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Serum endocan level and its prognostic significance in breast cancer patients

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ABSTRACT

Background: Endocan, known as endothelial cell specific molecule (ESM), is a novel endothelial dysfunction marker. The aim of this study is to examine the plasma endocan level and its prognostic significance in newly diagnosed breast cancer patients.
Methods: A total of 84 patients were enrolled the study. Plasma endocan level was measured by specific enzyme-linked immunosorbent assay (ELISA) kit. Ethical approval and informed consent were attained.
Results: At the time of diagnosis, 33 patients had stage 4 disease. The median plasma endocan level was 619.9 (min 259.9–2813.2) ng/L and its level was significantly higher in metastatic breast cancer group compared to non–metastatic breast cancer group. According to molecular sub-type of breast cancer, there is not statistical difference in plasma endocan level, but its level was higher in patients with Her-2 amplified and triple negative breast cancer (TNBC). Median follow-up time is 11 (1-30) months. Event free survival (EFS) was 15 months in patients with plasma endocan level lower than 620, while it was 4 months in patients with serum endocan level greater than 620 ($p = 0.016$). There was no difference between groups in terms of hypertension, age, Lymphovascular invasion (LVI), extra capsular extension (ECE), body mass index (BMI) and White blood cells (WBC), platelet count and plasma endocan level.
Conclusion: Plasma endocan levels higher than non metastatic breast cancer. Patients with high plasma endocan levels are short EFS. Further studies would be useful to assess endocan level as a prognostic factor in breast cancer.

1. Introduction

Breast cancer is the most common cancer in women. There are different types of breast cancer according to the molecular subtypes (such as luminal, her-2 amplified and TNBC). Neovascularization is also important for breast cancer as well as all cancers for metastasis and tumor invasion.

Endocan, known as endothelial cell specific molecule (ESM), is a dermatan sulphate proteoglycan. It is secreted especially renal, lung and tumor endothelium. ESM has also been evaluated as a biomarker of endothelial dysfunction. Plasma level of which has been shown to be elevated in sepsis, chronic kidney disease (CKD), lung injury and diabetic proliferative retinopathy (DPR).[1,2] Its secretion is regulated by some cytokines (such as TNF alpha, IL-1, VEGF-A, FGF-2).[1,3]

Plasma endocan level has been shown to increase in some cancers.[4,5] Its plasma level is shown positive correlation with in tumor recurrence and progression. It was used as a prognostic biomarker in some cancer types.

Studies on plasma endocan levels in breast cancer patients are very limited. Aim of this study was investigate between plasma endocan level and prognostic significance in newly diagnosed breast cancer patients.

2. Patient and methods

2.1. Patient and methods

Total of 84 newly diagnosed breast cancer women were included the study. This study is a cross sectional observational study. All patients were evaluated using standard form that

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included medical history and physical examination findings. Weight and height were measured. Estrogen (ER) and progesterone receptors (PR) nuclear staining ≥ 1% was accepted as ER and/or PR-positive by IHC evaluation according to the ASCO/CAP guidelines.[6] HER2 status was determined by immunohistochemical (IHC) staining. Tumors having a score of 3 (+) were considered as HER2-positive. Tumors scoring 2 (+) for HER2 expression were subsequently analyzed by fluorescence *in situ* hybridization (FISH) test and were considered as HER2-positive if HER2 amplification was present in FISH. There are different types of molecular subtypes of breast cancer.[7] Luminal subtype (A and B) is hormone receptor positive breast cancer. The most common type is luminal A with a low grade, low Ki 67 score, and good prognosis.[8] Luminal B subtype express more proliferation and her-2 gene and fewer ER-related gene.[9] Her-2 and TNBC subtypes are high grade and more aggressive types displaying high risk of systemic and local risk.[10,11]

