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Clinicopathological and immunohistochemical characteristics of breast cancer patients from Northeast India with special reference to triple negative breast cancer: A prospective study

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ABSTRACT

Background: Molecular pathogenesis of Triple-negative breast cancer (TNBC) is inconclusively documented from resource limited countries and hence there is a lack of available targeted therapy for clinical interventions. Compared to other breast cancer subtypes, TNBC is more aggressive, higher recurrence rate, and higher prevalence in younger premenopausal women. Sporadic literature indicates predominance of TNBC in all reported breast cancer cases from Northeast India.
Aim: This study was conducted to evaluate the candidature of panel of key molecular markers involved in the development and progression of TNBC for prognosis and futuristic tailored targeted therapy.
Materials and Methods: We analyzed the clinicopathological characterized and immunohistochemically screened the differential expression of key molecular markers involved in the development and progression of in TNBC cases vis-a-vis non-TNBC and autopsy-based control samples.
Results: TNBC tends to display at an early reproductive age and is more aggressive in nature. Further, the differential expression of 2 specific markers viz., epidermal growth factor receptor (EGFR) and FolR1 was higher in TNBC cases compared to controls and non-TNBC (both in terms of susceptibility and specificity), clinical staging in TNBC cases (severity) and mortality (outcome). Although Ki67 and vascular endothelial

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growth factor expression also correlated with severity and outcome of the disease but their differences in non-TNBC cases were not significantly differentiable compared to TNBC.
Conclusions: The study indicates that EGFR and FolR1 could serve as useful biomarkers to determine TNBC prognosis. Further studies will be needed to evaluate EGFR and Folate pathways in order to screen out the molecular targets which may be meaningfully used for clinical stratification, intervention, and treatment.

ARTICLE INFO

Keywords: Triple negative breast cancer; Prognosis; EGFR; FolR1

Introduction

Triple-negative breast cancer (TNBC) represents a subtype which is immunohistochemically defined by the lack of estrogen receptor (ER), progesterone receptor (PR) expression, and human epidermal growth factor receptor 2 (HER2).[1] TNBC is more prevalent among younger premenopausal women,[2] is biologically more aggressive, has higher recurrence and death rate than receptor-positive breast cancer cases (non-TNBC).[3,4] TNBCs superior sensitivity and responsiveness to chemotherapy had been well documented in many early and advanced diseases but the most efficacious chemotherapeutic regimen had not yet been determined which could provide maximum benefit to the patients thereby reducing the unnecessary toxicity to a minimum level. Thus, this subtype represents an important clinical challenge. TNBC is an important phenotype and accounts for approximately 15% of all breast cancer cases in a general population.[5] Several new promising chemotherapeutic drugs are under preclinical investigation such as polyadenosine-diphosphate ribosepolymerase inhibitors, antiangiogenic agents, epithelial growth factor receptor inhibitors, mTOR inhibitors, multityrosine kinase inhibitors etc but till date none of them are brought in day-to-day clinical practice. Several studies reported that TNBC is more common in women of African ancestry than women of other ethnicity.[3] Limited existing reports suggest that the incidence of TNBC in India varies from 12.5% to 29.8%.[6-8] A previous study from Dr B. Borooah Cancer Institute had reported a high prevalence of TNBC cases (31.9%) in Northeast Indian breast cancer patients,[9] which underlines the requirement of scientifically addressing to the issue. Despite various sporadic reports on association of several risk factor(s) in the epidemiology and genesis of TNBC,[2,3,10-20] it still remains poorly addressed as all these data are equivocal and inconclusive. Currently, there is no international definition of TNBC but the most appropriate approach of treatment would be identification of prognostic markers based on immunohistochemistry (IHC) criteria.[21] Available literature suggested that expression of some biomarkers indicative of proliferation (EGFR, folate receptor alpha [FolR1], Ki67), angiogenesis (vascular endothelial growth factor [VEGF]), growth factors (HER3), cell adhesion (E cadherin), basal markers (CK5/6, CK14), epithelial-to-mesenchymal transition (Vimentin), and apoptosis (Caspase 3) could possibly give a clear cut definition to TNBC.

