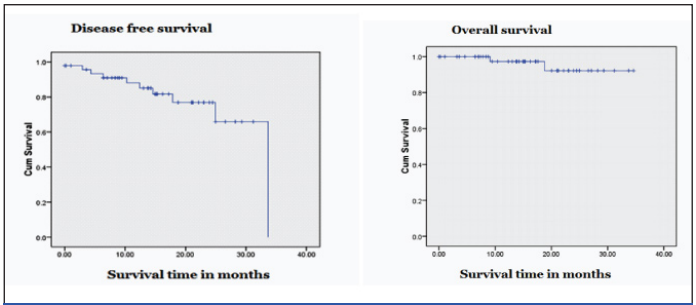
[Table/Fig-3]: Cases positive for individual markers.



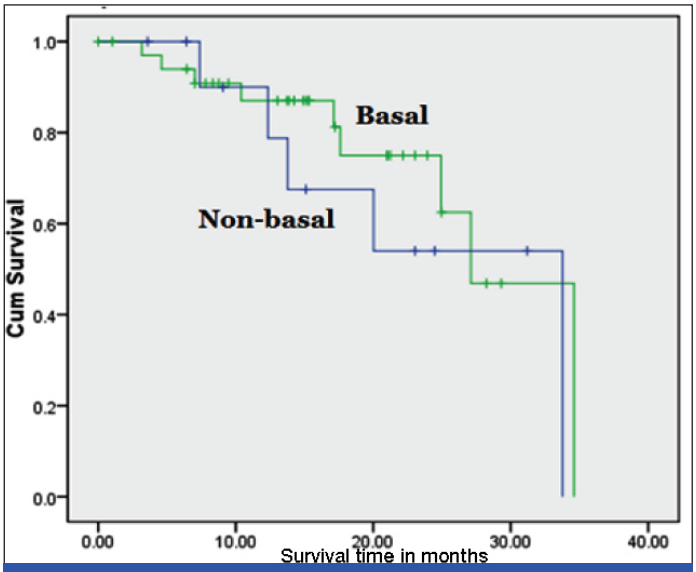
[Table/Fig-4]: Combination of two markers to identify TNBC.

Parameters	Basal Number of cases (%)	Non-basal Number of cases (%)	p-value
Age: Mean(SD)	47.29(46.17)	12.21 (10.21)	0.78
Age < 40 years > 40 years	 8(24) 26(76)	 2(17) 10(83)	0.93
Family history Present	 3(12)	 ---	0.81
Size <1cm 1-2cm >2cm	 --- 14(52) 13(48)	 2(25) 4(50) 2(25)	0.023
Lymph node involvement	28(80)	9(75)	0.644
Tubules No tubules <10% 10-75% >75%	 7(20) 23(66) 5(14) ---	 5(42) 5(42) 2(16) ---	0.28
Nuclear pleomorphism Moderate Marked	 28(80) 7(20)	 11(92) 1(8)	0.63
Shape of cells Polygonal Spindle	 34(97) 1(3)	 12(100) ---	1.00
Chromatin Hyperchromatic Vesicular Hyperchromatic and vesicular	 12(34) 10(29) 13(37)	 4(33) 4(33) 4(33)	0.95
Nucleoli Inconspicuous/absent Present Prominent	 2(6) 11(31) 22(63)	 1(8) 4(34) 7(58)	0.93
Cytoplasmic amount Moderate Abundant	 33(94) 2(6)	 8(67) 4(33)	0.04
Mitotic rate <10 10-40 >40	 6(17) 18(51) 11(32)	 5(42) 7(58) ---	0.09
Squamous metaplasia Present	 3(8.5)	 ---	0.72
Other metaplasias Present	 1(3)	 ---	1.00
Necrosis Present	 28(80)	 6(50)	0.10
Central acellular sclerosis Present	 5(14)	 1(8)	0.97
DCIS Present	 13(37)	 3(25)	0.68
Lymphocytic infiltrate Absent Mild Moderate Marked	 --- 20(57) 11(31) 4(12)	 1(8) 6(50) 3(25) 2(17)	0.35
Lymphovascular invasion Present	 25(71)	 9(75)	1.00
WHO subtype Invasive ductal carcinoma Others	 33(94) 2(6)	 11(92) 1(8)	1.00
Histological grade Grade II Grade III	 16(46) 19(54)	 5(50) 5(50)	0.55
Ishikawa subtypes Type A Type B Type C Type D	 6(17) 6(17) 22(63) 1(3)	 1(8) 1(8) 9(76) 1(8)	0.62