Research ethics committee approval was obtained. The blood samples were collected from April 2014 to December 2015. Prior to collection of blood samples, the research purpose was explained and the consent of patients was obtained. Ten milliliters of peripheral blood samples were obtained from all patients. Blood samples were centrifuged via ultracentrifuge at 2000 rpm for 10 min. Then each serum sample was a liquated in three-eppendorf tubes were stored in -86 °C freezers until processing. Plasma endocan level were measured by an enzyme-linked immunosorbent assay kit (ELISA) according to manufacturer's standard protocol (Sunred Biological Technologies Human ECSM1/ENDOCAN ELISA Kit, Catalog No.: 201-12-1978, China). Endocan levels were expressed as pg/m. Protein standards were diluted four times. As a result of dilution step, five standard dilutions were obtained. Prepared standard dilutions and serum samples were added in a 96-well plate. Each standards and serum samples were added duplicate. Serum samples were incubated with ECSM1/ENDOCAN antibody and Streptavidin-HRP, at the same time standards were just incubated with Streptavidin-HRP for 60 min at 37 °C. After this incubation step, test well plate was washed 5 times with washing solution. When washing process was finished, chromogenic reagents were added to each well and incubated for 10 min at 37 °C in unlit area. After last incubation step, stop solution was added in each well and according to standards concentration optical density of samples was measured under 450 nm wavelength with *SpectraMax® Plus*microplate reader device within 15 min.

2.2. Statistical analyses

The statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA) Summary data were presented as mean standard lead and median distribution analysis. The Kolmogorov-Smirnov test was used to assess the normalization of the distribution. Comparisons between groups were made using the Mann-Whitney *U* test. Also data on normal distribution are expressed as mean ± SD and were using a *t*-test. Categorical variables are expressed using the chi-square test or fisher's exact test, as appropriate. Correlation analyses were performed using spearmen or Pearson correlation test. Results related to $p < 0.05$ were assumed statistically significant. Event-free survival (EFS) was defined as time from diagnosis until relapse or progression. OS was measured from the date of registration to the study to the date of death from any cause. OS and EFS were estimated using the Kaplan-Meier method.

3. Results

Mean diagnostic age of patients were 52.5 ± 13.3 (27-84), and 54.7% of the patients are postmenopausal. Demographic features of patients are seen in Table 1. Total of 12 (14.3%) patients had diabetes mellitus (DM) and 25 (29.8%) patients had hypertension (HT). At the time of diagnosis, 33 patients had stage 4 disease. Invasive ductal cancer (IDC) and invasive lobular cancer (ILC) constituted 76.2% and 3.2% of patients respectively. Total of 75% of patients were hormone responsive. Median plasma endocan level was 619.9 (min 259.9–2813.2). Its level was higher in metastatic breast cancer group than non–metastatic breast cancer group (p = 0.045) (Table 2). According to the sub-type there is not statistical difference in plasma endocan level, but its level was higher in patients with Her-2 amplified breast cancer and TNBC. There was no difference between groups in terms of hypertension, age, LVI, ECE, BMI and WBC and plasma endocan level. Plasma endocan level positively correlated with serum CEA level. But no association was observed BMI, WBC, platelet level, diagnostic age or serum ca 15-3 level. The plasma endocan level was lower in diabetic patients. Median follow-up time was 11 (1-30) months. There was not death. Event free survival (EFS) was 15 months in patients with serum endocan levels lower than 620, while it was 4 months in patients with serum endocan levels greater than 620 (p = 0.016) (Fig. 1). Patients features were evaluated according to metastatic situation. When clinicopathological parameters were compared according to metastatic or non metastatic situation there were no significant

Table 1
Patients characteristics.

	n (%)
T1	13 (15.5)
T2	42 (50)
T3	13 (15.5)
T4	12 (14.3)
N0	26 (31)
N1	20 (23.8)
N2	17 (20.2)
N3	17(20.2)
Stage	
Stage 1	10 (11.9)
Stage 2	21 (25)
Stage 3	20 (23.8)
Stage 4	33 (39.2)
Hormonal situation	
ER+	61 (72.6)
PR+	51 (60.7)
Hormone receptor	
Positive	63 (75)
Negative	21 (25)
BMI	
<24.9	18 (21.4)
25–29.9	34 (40.5)
>30	32 (38.1)
Co-morbid disease	
DM	12 (14.3)
HT	25 (29.8)
Pathology	
IDC	64 (76.2)
ILC	3 (3.6)
MİX	13 (16.5)
Mucinous	2 (2.4)
Metaplastic	1 (1.2)
Neuroendocrine	1 (1.2)
Sub-type	
Luminal A	26 (31)
Luminal B	34 (40.5)
Her-2	11 (13.1)
TNBC	13 (15.5)
Age of diagnosis	
<40	15 (17.8)
40-49	19 (22.6)
50-59	23 (27.5)
60-69	18 (21.4)
>70	9 (10.7)

