CLINICAL UTILITY OF TENSIN 2 LEVELS AS A DIAGNOSTIC AND PROGNOSTIC BIOMARKER IN BREAST CANCER

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ABSTRACT

Background*: To discuss the diagnostic and prognostic potential of tensin-2 (TNS2) levels in patients with breast cancer (BC).*

Methods: *The study included sixty patients with BC and twenty healthy female controls for a comparative investigation of TNS2 protein and gene expression levels..*

Results: *Compared to the healthy controls, we found that the patient group showed a statistically significantly lower mean level of TNS2 protein ($p<0.001$) and higher mean level of TNS2 gene expression ($p=0.015$). Secondly, we examined the clinical utility of TNS2 levels as an indicator of invasiveness and aggressiveness in BC by comparing patient TNS2 levels by stage and grade. Although the measured mean values differed between the patients subgrouped by tumor biology, grade, and stage, we found that the differences were not statistically significant.*

Conclusion: *Our findings suggest that TNS2 levels can be utilized diagnostically and prognostically, and that there is a need for further studies with larger he mean values measured differed between the patient subgroups based on tumor elucidate the clinical value of TNS2 protein and gene expression levels as an early prognosticator of aggressiveness in BC and thus a useful criterion in treatment optimization.*

Keywords: *Breast cancer, novel biomarkers, tensins, TNS.*

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Introduction

Breast cancer (BC) is the most common and psychologically most challenging type of cancer in women[(1, 2)]. The ongoing efforts of extensive screening for prolonged survival and better outcomes have proven effective; early diagnosis and multidisciplinary therapeutic modalities reduced the BC mortality in Europe by 15% from 2002 to 2012[(2, 3)]. Nevertheless, it remains a problem that most genetic and biochemical cancer markers used in the treatment and follow-up processes cannot provide adequately accurate information about tumor invasiveness and aggressiveness. One of the potential biomarkers that have been studied in patients with BC is tensin-2 (TNS2). Tensins are intracellular focal adhesion molecules that participate in signal transduction by binding to actin filaments and are also involved in tumor cell motility, invasion, and metastasis[(4-7)]. There are four types of tensins, among which tensin-4 (cten) has been the most studied adhesion-regulatory molecule and shown to be effective in cell migration.

Similarly, downregulation of tensin-3 has been shown to have negative effects on cell motility and cytoskeletal destabilization[8]. TNS2, known to participate in regulating cell migration and proliferation, is the most controversial member of the family[9]. There have been inconsistent findings indicating that increased TNS2 expression can both inhibit and induce tumor development, depending on tissue type[10-12]. While downregulated TNS2 expression has been shown in invasive prostate, breast, and kidney cancer development[7, 13], TNS2 overexpression is thought to be inhibitory to cell transformation by blocking binding via Ras[14]. However, although the effects of TNS2 on cell proliferation, migration and invasiveness in cancer have been studied at the cellular level[12], further studies are needed to confirm the clinical significance of the reported findings.

Accordingly, in this paper we aimed to present our results that suggest that low levels of TNS2 protein observed in patients with BC could be used as a tumor biomarker and an early prognosticator of metastasis and invasion.

Methods

Patients

The study included a total of eighty participants. Of these, sixty were patients who were treated for diagnosed BC at the Istanbul University Oncology Institute between 1 January 2017 and 1 January 2018 (Group 1). The remaining twenty were healthy female volunteers recruited as the control group (Group 2). Patients with carcinoma in situ, previously diagnosed/treated BC, and a history of radiotherapy or chemotherapy received for other pathologies were excluded. Patient demographic data, radiological, biochemical and surgical findings, and pathological reports were reviewed.

Tumor classification was performed according to the World Health Organization criteria and staging based on histopathological characteristics was performed using the AJCC staging system (7th ed.). Patient blood samples were obtained preoperatively and before any adjuvant chemotherapy. After 30 minutes at room temperature, the samples were centrifuged at 4000 rpm for 10 minutes. The resultant serum was stored at -80°C until all tests were completed.

Study ethics

Based on prior ethics consultation (with MKT), this prospective study was designed and conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the ethical review board of the Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey (2016-1276). Written informed consent was obtained from all participants.

Determination of serum levels using ELISA test

The TNS2 serum levels were determined by the double-antibody sandwich method of ELISA (enzyme-linked immunosorbent assay). Antigen-specific antibodies were absorbed onto the inner wall of the measurement wells, into which samples were pipetted. During the incubation, all sample antigens were captured by the immobilized antibodies, forming antibody-antigen complexes. After substances other than the antibody-antigen complexes were removed by washing, the reagent (enzyme-labelled antibody) was pipetted into the measurement tube. The primary antibody-antigen-enzyme-labelled antibody complex was formed during the second incubation step. Substances other than this complex were removed by washing again before the enzyme substrate was introduced. Colored product formation, directly proportional to the serum antigen concentration, was measured spectrophotometrically. The TNS2 serum concentrations were determined from the standard graphic.

