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ORIGINAL ARTICLE

Characterization of immunohistochemical markers in triple negative breast carcinomas

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Summary

Purpose: *Triple negative (TN) breast carcinomas (estrogen receptor/ER, progesterone receptor/PR and HER-2/neu negative) constitute 15-25% of all breast carcinomas and have been correlated with aggressive behavior and poor prognosis. Our aim was to describe and characterize the immunophenotype of these tumors in a group of patients from Turkey.*

Methods: *We used the immunohistochemical markers CK5/6, CK14, EGFR, E-cadherin, p53 and androgen receptor. Formalin-fixed, paraffin-embedded tissues from 51 breast carcinoma patients (36 TN and 15 non TN) were included into this study.*

Results: *The mean values of the distribution of immunohistochemical markers in TN vs non-TN groups were as follows: CK5/6 78.4 vs 5.3%, CK14 84.8 vs 8%, EGFR 87.2 vs 8%, E-cadherin 96.9 vs 53.2%, p53 87.3 vs 7.3% and androgen receptor 89.5 vs 33.3% (all p-values<0.001). CK5/6 stained significantly different in the grade 2 and 3 cases (p=0.035) in the TN group.The other markers demonstrated no significant differences between grades.*

Conclusion: *TN breast carcinomas in Turkish patients express basal cytokeratins, and have high levels of p53 compared to non-TN breast carcinomas.*

Key words: *breast carcinoma, immunophenotype, triple negative*

Introduction

Breast carcinoma is one of the most common cancers in females worldwide with increasing incidence and TN breast carcinomas constitute 10-17% of all breast cancer cases [1]. Early diagnosis and aggressive multimodal treatment protocols have decreased the mortality rates [2]. Treatment methods are determined by using prognostic and predictive parameters like the patient's age, pathological tumor grade, menstrual status, status of hormone receptors and human epidermal growth factor receptor 2 (HER2) [2].

Treatment of this disease results in different clinical results even if the patients have the same laboratory and clinical profiles. There exists an obvious need for more data acquisition to understand the biology of this disease in order to improve the clinical outcome [2,3].

Recent studies about gene expression profiling suggested 5 subtypes of breast cancer which display different prognostic profiles: luminal A, luminal B, normal breast-like, HER2-overexpressing and basal-like subtype [4]. TN terminology is used for tumors that are uniformly negative for estrogen receptor (ER), progesterone receptor (PR) and HER2 and basal-like phenotype constitutes approximately 80% of all breast carcinomas that bear worse prognosis [5,6]. Previous studies showed that TN breast cancers have aggressive clinical and pathological features [7,8] . Several authors have reported racial and ethnic disparities in the clinical outcome and prognosis of TN breast carcinomas [9,10].

Carey et al.[10] reported that basal-like phenotype occurred more often in African-American

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women than in other racial groups and Bauer et al.[9] stated that TN breast cancer is more prevalent in non-Hispanic black compared with other ethnic groups.

There are few studies characterizing TN breast cancer in Asian populations and Middle East countries [11,12]. Kim et al. have examined the expression of CK5, CK14 and CK8/18, EGFR, c-kit, hormone receptors, p53, and HER2/neu in 776 Korean patients diagnosed with invasive breast carcinoma. Histologically, most basal-like breast cancers were invasive ductal carcinomas not otherwise specified (98 cases; 86.0%), with high nuclear and/or histologic grades, and most metaplastic carcinomas (6 of 8 cases; 75%) were of the basal-like subtype. The authors reported that the HER2/neu status was the most important prognostic factor [11].

El-Hawary et al. defined the luminal A subtype as the most prevalent (41.2%), followed by TN subtype (28.5%), then HER2-expressing subtype (19.4%) and luminal B subtype (13.9%). The most common histological subgroup was the infiltrating ductal carcinoma (83.2%), followed by the infiltrating ductal carcinoma (9.1%) and medullary carcinoma (3.2%). The authors concluded that the commonest molecular subtype of invasive breast carcinoma among Egyptian women was the luminal A subtype which had more favorable prognosis [12].

Our study investigated the immunohistochemical and histopathological charecteristics of TN and non-TN breast cancer in a group of Turkish patients.

Methods

This was a retrospective study that included 36 TN and 15 non-TN breast carcinoma patients who were diagnosed at the Pathology departments of Izmir Dokuz Eylul University and Hatay Mustafa Kemal University, Turkey, between 2005-2009. Excisional biopsy, incisional biopsy and modified radical mastectomy materials were used in this study.

The ethics committee on human research at our institution approved the protocol. Routine hematoxylin-eosin staining, and estrogen, progesterone, CerbB2, CK 5/6, CK 14, EGFR, E-cadherin, p53 and androgen receptor immunostains were performed on paraffin-embedded tissues.

Immunohistochemical staining was carried out by deparaffinization, dehydration and incubation in citrate buffer. A labeled streptavidin – biotin - peroxidase (immunoenzymatic) antigen detection system and AEC chromogen were used to observe the immunohistochemical reaction.