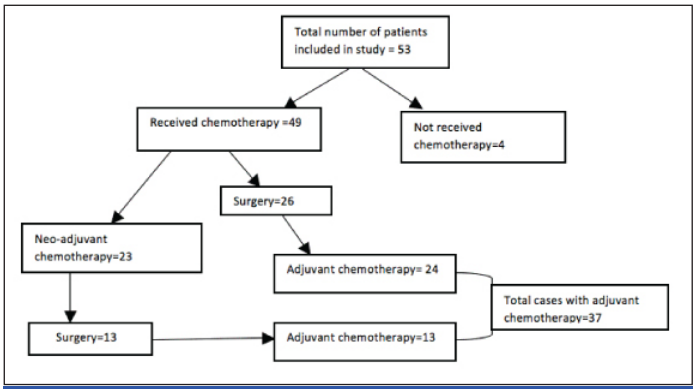
[Table/Fig-5]: Comparison of clinico-pathological features in basal and non basal TNBC.



[Table/Fig-6]: Disease free and overall survival outcomes in TNBC cases.



[Table/Fig-7]: Disease free and overall survival outcomes in TNBC cases basal TNBC.



[Table/Fig-8]: Disease free and overall survival outcomes in TNBC cases.

	Basal (Number of cases)	Non-basal (Number of cases)
Sites of metastases at presentation	Liver and lung (1) Liver (1) Bone marrow (1) Skin (1) Lymph nodes (1)	Lymph nodes (2) Skin (1) Chest wall (1) Bone marrow and lung (1)
Sites of metastases after presentation	Ovary (1) Lung (2) Skull (1) Brain, bone marrow and chest wall (1) Lymph node(1) Chest wall and lung (1)	Brain and bone marrow (1) Lymph node and lung (1) Bone marrow

[Table/Fig-9]: Metastases in basal and non basal TNBC.

TNBC patients are usually less than 40 years as compared to the non TNBC [29]. Our study did not show any statistical significance (p-value = 0.776) in age between basal and non-basal subtypes. The average size of TNBC in our study was 4.3± 2.56 cm. There was significant difference in tumour size between basal and non-basal tumours in our study (p-value = 0.023), which is also similar to previous studies [28–30]. Positive family history of breast carcinoma was seen only in the basal like subtype.

The most common histological subtype in our study was that of infiltrating ductal carcinoma (NOS), similar to other studies [2–5,31].

In the present study, 3 cases (6%) showed focal squamous cell differentiation. All cases in our study were classified as grade II or III and none were classified as grade 1. There was no significant difference between basal and non-basal TNBC (p-value = 0.546), in congruence with literature [29,30,32].

Majority (37.8 %) of our cases had a predominantly pushing border, 31.1% had both pushing and focally infiltrating borders and 31.1% cases had an infiltrating border. Most studies have shown TNBC to have a pushing non-infiltrative border of invasion [29,32].

Central acellular sclerosis was seen in 12% of TNBC cases as compared to 27.9% in a study done by Choi et al., [33]. Central acellular sclerosis and necrosis was more common in basal (14% and 80%) than non-basal (50% and 8%), but was not statistically significant.

In our study, 98% cases had shown lymphocytic infiltrate, of which 54% had mild infiltrate, 33% had moderate infiltrate and 11% had a marked infiltrate. There was no statistically significant difference in the degree of lymphocytic infiltrate between basal and non-basal tumours (p-value-0.346). Literature has shown that most TNBC cases with a dense lymphocytic infiltrate either intra-tumoural or within the vicinity of the tumour [29,32].

Of 17 cases of DCIS in our study, 8 (47%) cases were found to be triple negative, of which 3 cases were found to be basal like, both in the DCIS and invasive components. Literature has shown that TNBC and basal like cancers often lack an in-situ component due to rapid disease progression [32]. Ishikawa et al., found 29 cases (30%) of DCIS out of 97 cases of TNBC [10]. Thike et al., found 295 (45.2%) cases of DCIS out of 653 TNBC cases [34]. Doval et al., found 35.6% cases of DCIS among TNBC cases [35].