The TNS2 mRNA levels were determined using a monophasic phenol and guanidine thiocyanate solution. Chloroform was added for the serum RNA isolation solution before incubation on ice for 5 minutes and then centrifuge at 4°C and 11000 rpm for 15 minutes. The RNA phase was placed into the propanol-containing tube and centrifuged again (4°C, 11000 rpm, 10 min). The RNA-containing pellet was washed with 75% alcohol, dried at room temperature, and dissolved in RNase-free water. cDNA synthesis from total RNA was carried out by use of a commercially available kit.

The serum expression determination was performed using GAPDH as internal control and SYBR Green fluorescent molecule.

Temperature (°C)	Duration (min)
42	10
50	60
70	10

Table 1: The cDNA synthesis conditions.

The expression levels were measured by the LightCycler® 480 real-time PCR system. (Table 1).

Six µL total RNA was used in real-time PCR for TNS2 gene expression. The primers CCTCAAAGGCGATGTCATGG and GCTGCCTTTGATCTTCTCGG were used as the forward and reverse primers, respectively.

Statistical analysis

Statistical analyses were performed using SPSS v. 22. Variables were investigated for normal distribution graphically and analytically (by the Kolmogorov-Smirnov/Shapiro-Wilk tests). The Mann-Whitney U and Kruskal–Wallis tests were used accordingly. ROC analyses were performed to assess the diagnostic usability of the parameters. The results were evaluated at a 95% confidence interval. $p<0.05$ was considered statistically significant.

Results

In 28 all patients, the tumor was localized in the right breast. In 15 patients, the tumor diameter was less than 2 cm. Pathologically, 45 patients had invasive ductal carcinoma. Clinical and pathological patient characteristics are presented in Table 2.

Although there was no statistically significant difference between the two groups in terms of mean age ($p=0.496$), the mean TNS2 protein level was significantly lower in the patient group ($p<0.001$) while the mean TNS2 gene expression level was significantly higher for the healthy controls ($p=0.015$) (Table 3).

We also evaluated in Group 1 by subgrouping for tumor diameter, lymph node involvement, pathological type, lymphovascular invasion, grade, and stage characteristics. In terms of mean TNS2 protein and gene expression levels, there was no significant difference between the patients whose tumor diameter was ≤2 and >2 cm. Of the sixty patients, 43 (71.6%) had invasive ductal carcinoma and the mean levels did not differ significantly in terms of pathological type ($p>0.05$). Nineteen patients (31.6%) showed lymphovascular invasion (LVI), a well-known pathological risk factor for neoplasm invasiveness, although there was again no significant difference between the LVI-positive and -negative patients ($p>0.05$).

With respect to tumor grade, which is an important determinant of disease-free and overall survival, 39 patients (65%) had grade 3 tumors. The mean TNS2 protein and gene expression levels were statistically insignificantly higher in the Grade 1-2 and Grade 3 subgroups, respectively.

	n
Number of patients	60
Age	
<50 years / ≥50 years	25/35
Localization of lesion	
Right/left	28/32
Tumor diameter	
<2cm/≥2 cm	15/45
Histopathology	
Invasive ductal carcinoma	45
Invasive lobular carcinoma	8
Others	7
Lymphovascular invasion presence	26
Necrosis presence	24
Molecular subtype	
Luminal	40
Non luminal	20
Grade	
I	5
II	20
III	35
pN status	
pN0	25
pN-positive	35
Disease stage	
Stage 1	15
Stage 2	37
Stage 3	4
Stage 4	4

Table 2: Clinical and pathological patient characteristics.

Twenty-three patients (38%) had axillary lymph node metastasis (LNM) while no lymph node or distant metastasis was observed in the other 37 patients. The LNM-negative patients showed an insignificantly higher mean level of TNS2 protein and lower mean level of TNS2 gene expression.

Tumor stage is the main factor determining disease-free and overall survival, and 48 (75%) of our patients had stage 1 or 2 cancer.

	Group 1 (n=60)	Group 2 (n=20)	p
Age (year)	51.86±10.48	49.3±10.82	0.353
Level of TNS2 protein (ng/ml)	265,33 (38,7-975,62)	832.84 (113-1045)	<0.001
Level of TNS2 gene expression	0.85 (0,01-2,22)	0.26 (0,05-0,62)	0.015

Table 3: Comparison of the two groups (mean±SD).