Epidermal growth factor receptor (EGFR), a tyrosine kinase receptor on cell surface, plays crucial role in mediating tumor proliferation, differentiation, migration, angiogenesis, and apoptosis.[22] EGFR protein was found to be expressed in majority of TNBC and was correlated with poor prognosis.[10,23,24] These findings advocate that EGFR could be a strong candidate for new tailored therapeutic interventions against TNBC management. FolR1 is a membrane-bound protein and overexpression of FolR1 may confer a growth advantage to tumors by increasing folate uptake and may affect cell proliferation.[12,25] FolR1 has been shown to be selectively up-regulated in several types of solid human cancers including TNBC.[11,12] The Ki67 is a nonhistone DNA-binding nuclear protein present during G1, S, G2, M phases of the cell cycle but not during the resting phase (G0) and necessary for cellular proliferation.[14] It has been proposed in various reports that Ki67 might be a potent prognostic marker for breast cancer in general[13] and TNBC[14] in particu-

lar. VEGF is a primary mediator of angiogenesis in normal and tumor tissues.[26] Preliminary data also revealed that VEGF was overexpressed in TNBC tissue as compared to non-TNBC tissue.[15] Receptor tyrosine protein kinase erbB-3 (HER3) is one of the members of the human EGFR/HER family. Several clinical studies interpret that the overexpression of HER3 in breast cancer including TNBC.[16] However, the prognostic significance of HER3 expression in TNBC had been poorly documented.[27] E-cadherin plays a prominent role in the formation of cell-to-cell adhesion in epithelial tissues.[17] Very scarce reports[14] showed that reduced E-cadherin expression was an independent prognostic factor in TNBC. Cytokeratin 5/6 (CK5/6) and Cytokeratin 14 (CK14) represents one of the large numbers of high molecular weight basal cytokeratins mainly found in the myoepithelial cell layers of stratified epithelium.[28] TNBC has highly diversified pathogenesis; therefore, its further stratification would be necessary in order to understand the molecular etiology and prediction of treatment. Vimentin is a basic epithelial-mesenchymal transition (EMT) marker.[18] Overexpression of vimentin had been reported in various breast cancer patients and cell lines including TNBC.[29,30] Caspase-3 is a central member of the cysteine protease family which plays a pivotal role in mediating apoptotic pathways.[19,20] Scanty reports suggest contradictory reports either high or no significant expression of caspase-3 in breast cancer tissue sections.[31,32]

Available literature and data on molecular mechanism(s) associated with cancers of different organ etiologies including some sporadic reports on TNBC suggest the association of deregulation of multiple lead pathways including those involved in proliferation, angiogenesis, cell adhesion, EMT, apoptosis etc, which are hallmark of cancer development and progression. Therefore, the aim of this study was to analyze the differential expression of key molecular markers involved in the development and progression of TNBC samples. It is expected that the study results would highlight the role played by key signal transducing molecules marker in the development of TNBC, for evaluating the status of the disease, and would be useful in planning treatment regime for TNBC.

Materials and methods

Patient enrolment and stratification

The present study was planned and conducted on formalin fixed paraffin embedded (FFPE) tissue blocks of breast cancer patients (N = 249) who had undergone surgical resection at Dr B. Borooah Cancer Institute, Regional Cancer Center, Guwahati, Assam, India between 2013 and 2018. Clinicopathological details, patient history, course of treatment, ER-PR-HER2 status, relapse, and mortality status etc was filled up from hospital data and updated on a regular basis. The FFPE blocks of these breast cancer cases were segregated and stratified into 3 different cohorts namely TNBC (N = 69) and non-TNBC (N = 180) based on immunohistochemical findings, along with representative number of autopsy based controls (N = 20). "TNBC" subgroup includes all those breast cancer cases which were ER, PR, and HER2/neu negative while "non-TNBC" subgroup consists of those breast cancer cases that were positive for any of these markers.