In our study, majority of TNBC had a Type C (67.3%) pattern which corresponds to invasive ductal carcinoma (NOS) according to Ishikawa classification. There was no statistically significant difference in the Ishikawa histological types between the basal and non-basal subtype (p-value-0.623).

Of 53 cases of TNBC in our study, 35 cases (74.4%) were of basal like subtype. Studies show the basal like subtype to be the most common in TNBC. Bertucci et al., identified 71.5% basal like subtypes among TNBC [36]. Rakha et al., used a combination of 4 basal markers and identified 71% of basal like types among TNBC [37].

The combination of CK5/6 and EGFR in our study identified all basal like TNBC cases. Nielsen et al., used a panel of 4 markers, ER, PR, EGFR, CK5/6 to identify basal subtype and found a sensitivity and specificity of 76% /100% [6]. Similarly, Thike et al., used a panel of 3 markers, CK14, EGFR, 34ße12 and found a sensitivity and specificity of 78%/100% [8]. Majority of TNBC are found to over-expresses EGFR and could respond to anti-EGFR therapies [37–39].

Similar to findings of Rakha et al., our study also has not shown any significant morphological differences between basal and non basal subtypes except for the amount of cytoplasm (p-value= 0. 49) [40].

TNBC are associated with a worse prognosis, develop early and are seen more often in premenopausal women. A characteristic peak of recurrence is seen within first 3 years after initial treatment and declines rapidly thereafter. In contrast, in ER positive breast cancers, more than 50% of recurrences are seen between 5 and 10 years after the first surgery. After 5 years of therapy the survival rates equals those of the non-TNBC subtypes. This suggests that the poor prognosis of TNBC may be due to effects that occur during the first 5 years after surgery [37–39].

TNBC are characterized by higher relapse rates compared to ER positive breast cancers, including an increased risk of loco-regional recurrence, lung, and brain involvement, but lesser risk for recurrence in bone. After metastatic relapse, survival is shorter

in TNBC compared to other subtypes, due to their predilection for visceral and lung metastasis compared with ER-positive breast cancers that are more likely to relapse in bone and skin [37–39].

LIMITATION

The sample size of our cases was relatively small due to a short study period. A few cases in our study were received as consults without paraffin blocks. Therefore basal immunohistochemical markers could not be performed on those cases. Survival of Ishikawa subtypes in basal and non- basal TNBC could not be analyzed due to small numbers in each subtype.

CONCLUSION

Frequency of TNBC in our institution was found to be 22.2%. Identifying TNBC, only by the use of ER, PR and HER2 is not enough, as the poor prognostic outcomes in TNBC is mainly accounted by the basal like subtype, which dominates the majority of TNBC.

Our study has demonstrated that high grade morphological features, hormonal markers with additional use of basal markers can help identify the basal like subtype of TNBC, thereby predicting breast cancer survival. The combination of CK5/6 and EGFR identified all cases of the basal subtype. EGFR in addition also has potential therapeutic implications and can benefit these patients.

ACKNOWLEDGEMENTS

We would like to thank the laboratory technicians and Clinicians at CMC Vellore for their valuable contribution and guidance towards this study. We would also like to thank the IRB board at CMC for providing the financial assistance for this project.