Table 2
Association of serum endocan levels with clinical and pathological variables in breast cancer patients.

Parameters	n	Plasma endocan level	p
Stage			
Stage 1–3	51	547.6	**0.045**
Stage 4	33	695.3	
Sub-type			
Luminal A	25	581	0.41
Luminal B	34	611	
Her-2	11	678	
TNBC	14	595	
DM			
Yes	12	466	**0.007**
No	72	681	
HT			
Yes	25	542	0.16
No	59	671	
Menopause			
Premenopausal	34	673	0.47
Premenopausal	4	1312	
Postmenopausal	46	602	
Grade			
Grade1	42	581	0.2
Grade 2-3	41	683	
BMI			
<24.9	18	774	0.2
25–29.9	34	549	
>30	32	649	
LVI			
Yes	36	707.3	0.11
No	48	575.3	
ECE			
Yes	25	756.6	0.09
No	59	585.6	
WBC			
>10.000	10	671	0.56
<10.000	74	611	
Age			
<55	50	690	0.19
>55	34	564	

Abreviations: Lymphovascular invasion (LVI), extra capsular extension (ECE), body mass index (BMI) and White blood cells (WBC).

difference. Only CEA and Ca15-3 levels were higher in metastatic group than non metastatic group (Table 3).

4. Discussion

We have shown that plasma endocan level is higher in metastatic group than non metastatic group by means of in this study. Also we found that patients with high plasma endocan levels were poor prognosis. According to the sub-type analysis, there was not statistical difference in plasma endocan levels but the level was higher in patients with Her-2 amplified breast cancer and TNBC.

Endocan is secreted from vascular endothelium by stimuli of inflammatory cytokines, and regulates cellular adhesion, migration and proliferation. It also regulates relationships between leukocyte function associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM).[12] It is encoded by (5q11.2) gene.

Plasma endocan level has been shown to increase in some cancers.[13,14] High endocan mRNA levels were significantly higher in gastric tumor than non–tumor tissue.[4] Also plasma endocan level significantly higher in HCC patients than control group.[13]

Endocan was accepted as a biomarker of tumor progression.[3] Its level is correlated with HGF/SF (hepatocyte growth factor/scatter factor), which is associated with tumor development and progression. High endocan levels have been shown to be worse prognostic and related to metastasis in some cancers. In a study on HCC patients, high mRNA levels of ESM were associated with tumor node metastasis, vascular invasion and metastasis.[13] In another study with gastric cancer, plasma endocan level was found to be higher in patients with advanced stage than others in early stages.[4] In our study, it was shown that plasma endocan levels were higher in patients with metastatic breast cancer than non-metastatic patients. Also patients with high plasma endocan levels developed recurrence or progression in a shorter time.

Angiogenesis has a key role in many tumors. It is important in tumor development and progression, and shows tumor aggressiveness. Endocan is accepted as an angiogenesis marker. It is highly secreted in the proliferative endothelium. Increased level of plasma