IHC assay

Slides with tissue segments of breast cancer samples and control samples were taken for immunohistochemical study of markers indicative of proliferation (EGFR [*BioGenex US*], FolR1 [*Abcam US*], Ki67 [*BioGenex US*]), angiogenesis (VEGF [*BioGenex US*]), growth factors (Her3 [*BioGenex US*]), cell adhesion (E-Cadherin [*Thermoscientific US*]), basal markers (CK5/6 [*Thermo-scientific US*], CK14 [*Thermoscientific US*]), EMT (Vimentin [*BioGenex US*]), apoptosis (Caspase3 [*Thermoscientific US]*) protein expression. The standard protocol for IHC as instructed in the kit, for the expression study of a protein was performed using IHC Detection System. The specimens that were

embedded in paraffin blocks were cut into 5-μm sections on poly-L-lysine coated slides. IHC was performed using the IHC detection system (*BioGenex US*). Briefly, the sections were deparaffinized and subjected to antigen heat retrieval in a citrate buffer (pH 6.0) at 90°C for 30 minutes. Endogenous peroxidase activity and nonspecific binding were blocked by incubation with a peroxide block and a power block, respectively, using an IHC kit. The slides were then incubated sequentially with primary antibodies overnight at 4°C and then with their respective secondary antibodies for 1 hour at room temperature. Diaminobenzidine hydrochloride was used as a chromogen. Subsequently, the sections were counterstained with hematoxylin and mounted using DPX mounting media. IHC scoring was performed as previously described. Briefly, the tumor staining was examined by a senior oncopathologist.

Statistical analysis

The statistical analysis was performed using standard statistical software SPSS version 13.0 (SPSS, Inc., Chicago, IL) with probability of <0.05 was regarded to be statistically significant. The cases were represented as numbers and percentages. The Odds ratio, Chi Square, and Wilcoxon Signed Ranked Test was used to analyze the differences.

Results

Demographical and clinical profile of enrolled subjects

All the enrolled FFPE tissue samples were histopathologically and immunohistochemically segregated into 2 categories namely TNBC (N = 69) and non-TNBC (N = 180) cohorts along with autopsy based controls. "TNBC" subgroup includes all those breast cancer cases which were ER, PR, and HER2 negative while "non-TNBC" subgroup consists of those breast cancer cases that were positive for any of these markers. The clinicopathological profile of breast cancer samples are tabulated in Table 1. Data reflects that TNBC affects women of reproductive age group ($P < 0.001$) and premenopausal women ($P = 0.002$) compared to non-TNBC cases. TNBC was also associated with recurrence compared to non-TNBC cases ($P = 0.005$).

The differential protein expression of multiple molecular markers indicative of proliferation, angiogenesis, growth factors, cell adhesion, basal markers, epithelial-to-mesenchymal transition, and apoptosis namely; EGFR, FolR1, Ki67 (Fig 1); VEGF, Her3, E-Cadherin, CK5/6, CK14, Vimentin, and Caspase3 (Fig 2) was studied in TNBC, non-TNBC, and autopsy based control FFPE tissue samples using IHC detection system. The expression was graded as strong (+++), moderate (++), low (+) or no expression (−) in all the controls, TNBC (Infiltrating Ductal Carcinoma (IDC) II [N = 36] and IDC III [N = 33]), non-TNBC, and autopsy based control cases. The results of IHC based expressions of EGFR, FolR1, Ki67, VEGF, Her3, E-Cadherin, CK5/6, CK14, Vimentin, and Caspase3 were tabulated in Table 2.

The details of difference in expression of specific markers based on IHC analysis and scoring by a registered senior oncopathologist is provided in Table 2.

Strong Ki67 ($P = 0.098$), EGFR ($P = 0.021$), VEGF ($P = 0.262$), and FolR1 ($P = 0.021$) expression was observed in TNBC cases compared to autopsy-based controls. The strong Ki67 (odds ratio [OR] = 2.255 [0.849-5.991] at 95% confidence interval [CI], $P = 0.154$), EGFR (OR = 3.170 [1.158-8.676] at 95% CI, $P = 0.035$), VEGF (OR = 2.169 [0.701-6.718] at 95% CI, $P = 0.274$) and FolR1expression (OR = 3.170 [1.158-8.676] at 95% CI, $P = 0.035$) was found to be associated with increased susceptibility to the development of TNBC compared to autopsy-based controls. The expression of the other evaluated markers in the study was comparative between TNBC and autopsy-based controls, with strong expression of selective markers being observed in few subjects only.