REFERENCES

[1] Wahba HA, El-Hadaad HA. Current approaches in treatment of triple-negative breast cancer. *Cancer Biol Med.* 2015;12(2):106–16.
[2] Anders C, Carey LA. Understanding and treating triple-negative breast cancer. Oncol Williston Park N. 2008;22(11):1233–39; discussion 1239–40, 1243.
[3] Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2007;13(15 Pt 1):4429–34.
[4] Rakha EA, Tan DSP, Foulkes WD, Ellis IO, Tutt A, Nielsen TO, et al. Are triple-negative tumours and basal-like breast cancer synonymous? *Breast Cancer Res*. 2007;9(6):404.
[5] Rakha EA, Ellis IO. Triple-negative/basal-like breast cancer: review. *Pathology*. 2009;41(1):40–47.
[6] Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res*. 2004;10(16):5367–74.
[7] Naidoo K, Pinder SE. Immunohistochemistry for Triple-Negative Breast Cancer. *Methods Mol Biol Clifton NJ*. 2016;1406:39–51.
[8] Thike AA, Iqbal J, Cheok PY, Chong APY, Tse GM-K, Tan B, et al. Triple negative breast cancer: outcome correlation with immunohistochemical detection of basal markers. *Am J Surg Pathol*. 2010;34(7):956–64.
[9] Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res*. 2008;14(5):1368–76.
[10] Ishikawa Y, Horiguchi J, Toya H, Nakajima H, Hayashi M, Tagaya N, et al. Triple-negative breast cancer: histological subtypes and immunohistochemical and clinicopathological features. *Cancer Sci*. 2011;102(3):656–62.
[11] Thike AA, Cheok PY, Jara-Lazaro AR, Tan B, Tan P, Tan PH. Triple-negative breast cancer: clinicopathological characteristics and relationship with basal-like breast cancer. *Mod Pathol*. 2010;23(1):123–33.
[12] Baba AI, Câtoi C. Comparative Oncology [Internet]. Bucharest: The Publishing House of the Romanian Academy; 2007. Chapter 3, TUMOUR CELL MORPHOLOGY. Available from: http://www.ncbi.nlm.nih.gov/books/NBK9557/
[13] Hammond MEH, Hayes DF, Wolff AC, Mangu PB, Temin S. American society of clinical oncology/college of american pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Oncol Pract*. 2010;6(4):195–97.
[14] Rich JT, Neely JG, Paniello RC, Voelker CCJ, Nussenbaum B, Wang EW. A practical guide to understanding Kaplan-Meier curves. *Otolaryngol Head Neck Surg*. 2010;143(3):331–36.
[15] Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology*. 1991;19(5):403–10.
[16] Ambroise M, Ghosh M, Mallikarjuna VS, Kurian A. Immunohistochemical profile of breast cancer patients at a tertiary care hospital in South India. *Asian Pac J Cancer Prev*. 2011;12(3):625–29.
[17] Sen S, Gayen R, Das S, Maitra S, Jha A, Mahata M. A clinical and pathological study of triple negative breast carcinoma: experience of a tertiary care centre in eastern India. *J Indian Med Assoc*. 2012;110(10):686–89, 705.
[18] Krishnamurthy S, Poornima R, Challa VR, Goud YGB. Triple negative breast cancer - our experience and review. *Indian J Surg Oncol.* 2012;3(1):12–16.
[19] Raina V, Deo S, Shukla N, Mohanti B, Gogia A. Triple-negative breast cancer: An institutional analysis. *Indian J Cancer.* 2014;51(2):163.
[20] Rao C, Shetty J, Prasad KH. Immunohistochemical profile and morphology in triple - negative breast cancers. *J Clin Diagn Res*. 2013;7(7):1361–65.
[21] Gaopande V, Joshi S, Kulkarni M, Dwivedi S. A clinicopathologic study of triple negative breast cancer. *J Sci Soc*. 2015;42(1):12.
[22] Sharma M, Sharma JD, Sarma A, Ahmed S, Kataki AC, Saxena R, et al. Triple negative breast cancer in people of North East India: critical insights gained at a regional cancer centre. *Asian Pac J Cancer Prev*. 2014;15(11):4507–11.
[23] Verma S, Bal A, Joshi K, Arora S, Singh G. Immunohistochemical characterization of molecular subtypes of invasive breast cancer: a study from North India. Acta *Pathol Microbiol Immunol Scand*. 2012;120(12):1008–19.
[24] Kalwar A, Sharma N, Kapoor A, Kumar N, Satyanarayan, Sharma B. Five year retrospective survival analysis of triple negative breast cancer in North-West India. *Indian J Cancer*. 2013;50(4):330-32.