Staining was performed with mouse monoclonal antibody (Cell Marque Corp. USA) for CK5/6, with mouse monoclonal antibody (Novocastra-Leica Biosystems, Newcastle, United Kingdom) for E-cadherin, CK 14 and EGFR, with mouse monoclonal antibody (ScyTek Laboratories, UT, USA) for p53 and with mouse monoclonal antibody (Biocare Medical, LLC, CA, USA) for androgen receptor.

For the evaluation of CK5/6, CK 14, EGFR, E-cadherin, p53 and androgen receptor we counted the positively stained cells included in at least 5 dense stained fields, at a magnification of ×400 by DP2-BSW programme with Olympus BX53 light microscope. The evaluation of the immunohistochemical markers was performed by two pathologists as follows: ER and PR were categorized as negative (0%), low positive (1-10 %) and positive (>10%). HER2 positivity was based on the CAP (Canadian Association of Pathologists) guidelines (2007); only tumors with complete strong membranous staining of at least 30% of cells were considered as positive [6].

The results of all other immunohistochemical markers were evaluated as continuous variables based on the proportion of tumor cells with positive staining (1-100%), regardless of staining intensity according to Nofech-Mozes et al. study [6].

Statistics

Variables were expressed as mean ± standard deviation (SD) or as percents if categorical. For comparisons of the findings the Mann Whitney and Kruskal Wallis tests were performed. Statistical evaluations were performed using the SPSS 16,0 for Windows program and $p<0.05$ was considered statistically significant.

Results

The majority of TN cases were invasive ductal carcinomas (44.4%), followed by mixed carcinomas (invasive ductal and invasive lobular/secretory breast carcinoma; 27.7%), medullary carcinomas (11.1%), invasive lobular carcinomas (8.3%) and metaplastic carcinomas (8.3%). The staining pattern of TN cases is shown in Figure 1.

All of the cases were grouped according to histologic grade (Table 1). Nine (25%) were grade 2 and 27 (75%) grade 3 in the TN group, whereas 3 (20%) were grade 1, 8 (53.3%) grade 2 and 4 (26.7%) grade 3 in the non-TN group. CK5/6 stained significantly different in grade 2 and 3 cases ($p=0.033$) in the TN group. The other markers demonstrated non significant differences between grades ($p>0.05$).

Table 1. Pathologic grade in triple negative and non-triple negative cases

Grade	*Triple negative N (%)*	*Non-triple negative N (%)*
I	0 (0)	3 (20)
II	9 (25)	8 (53.3)
III	27 (75)	4 (26.7)

Table 2. The distribution of immunohistochemical staining among triple negative and non-triple negative cases

Immunohistostaining	*Triple negative Mean (range)*	*Non-triple negative Mean (range)*
CK 5/6	78.4 (0-100)	5.3 (0-30)
CK 14	84.8 (0-100)	8.0 (0-40)
EGFR	87.2 (40-100)	8.0 (0-30)
E-cadherin	96.9 (75-100)	53.2 (0-98)
p53	87.3 (0-100)	7.3 (0-60)
Androgen receptor	89.5 (70-100)	33 (0-90)

The mean values of the distribution of immunohistochemical markers in TN vs non-TN groups were as follows: CK5/6 78.4 vs 5.3%, CK14 84.8 vs 8%, EGFR 87.2 vs 8%, E-cadherin 96.9 vs 53.2%, p53 87.3 vs 7.3% and androgen receptor 89.5 vs 33.3% (all p-values <0.001; Table 2, Figure 2).

Discussion

Breast carcinomas are morphologically heterogeneous tumors and it is difficult to determine their clinical course due to different responses to treatment modalities . For this reason, tumors which do not benefit from conventional treatment methods should be reclassified with additional markers [7]. Besides, there are ethnic and geographic variations in various types of breast carcinoma. Recent studies conducted by Carey et al. and Umemura et al. demonstrated strong immunoreactivity for p53, vimentin, EGFR and Ki67 in TN breast carcinomas [10,13].

We investigated the TN breast carcinomas in our regions by applying CK5/6, CK14, EGFR, E-cadherin, p53 and androgen receptor and comparing them with non-TN tumors.

TN breast cancers were defined mainly by histology and grade prior to immunohistochemistry and expression profiling. The majority of our cases were invasive ductal carcinoma (44.4%) but the spectrum was wide despite the small number of our patients. Similar to our study Nofech-Mozes et al. and Williams et al. described the vast majority of ductal histology (92% and 91%, respectivelly) in their series [6,14].