The markers which were prominently highly expressed in TNBC compared to non-TNBC cases were EGFR ($P = 0.007$), VEGF ($P = 0.273$), and FolR1 ($P = 0.030$). The strong expression

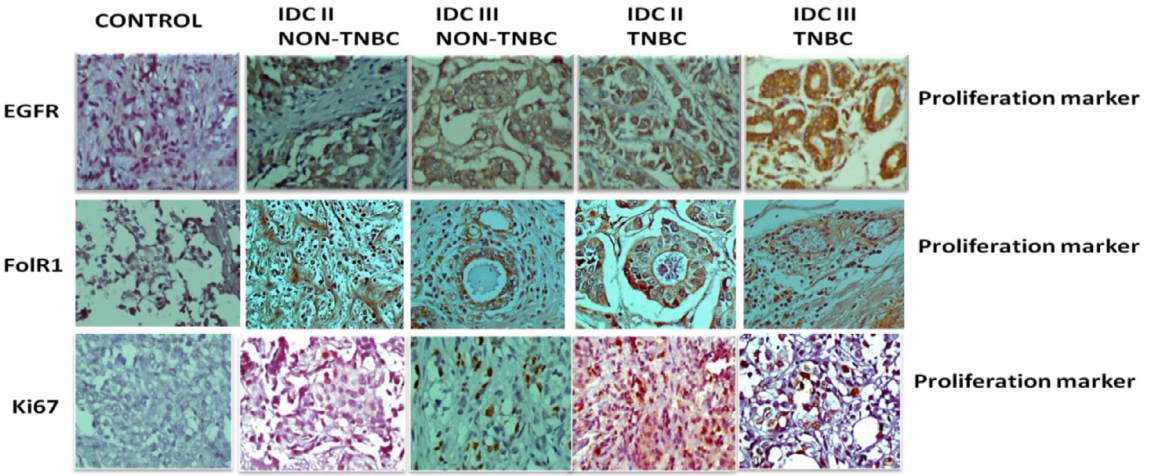

Fig. 1. Representative Immunohistochemistry images of EGFR, FolR1 and Ki67 expression in autopsy based control, non-TNBC and TNBC.