Similarly, the low-stage patients showed statistically insignificantly higher mean levels of TNS2 protein and lower mean levels of TNS2 gene expression. All these and other patient subgroup findings are summarized in Table 4.

	TNS2 protein level (ng/ml)	p	TNS2 gene expression level	p
Tumor diameter [a]				
≤2 cm	186.96±159.70	0.209	0.71±0.76	0.469
>2 cm	291.45±280.21		0.89±0.73	
pN status [a]				
pN0	287.33±295.66	0.589	0.81±0.77	0.794
pN-positive	247.88±227.81		0.87±0.72	
Pathological type [b]				
Invasive ductal carcinoma	225.79±199.10	0.120	0.76±0.68	0.281
Invasive lobular carcinoma	355.01±414.02		1.22±0.69	
Other	418.15±388.10		1.10±0.97	
Lymphovascular invasion [a]		0.832		0.362
LVI-positive	305.68±269.94		0.72±0.77	
LVI-negative	286.52±290.57		0.94±0.71	
Molecular subtype [a]		0.210		0.784
Luminal	295.44±270.51		0.83±0.71	
Non-luminal	197.58±221.19		0.89±0.80	
Grade [b]				
1	345.29±390.13	0.558	0.65±0.80	0.864
2	306.52±294.98		0.84±0.71	
3	235.55±260.12		0.87±0.78	
Pathological stage [b]				
1	189.00±166.62		0.77±0.76	
2	315.99±292.56	0.317	0.82±0.76	0.862
3	241.38±246.74		0.96±0.64	
4	112.98±50.54		1.12±0.73	

Table 4: Patient TNS2 protein and gene expression levels as analyzed based on subgrouping by tumor characteristics.
[a]*Mann-Whitney U test*
[b]*Kruskal-Wallis test*

The TNS2 cut-off value for the ELISA test was 183.96 (ng/ml), with 76.9% sensitivity and 78.9% specificity. The TNS2 cut-off value for the PCR method was found to be 0.33, at which 63% sensitivity and 63% specificity were observed (Table 5) (Figure 1-2). We found no statistically significant correlation between the TNS2 ELISA and PCR methods (p=0.086).

	AUC	p	Sensitivity	Specificity	Cut-off value
TNS2 ELISA	80.9	0.001	76.9%	78.9%	183.96
TNS2 PCR	67.5	0.001	63.5%	63.2%	0.33

Table 5: Sensitivity and specificity at the cut-off points.

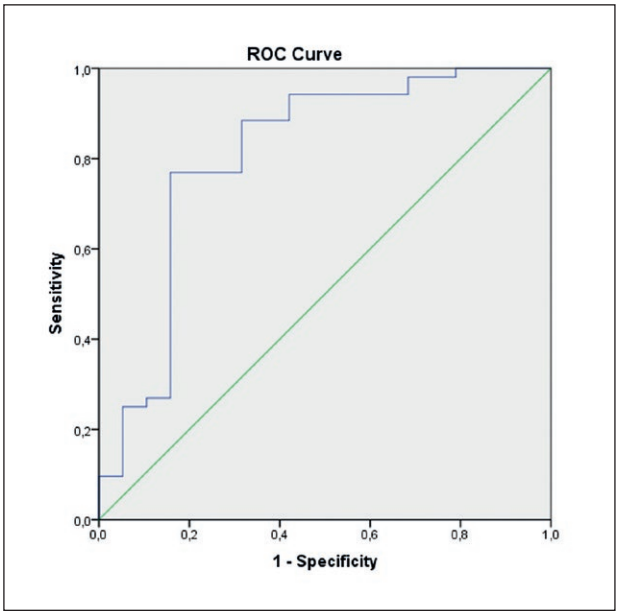

Figure 1: TNS2 cut-off values for the ELISA.

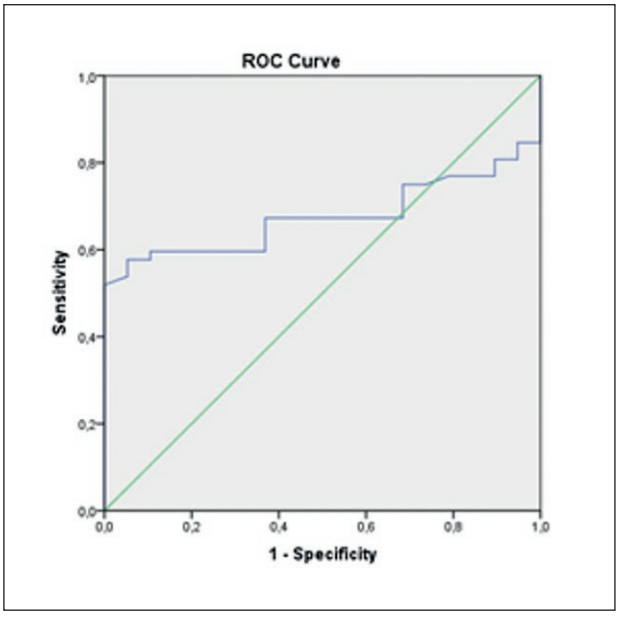

Figure 2: TNS2 cut-off values for the PCR methods.