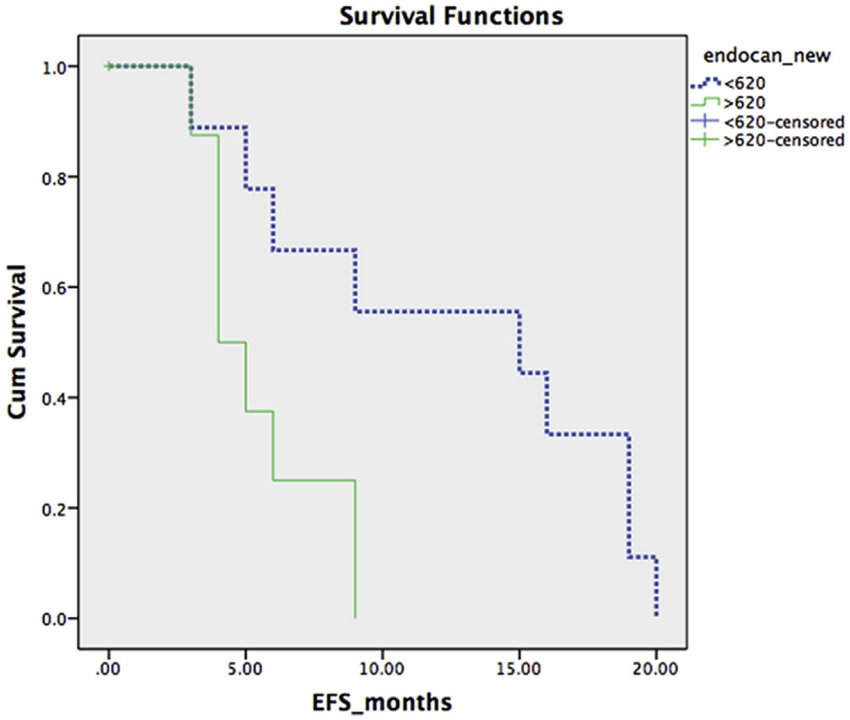

Fig. 1. Kaplan–Meier curves for Event free survival (EFS) of serum endocan levels.

Table 3
Clinicopathological parameters according to the metastatic status.

	Metastatic	Non-metastatic	p
No of patients	33	51	
Age, year	51 ± 12.4	53.1 ± 13.9	0.65
BMI, kg/m^2	28.9 ± 5.5	28.5 ± 5.4	0.73
Presence of hypertension [n, (%)]	7 (21%)	18 (35%)	0.16
Presence of diabetes [n, (%)]	4 (12%)	8 (24%)	0.75
Menaupose [n, (%)]			
Premenauposel	12 (36%)	22 (43%)	0.72
Postmenauposel	18 (54%)	28 (54%)	
LVI	16	20	0.27
CEA	0.8–3400 (2.6)	0.6–4.7 (1.3)	0.0001
Ca15-3	4.7–719 (23)	4.9–20.7 (12.3)	0.0001
Grade			
Grade 1	13 (39.4%)	29 (56.9%)	0.11
Grade 2-3	20 (60.6%)	22 (43.1%)	
Presence of estrogen receptor [n, (%)]	24 (72.7%)	37 (72.5%)	0.98
Presence of progesterone receptor [n, (%)]	20 (60.6%)	31 (60.8%)	0.98
Presence of Her2 [n, (%)]	9 (27.3%)	18 (35.3%)	0.44

endocan is correlated plasma VEGF level[14,15] This is associated with tumor hypoxia and progression. Endocan plays a role in cell proliferation, migration and tube formation and has proangiogenic properties.[16] Her-2 amplified and TNBC cases are aggressive breast cancer subtypes and these sub-types have the risk of rapid progression and metastasis. In our study, it was shown that plasma endocan level was higher in Her-2 amplified subtypes and TNBC sub-types, but not statistical significance.

Interestingly, serum endocan levels were found to be lower in diabetes mellitus patients than those of non-diabetics. It was seen that 12 diabetic patients had partially regulated diabetes (median HbA1c = 6.6 (5–10.7)).

One of the limitations of our study is the low number of patients and lack of control group without breast cancer. The duration of follow-up of our patients was limited.

As a result, plasma endocan level was elevated in metastatic breast cancer. High plasma endocan level at diagnosis is correlated with poor prognosis. There is not any statistical difference in plasma endocan levels across sub-types of breast cancer. Further studies would be useful to assess the interaction between breast cancer and serum endocan level.

Conflicts of interest

We declare that there are no conflicts of interest associated with this work.

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