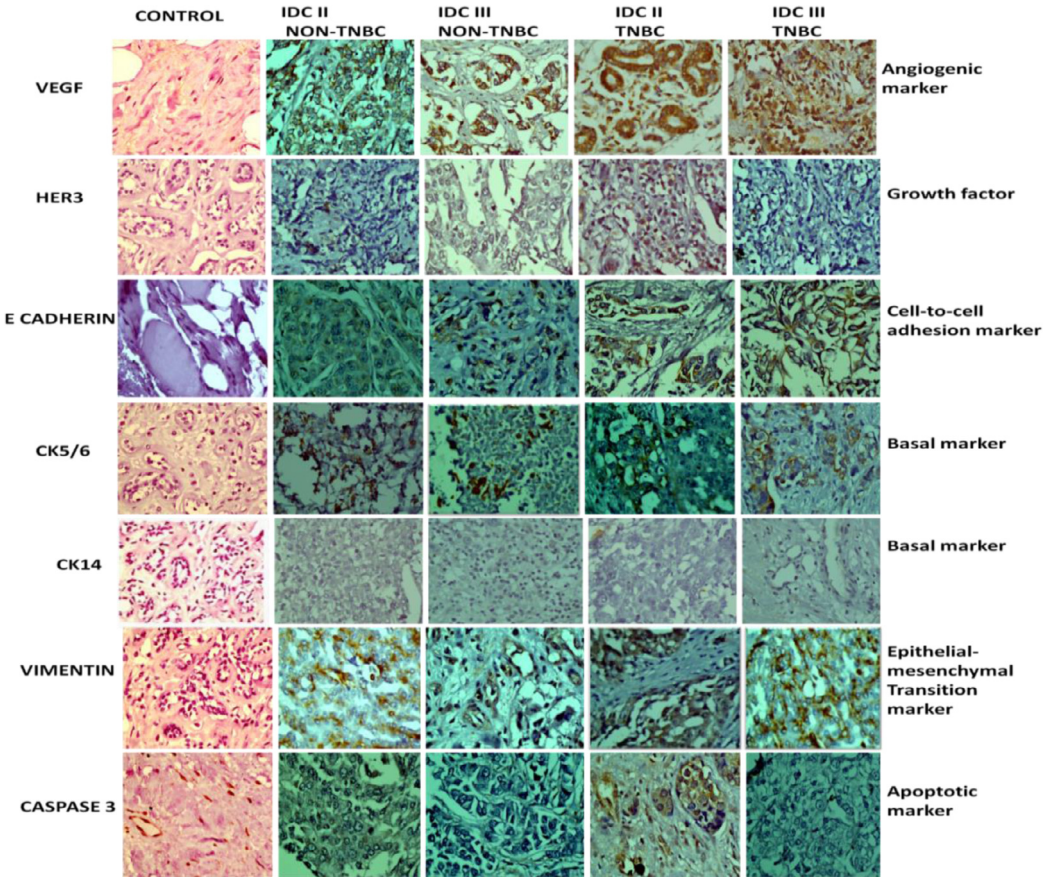

Fig. 2. Representative immunohistochemistry images of VEGF, Her3, E-Cadherin, CK5/6, CK14, Vimentin and Caspase3 expression in autopsy based control, non-TNBC, and TNBC.

Table 1
Clinicopathological profile of breast cancer samples in the study.

Parameters	Number of cases [%age]		
	Non-TNBC (N = 180)	TNBC (N = 69)	*P* value
Age			
Above 45	135 [75.00]	26 [37.68]	ref
Below 45	45 [25.00]	43 [62.32]	$P < 0.001$
Parity			
Less than 3	119 [66.11]	40 [57.97]	ref
More than 3	61 [33.89]	29 [42.03]	0.232
Menopausal status			
Postmenopausal	65 [36.11]	11 [15.94]	ref
Premenopausal	115 [63.89]	58 [84.06]	0.002
History of breast cancer			
Negative	180 [100]	66 [95.65]	ref
Positive	0 [0]	3 [4.35]	0.005
Grade			
I and II	30 [16.67]	12 [17.39]	ref
III	150 [83.33]	57 [82.61]	0.892
Histological type			
IDC	180 [100]	69 [100]	NA
ILC	0 [0]	0 [0]	
Medullary	0 [0]	0 [0]	
Mucinous and tubular	0 [0]	0 [0]	
Other	0 [0]	0 [0]	
Stage			
I and II	29 [16.11]	7 [10.14]	ref
III and IV	135 [75.00]	58 [84.06]	0.976
Unknown	16 [8.89]	4 [5.80]	NA
Mortality			
Alive	88 [48.89]	27 [39.13]	ref
Dead	92 [51.11]	42 [60.87]	0.168
T stage			
T1 and T2	13 [7.22]	16 [23.19]	ref
T3 and T4	167 [92.78]	53 [76.81]	$P < 0.001$
N stage			
N0 and N1	57 [31.67]	21 [30.44]	ref
N2 and N3	123 [68.33]	48 [69.56]	0.852
Distant metastasis			
Absent	59 [32.78]	38 [55.07]	ref
Present	106 [58.89]	27 [39.13]	0.002
Unknown	15 [8.33]	4 [5.80]	NA

Screening for panel of molecular markers for TNBC based on differential expression analysis of selective indicators for proliferation, angiogenesis, growth factors, cell adhesion, basal markers, epithelial-to-mesenchymal transition and apoptosis compared to non-TNBC and autopsy based controls
*Cases represented as numbers [%age].

of EGFR (OR = 3.723 [1.366-10.145] at 95% CI, $P = 0.010$) and FolR1 (OR = 2.717 [1.083-6.817] at 95% CI, $P = 0.043$) was significantly associated with increased risk of TNBC compared to non-TNBC cases, while the strong expression of VEGF was also associated with increased risk of TNBC compared to non-TNBC cases (OR = 1.768 [0.634-4.926] at 95% CI, $P = 0.309$). In TNBC cases, the Wilcoxon signed rank test results showed that higher EGFR expression correlated with higher VEGF expression ($P = 0.007$). Higher EGFR expression also correlated with higher FolR1 expression ($P = 0.034$). However, no statistically significant correlation was noted between FolR1 and VEGF expression ($P = 0.257$) and EGFR and Ki67 expression ($P = 0.180$).