[25] Wang Y, Yin Q, Yu Q, Zhang J, Liu Z, Wang S, et al. A retrospective study of breast cancer subtypes: the risk of relapse and the relations with treatments. *Breast Cancer Res Treat*. 2011;130(2):489–98.
[26] Kim M-J, Ro JY, Ahn S-H, Kim HH, Kim S-B, Gong G. Clinicopathologic significance of the basal-like subtype of breast cancer: a comparison with hormone receptor and Her2/neu-overexpressing phenotypes. *Hum Pathol*. 2006;37(9):1217–26.
[27] Kurebayashi J, Moriya T, Ishida T, Hirakawa H, Kurosumi M, Akiyama F, et al. The prevalence of intrinsic subtypes and prognosis in breast cancer patients of different races. *Breast Edinb Scotl*. 2007;16 Suppl 2:S72–77.
[28] Boyle P. Triple-negative breast cancer: epidemiological considerations and recommendations. *Ann Oncol*. 2012;23 Suppl 6:vi7–12.
[29] Fulford LG, Easton DF, Reis-Filho JS, Sofronis A, Gillett CE, Lakhani SR, et al. Specific morphological features predictive for the basal phenotype in grade 3 invasive ductal carcinoma of breast. *Histopathology*. 2006;49(1):22–34.
[30] Cakir A, Gonul II, Uluoglu O. A comprehensive morphological study for basal-like breast carcinomas with comparison to nonbasal-like carcinomas. *Diagn Pathol*. 2012;7:145.
[31] Reis-Filho JS, Tutt ANJ. Triple negative tumours: a critical review. *Histopathology*. 2008;52(1):108–18.
[32] Badve S, Dabbs DJ, Schnitt SJ, Baehner FL, Decker T, Eusebi V, et al. Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. *Mod Pathol*. 2011;24(2):157–67.
[33] Choi J, Jung W-H, Koo JS. Clinicopathologic features of molecular subtypes of triple negative breast cancer based on immunohistochemical markers. *Histol Histopathol*. 2012;27(11):1481–93.
[34] Thike AA, Iqbal J, Cheok PY, Tse GM-K, Tan PH. Ductal carcinoma in situ associated with triple negative invasive breast cancer: evidence for a precursor-product relationship. *J Clin Pathol*. 2013;66(8):665–70.
[35] Doval DC, Sharma A, Sinha R, Kumar K, Dewan AK, Chaturvedi H, et al. Immunohistochemical Profile of Breast Cancer Patients at a Tertiary Care Hospital in New Delhi, India. *Asian Pac J Cancer Prev*. 2015;16(12):4959–64.
[36] Bertucci F, Finetti P, Cervera N, Esterni B, Hermitte F, Viens P, et al. How basal are triple-negative breast cancers? *Int J Cancer*. 2008;123(1):236–40.
[37] Rakha EA, El-Sayed ME, Green AR, Lee AHS, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. Cancer. 2007;109(1):25–32.
[38] Hudis CA, Gianni L. Triple-negative breast cancer: an unmet medical need. *The Oncologist*. 2011;16 Suppl 1:1–11.
[39] Yamamoto Y, Ibusuki M, Nakano M, Kawasoe T, Hiki R, Iwase H. Clinical significance of basal-like subtype in triple-negative breast cancer. *Breast Cancer*. 2009;16(4):260–67.
[40] Rakha EA, Elsheikh SE, Aleskandarany MA, Habashi HO, Green AR, Powe DG, et al. Triple-Negative Breast Cancer: Distinguishing between Basal and Nonbasal Subtypes. *Am Assoc Cancer Res*. 2009;15(7):2302–10.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, CMC, Vellore, Tamil Nadu, India.
2. Professor, Department of Pathology, CMC Vellore, Tamil Nadu, India.
3. Professor and Head of Department, Department of Radiation Therapy, CMC Vellore, Tamil Nadu, India.
4. Professor and Head of Department, Department of Medical Oncology, CMC Vellore, Tamil Nadu, India.
5. Professor, Department of Endocrine Surgery, CMC Vellore, Tamil Nadu, India.
6. Professor and Head, Department of Endocrine Surgery, CMC Vellore, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Marie Therese Manipadamn,
Professor, Department of General Pathology, 4th floor, ASHA Building, Christian Medical College and Hospital,
Ida Scudder Road, Vellore-632004, Tamil Nadu, India.
E-mail: mtm2005@cmcvellore.ac.in

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Apr 03, 2016**
Date of Peer Review: **Jun 14, 2016**
Date of Acceptance: **Jul 26, 2016**
Date of Publishing: **Sep 01, 2016**