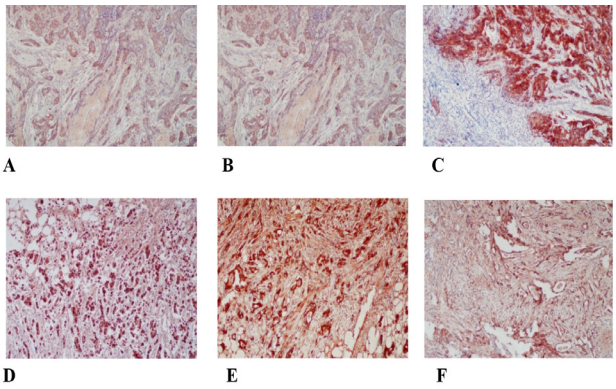

Figure 1. Expression of diverse markers in triple negative breast carcinomas: **A**: CK5/6 (x200), **B**: CK14 (x100), **C**: EGFR (x100), **D**: p53 (x100), **E**: E-cadherin (x100), **F**: androgen receptor (x100).

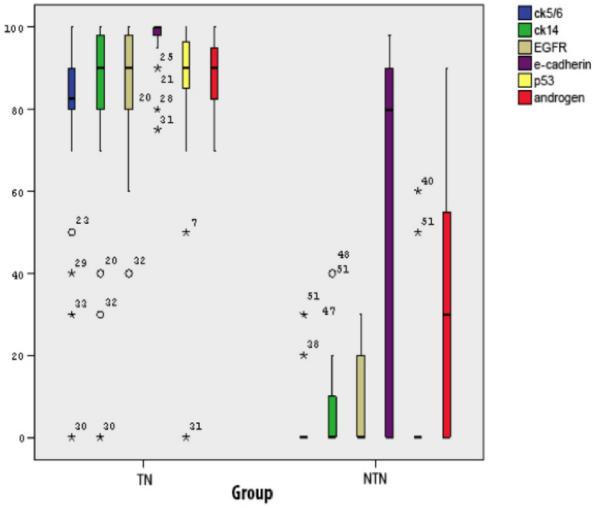

Figure 2. Distribution of immunohistochemical staining of triple negative (TN) and non-triple negative (NTN) cases (all p-values < 0.001).

There are a number of studies showing the relationship between of histologic grade and hormone negativity in breast carcinomas [7,10,15,16]. Carey et al. examined hormone receptor negative tumors and found that 26% of cases were TN and that these tumors were mainly of high histologic grade (grade 3) [10]. Dabbs et al. reviewed morphologically and stained 16 TN breast carcinomas and reported that all tumors were of high grade according to the Nottingham score 9/9 [16].

Similar to them all of our TN cases were high grade and grade 3 cases constituted the majority of them (75%). TN carcinomas are highly proliferative breast tumors and could be identified by basal cytokeratin expressions [6,7,17,18]. Rakha et al. examined a series of 1944 patients and reported positive immunohistochemical expression

of CK5/6 and/or CK14 in 157 (55.7%) TN cases [7]. Similarly Toyoma et al. examined all their TN breast carcinomas and found 31% positive for EGFR, 52% positive for CK5/6 and 55% positive for CK14 [2]. We also determined high positive rates in our study with 78.4% in CK5/6 and 84.8% in CK14.

Nofech- Mozes et al. followed 132 TN breast cancer patients of whom 116 expressed CK5/6; this rate was more common (87.9%) compared to ER (6.1%) and HER2 (16.8%) positive cancers, like in our cases [6]. In another study Yamamato et al. found positive expression of CK5/6 or EGFR in 15 (31.3%) and 16 (33.3%) respectively in their 48 cases of TN cancer [19].

Siziopikou et al. investigated CK5/6 and EGFR expressions in 271 patients (48;18% of them were TN). Of these cases 32 (67%) were CK5/6 positive and 22 (69%) EGFR positive [20]. In another study Collins et al. searched the frequency of EGFR and basal cytokeratin expressions in TN breast cancers with or without BRCA-1 mutations and found high rates of positivity, pointing out the association of basal-like phenotype with basal cytokeratins and/or EGFR expression [21].

Rakha et al. reported E-cadherin positive expression in 179 (65%) and androgen receptor in 36 (13%) TN breast carcinomas and in 754 (72.5%) and in 1000 (73%) in non-TN carcinomas [7]. In our series, the distribution rates of these two markers were 96.9% and 89.5% in TN vs 53.2% and 33.3% in non-TN breast carcinomas (p>0.05), which shows the low utility of these markers for distinguishing basal-phenotype tumors. p53 is a poor prognostic marker with high expression levels in TN breast carcinomas [7,15,17]. Rhee et al. determined high levels of p53 expression in their study composed of 136 TN breast cancers compared non-TN cases in Korean population [15]. We also determined diffuse p53 immunoreactivity in 87% of the TN group compared to 7.3% in the non-TN patient group, which could indicate poor prognosis in these tumors of Asian countries.

In summary, despite the small number of our cases, TN carcinomas in Turkish patients also express basal cytokeratins, have high levels of p53 proving their aggressive behavior and CK5/6 is the major immunohistochemical marker correlated with higher histologic grade. Further studies with larger number of cases and gene expression analyses from different regions would enlighten our questions about the nature and behavior of this group of breast carcinomas.

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