In all breast cancer samples, differential expression of Ki67, EGFR, FolR1, VEGF, Her3, E-Cadherin, CK5/6, CK14, Vimentin, and Caspase3 was correlated with severity grade of the disease. The differential expression level of EGFR ($P = 0.259$) and FolR1 ($P = 0.339$) was found to

Table 2
Details of IHC based scoring for evaluated markers.

Markers	Subjects	N	Strong (+++)	Moderate (++)	Low (+)	No expression (-)
EGFR	Controls	69	6[8.70]	8[11.59]	25[36.23]	30[43.48]
	Non-TNBC	180	14[7.78]	20[11.11]	79[43.89]	67[37.22]
	TNBC	69	16[23.19]	22[31.88]	18[26.09]	13[18.84]
FolR1	Controls	69	6[8.70]	7[10.14]	11[15.94]	45[65.22]
	Non-TNBC	180	18[10.00]	31[17.22]	72[40.00]	59[32.78]
	TNBC	69	16[23.19]	23[33.33]	17[24.64]	13[18.84]
Ki67	Controls	69	7[10.14]	9[13.04]	29[42.03]	24[34.79]
	Non-TNBC	180	32[17.78]	40[22.22]	63[35.00]	45[25.00]
	TNBC	69	14[20.29]	10[14.49]	27[39.13]	18[26.09]
VEGF	Controls	69	5[7.25]	7[10.14]	25[36.23]	32[46.38]
	Non-TNBC	180	16[8.89]	29[16.11]	52[28.89]	83[46.11]
	TNBC	69	10[14.49]	10[14.49]	23[33.33]	26[37.69]
Her3	Controls	69	2[2.90]	6[8.70]	6[8.70]	55[79.70]
	Non-TNBC	180	29[16.11]	20[11.11]	52[28.89]	79[43.89]
	TNBC	69	0[0.0]	7[10.14]	14[20.29]	48[69.57]
E-Cadherin	Controls	69	2[2.90]	4[5.80]	7[10.14]	56[81.16]
	Non-TNBC	180	2[1.11]	7[3.89]	32[17.78]	139[77.22]
	TNBC	69	0[0.0]	9[13.04]	11[15.94]	49[71.02]
CK5/6	Controls	69	1[1.45]	4[5.80]	7[10.14]	57[82.61]
	Non-TNBC	180	0[0.0]	7[3.89]	9[5.00]	164[91.11]
	TNBC	69	0[0.0]	7[10.14]	17[24.64]	45[65.22]
CK14	Controls	69	2[2.90]	4[5.80]	0[0.00]	63[91.30]
	Non-TNBC	180	2[1.11]	2[1.11]	12[6.67]	164[91.11]
	TNBC	69	7[10.14]	3[4.35]	24[34.78]	35[50.73]
Vimentin	Controls	69	2[2.90]	7[10.14]	14[20.29]	46[66.67]
	Non-TNBC	180	7[3.89]	23[12.78]	52[28.89]	98[54.44]
	TNBC	69	3[4.35]	12[17.39]	30[43.48]	24[34.78]
Caspase3	Controls	69	1[1.45]	3[4.35]	13[18.84]	52[75.36]
	Non-TNBC	180	11[6.11]	16[8.89]	20[11.11]	133[73.89]
	TNBC	69	3[4.35]	9[13.04]	23[33.33]	34[49.28]

Cases represented as numbers [%age].