Discussion

In BC, death principally occurs as a result of multiple organ dysfunction due to systemic spread. As in various malignancies, the most important factors for survival are early diagnosis and patient age at the time of diagnosis, which are highly relevant to cancer cell invasiveness and motility[15].

Although systemic spread is affected by many factors, the invasion and metastasis ability of cancer cells provide important insights into the aggressiveness of the disease, even in cases of tumors diagnosed at a very early stage of development. The ongoing cancer research on invasion and metastasis is primarily focused on cell adhesion and apoptosis. Besides their structural functions, tensins as intracellular adhesion molecules also participate in signal transduction and connection of the extracellular matrix with the actin cytoskeleton. Low levels of tensins have been shown to be effective on the invasion and aggressiveness characteristics as well as on tumor development. However, despite the many experimental studies demonstrating the link between TNS2 levels and invasiveness in BC, there currently appears to be only one study that deals with the potential clinical utility of this link[14].

In our study, we first aimed to investigate the diagnostic potential of TNS2 protein and gene expression levels. Compared to healthy controls, we found that our patient group showed a statistically significantly lower mean level of TNS2 protein and higher mean level of TNS2 gene expression. We think that the potential contribution of these data to early diagnosis warrant further studies with larger samples. Secondly, we investigated the clinical utility of TNS2 levels as an indicator of invasiveness and aggressiveness in BC by comparing patient TNS2 levels by stage and grade. Although the measured mean values differed between the patients subgrouped by tumor biology, grade, and stage, we found, contrary to expectations, that the differences were not statistically significant. We attributed this result to the low number of the patients included and possible heterogeneities between the subsamples.

Also, we observed that the lower levels of TNS2 protein detected in the patient group were more declined as stage and grade became more advanced, though again statistically insignificantly. We think that the numerical decline in the TNS2 protein measurements despite the TNS2 gene overexpression could be explained by analysis problems due to various cellular factors affecting protein production and determination problems due to different post-transcriptional product half-lives[16, 17]. Tensins have been suggested to suppress invasion and metastasis by the stabilizing effect of the integrin adhesion complex formed by increased cell adhesion and cytoskeleton reorganization, i.e., to function as a cell motility regulator or "metastasis regulator"[12, 18, 19]. Antioxidant chemicals and nutrients thought to show a chemopreventive effect by increasing tensin levels are recommended again many types of cancer[18]. Downregulation of tensins as signal transduction molecules and connectors between the extracellular matrix and actin molecules in the cytoskeleton may serve as a functional marker of malignant transformation. Studies at the cellular level have suggested that tensins could also serve as an indicator in determining the invasion ability/potential of tumors[12, 13]. Clinical studies have shown that low TNS2 protein levels in breast[12, 13], prostrate[12], and lung[13] cancers are generally effective on tumor invasiveness and prognosis, although these findings are controversial in some malignancies, as in the case of hepatocellular carcinoma (HCC). In HCC, TNS2 downregulation and TNS2 overexpression were reported to be a cellular marker for aggressive tumor behavior by Ryschich et al.[20] and Yam et al.[21], respectively. Our findings are also supported by the studies reporting that increased TNS2 protein levels are regulatory to the declined insulin receptor substrate-1 levels shown in patients with grade 3 BC and poor prognosis[22, 23]. In conclusion, tensins are molecules known to be effective on cancer behavior. However, although decreased TNS2 protein levels and increased TNS2 gene expression are described as risk factors for tumor development, the literature lacks clinical studies that investigate and demonstrate how these are relevant to invasiveness and aggressiveness in BC. Our findings suggest that TNS2 levels can be utilized diagnostically and prognostically.

Also, we showed that our patient subgroups based on tumor characteristics exhibited higher levels of TNS2 gene expression and lower levels of TNS2 protein with more advanced cancer stages and grades, although the intergroup differences showed no statistical significance, possibly due to the small sample size and heterogeneous subject distribution. Further studies with more patients can better elucidate the clinical value of TNS2 protein and gene expression levels as an early prognosticator of aggressiveness in BC and thus a useful criterion in treatment optimization.

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Statement of Ethics:
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Author Contributions:
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