be higher in IDC III cases compared to IDC II cases indicating its association with higher staging and severity in breast cancer samples. However, differential expression of Ki67 ($P = 0.467$), Her3 ($P = 1.0$), E-cadherin ($P = 1.0$), CK5/6 ($P = 1.000$), CK14 ($P = 0.495$), Vimentin ($P = 0.637$), VEGF ($P = 0.628$), and Caspase3 ($P = 1.000$) protein molecules was found to be comparative. The markers next evaluated for their correlation of disease severity in TNBC cases. The differential expression level of EGFR ($P = 0.241$), FolR1 ($P = 0.280$), and VEGF ($P = 0.540$) was found to be higher in IDC III TNBC cases compared to IDC II cases indicating its association with higher staging and severity in TNBC. However, differential expression of Ki67 ($P = 0.495$), Her3 ($P = 0.816$), E-cadherin ($P = 1.0$), CK5/6 ($P = 0.923$), CK14 ($P = 0.695$), Vimentin ($P = 0.637$), and Caspase3 ($P = 0.788$) was found to be comparative. When all breast cancer samples (TNBC and non-TNBC) cases were considered, higher VEGF ($P = 0.273$), EGFR ($P = 0.067$), and FolR1 ($P = .103$) expression was found to be associated with mortality of the disease. Further, upregulated FolR1 (OR = 43.313 [3.75-0.325] at 95% CI, $P = 0.586$), VEGF (OR = 17.894 [2.000-50.224] at 95% CI, $P = 0.602$) expression was also found to be associated with increased risk of mortality.

In TNBC cases, higher Ki67 ($P = 0.273$), EGFR ($P = 0.058$), FolR1 ($P = 0.046$), VEGF ($P = 0.326$), CK14 ($P = 0.273$), and Vimentin ($P = 0.450$) was found to be associated mortality of the disease. Caspase 3 ($P = 1.000$), E-Cadherin ($P = 1.000$), CK5/6 ($P = 1.000$), and Her3 ($P = 1.000$) expression did not show any association with the mortality of the disease. Further, upregulated EGFR (OR = 2.800 [0.255-30.703] at 95% CI, $P = 0.113$), FolR1 (OR = 12.08 [4.176-35.682] at 95% CI, $P = 0.212$), VEGF (OR = 2.500 [0.410-15.23] at 95% CI, $P = 0.386$, $P = 1.000$) protein expression was found to be associated with increased risk of mortality.

Discussion

TNBC accounts for around 15% of all breast cancer cases globally,[5] but TNBC phenotype is prevalent in a larger proportion of patients in people of northeast India (31.9%) of total breast cancer patients.[9] Given that TNBC is particularly prevalent amongst younger premenopausal women,[2] biologically more aggressive in nature, is associated with poor prognosis and higher death rate than non-TNBC[3, 4, 33] and lacks of specific therapeutic agents, this subtype thus represents an important clinical challenge, thereby making better understanding of TNBC necessary for identification and development of efficacious chemotherapeutic regimen which could provide maximum benefit to the patients reducing the unnecessary toxicity to a minimum level. In spite of several reports revealing the involvement of various signaling molecules in the developmental mechanisms of TNBC, the pathogenesis of TNBC is still unanswered; which holds key for development of targeted therapies. Therefore, the aim of this study was to analyze the differential expression of key molecular markers involved in the development and progression of TNBC vis-a-vis non-TNBC and controls.

The present study was initiated with the enrolment of FFPE tissue blocks of breast cancer patients (N = 249) who had undergone surgical resection at BBCI. All the enrolled FFPE tissue samples were histopathologically and immunohistochemically segregated into 2 categories namely TNBC (N = 69) and non-TNBC (N = 180) cohorts along with autopsy-based controls with the help of a registered pathologist. The mean age of the TNBC patients was found to be 40 ± 11 years with the disease being most predominantly present in the age group of 40-45 years followed by 35-40 years, which suggested that TNBC was most prevalent in women of reproductive cycle. Similar reports have recently been demonstrated by Sharma M et al, 2014[9] in North-eastern Indian population. Further, all the TNBC cases (N = 69) were clinically staged as IDC (IDCII [n = 36 cases] [52.17%] and IDC III [n = 33 cases] [47.83%]). The mean age of our TNBC patients was comparative, and in agreement to the one documented by Sharma M et al, 2014[9] and Carey LA et al, 2006.[3] This was concurrent with the studies by Tan GH et al, 2009[6] who also observed that TNBC was common in age >40 years. All the TNBC cases were either grade II (36/69) or grade III (33/69). TNBC in India has been reported to be commonly present at an early age and associated with high grade large tumors and high rate of node positivity, IDC NOS being the most common histological subtype in TNBC[9] which is concurrent with the present study which also showed that majority of the cases of TNBC are from the reproductive age group and were histopathologically IDC positives.

Based on available literature, the study was initiated by studying a panel of molecular markers for its probable involvement in TNBC development and progression to severe stage as well as evaluates the prognostic significance and specificity of the differential expression of these markers. Although a lot of molecules were considered to be included in clinical practice such as CK5/6, CK14, E-Cadherin, Ki67, Vimentin, Her3, Caspase 3, FolR1, VEGF, EGFR but till date no marker is yet ready for routine use. Thus, the present study was to analyze the differential expression of these key molecular markers which are known indicators for proliferation (Ki67, EGFR, and FolR1), angiogenesis (VEGF), growth factors (Her3), cell adhesion (E-Cadherin), basal markers (CK5/6 and CK14), epithelial-to-mesenchymal transition (Vimentin), and apoptosis (Caspase3) in TNBC, non-TNBC cases and autopsy based controls FFPE tissue blocks.

The differential expression analysis for the panel of markers indicated a difference in the expression pattern for Ki67, EGFR, VEGF, and FolR1; with differential EGFR and FolR1 expression being associated with both disease susceptibility and progression to severity in TNBC cases, as well as with poor clinical outcome and mortality status. The EGFR expression correlated with VEGF expression which in turn was found to be associated with poor clinical outcome; thereby underlining the significance of differential EGFR expression in TNBC pathogenesis. EGFR overexpression had been reported in many human cancers and found to be correlated with poor clinical prognosis including breast cancer.[7] Our findings were supported by various reports which states that EGFR protein was frequently elevated in TNBC and also reported that there was a likely association between high EGFR levels and poor tumor differentiation and grade and poor disease outcome.[8] There are few scanty contradictory reports in the literature which

suggested no correlation of EGFR overexpression with TNBC.[34,35] Our results was consistent with Linderholm et al[15] which shows that high expression of VEGF was significantly associated with TNBC tumors with high histological grade and poor clinical outcome in women with early-stage TNBC. Although few published reports have failed to observed any association of FolR1 expression TNBC tumors with these clinicopathological parameters in specific populations[12,36]; significant upregulation of FolR1 expression observed in the TNBC cases of the studied cohort which is consistent with the findings from other groups who had reported that the overexpression of FolR1 is significantly associated with TNBC tumors with higher histological grade and poor clinical outcome as compared to non-TNBC tumors and normal breast tissues.[11,37] The difference in data may be attributed to the population differences, which is also suggestive of the importance of studying the folate pathway alterations associated with the downstream manifestations of altered folate status due to the differential FolR1 expression.

Conclusion

In conclusion, the differential expression of 2 specific markers viz., EGFR and FolR1 was higher in TNBC cases compared to controls and non-TNBC (both in terms of susceptibility and specificity), clinical staging in TNBC cases (severity) and mortality (outcome). Although Ki67 and VEGF expression also correlated with severity and outcome of the disease but their differences in non-TNBC cases were not significantly differentiable compared to TNBC. Therefore, EGFR and FolR1 could serve as useful biomarkers to better determine TNBC prognosis. Further studies will be needed to evaluate EGFR and Folate pathways in order to screen out the molecular targets which may be meaningfully used for clinical intervention and treatment.

CRediT authorship contribution statement

Rizwana Sultana: Methodology, Investigation, Visualization, Writing - original draft. **Amal Ch. Kataki:** Supervision. **Bibhuti Bhusan Barthakur:** Conceptualization. **Anupam Sarma:** Validation. **Sujoy Bose:** Formal analysis, Writing - review & editing.

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Disclosures

There are no conflicts of interest.

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