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Is Endocan a Novel Prognostic Marker for Colorectal Cancer?

Korkmaz UB[1], Akyol M[2], Alacacioglu A[3], Salman T[3]*, Varol U[3], Küçükzeybek Y[3], Oflazoglu U[3] and Uzum Y[1]

[1]Department of Internal Medicine, Izmir Katip Celebi University, Ataturk Education and Research Hospital, Turkey
[2]Department of Medical Oncology, University of Health Sciences MD, Suat Seren Chest Diseases and Surgery Training and Research Hospital, Turkey
[3]Department of Medical Oncology, Izmir Katip Celebi Univercity, Ataturk Education and Research Hospital, Turkey

Abstract

Purpose: The present study aimed to investigate the relationship between pretreatment serum Endocan levels and pretreatment serum VEGF levels in colorectal cancer cases, as well as colorectal cancer prognosis.

Methods: This study included a control group of 16 individuals and a patient group of 67 cases with colorectal cancer. Serum VEGF and Endocan levels were determined with enzyme-linked immunosorbent assay (ELISA) kits used for scientific research purposes.

Results: The examinations showed no significant relations between serum endocan level and prognosis. Also, no significant difference was detected between the patient group and control group in terms of height, weight, age or BMI levels. The examinations indicated no significant difference between the groups except for the VEGF level. VEGF levels in metastatic colorectal cancer cases were significantly higher than the control group and the tumor-free colorectal cancer cases (p: 0.005, p: 0.038; respectively).

Conclusion: This study showed that there was no significant relationship between pretreatment Endocan levels and VEGF levels, as well as prognosis.

Keywords: Colorectal cancer; Endocan; VEGF

Introduction

Colorectal cancer is the second most common type of cancer in women and the third one in men. In terms of worldwide prevalence, it ranks third [1]. The prognosis of colorectal cancer is dependent on the stages in the TNM system. The development of tumors and metastases depend on a delicate balance between endogenous angiogenic factors, which cause the formation of new blood vessels, and anti-angiogenic factors [2]. The process of angiogenesis consists of a multitude of sequential and interconnected steps including positive and negative regulators [3]. Today, it is known that angiogenesis is not only essential for tumor growth but also is responsible for the cancerous transformation of a premalignant tumor, circulation of cancer cells, and the transformation of micro-metastases into typical metastatic lesions [4]. Without doubt, the vascular endothelial growth factor (VEGF) is the most important molecule that plays a role in the angiogenetic process [5,6]. VEGF does not only induce the proliferation of endothelial cells but also increases the vascular permeability and causes the formation of a fibrin matrix that enables stromal cell invasion by increasing the extravasation of proteins through tumor vessels [7]. The data provided by preclinical and clinical studies indicate that VEGF is the predominant angiogenic factor in colorectal cancer [8]. A positive correlation was detected between increased VEGF levels and lymph node involvement, and distant organ metastasis [9].

Endocan (Endothelial cell-specific molecule-1) is a 50-kDa dermatan sulfate released from activated vascular endothelial cells including tumor cells [10]. In humans, it is mostly secreted by the lung endothelium, followed by renal and gastrointestinal channel endothelia with lower rates [11]. Endocan plays a role in the regulation of a series of biological processes such as adhesion, migration, proliferation and neovascularization [12]. Recent studies suggest that endocan expression is associated with tumor neovascularization, angiogenic transition in stem cells, and endothelial-mesenchymal transition process such as arterial wall remodeling [13]. Endocan synthesis is increased by VEGF-A, VEGF-C, IL-1, TNF-a, TGF-B1, and FGF-2; and it is reduced by interferon-γ and phosphatidylinositide 3-kinases (PI3K) [10].

The present study aimed to investigate the pretreatment Endocan levels in colorectal cancer cases with their VEGF levels, as well as its relationship with prognosis.

Patients and Methods

Study participants and blood sampling

This study included a control group of 16 individuals and a patient group of 67 colorectal cancer cases who referred to the Medical Oncology Outpatient Clinic at Izmir Katip Celebi University Ataturk Research and Training Hospital from January 2012 to December 2015. The patient's pre-treatment Endocan and VEGF levels, as well as body mass index (BMI), body fat index, fat-free body mass, and total body water scores were recorded.

We formed a control group of healthy individuals with similar demographic characteristics and measured their body mass index (BMI) scores, Endocan and VEGF levels [14]. The inclusion criteria were as follows: No pregnancy or breastfeeding, no other malignancies except colorectal cancer and no record of prior treatment for colorectal cancer.

We received their verbal informed consent prior to the study. The BMI was calculated in kg/m^2. Anthropometric measurements and bioelectric impedance analysis of the patients were performed at the

***Corresponding author:** Tarık Salman, Department of Medical Oncology, Izmir Katip Celebi Univercity, Ataturk Education and Research Hospital Associate Professor, 35360 Izmir, Turkey, Tel: 905066268179, 902322431530; E-mail: drtariksalman@yahoo.com

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beginning (basal). Body composition [total body water (TBW), fat-free mass (FFM), fat mass (FM), percent body fat] was measured by bioelectrical impedance analysis using TANITA BC-420MA scale. One nurse performed the measurements for all patients.

Assessment of endocan and VEGF levels

Two tubes of ethylenediamine tetra-acetic acid (EDTA) venous blood were extracted from the patients after 8-12 hours of fasting, in the morning (08:00-09:00 AM), before chemotherapy [15]. After half an hours rest, the blood samples were centrifuged at 2000 g for a period of 10 minutes. Separated serum samples were portioned into closed Eppendorf tubes and saved at -20°C throughout the study of tests. Serum VEGF and Endocan levels were determined with enzyme-linked immunosorbent assay (ELISA) kits used for scientific research purposes.

After the serums were solved at room temperature, the deposits of protein molecules were mixed with a vortex and the sample was homogenized [16]. The patient serums were studied following the procedures specified in ELISA kits. After the study procedures, the microplate was checked at 450nm wave-length at the ELISA reader to calculate concentrations.

Statistical analysis

The SPSS 21.0 Inc (IL, USA) was used for the statistical analyses of the research findings. Descriptive analyses were presented using mean and standard error (S.E.M.) for variables. Due to the non-normal distribution of variables, non-parametric tests were conducted to compare those parameters. The Mann-Whitney U-test was used to compare the parameters between control and patient groups. In independent groups, distribution and variance analyses were performed when it included more than two groups. We used the one-way ANOVA test for groups with normal distribution and variance and used Kruskal-Wallis test for groups without normal distribution. The correlation of Endocan, VEGF and overall survival rates with other variables was analyzed with Pearson's correlation test. A p-value o less than 0.05 were taken to indicate statistical significance.

Results

Twenty-six of the patients had rectal cancer, and 41 of them were diagnosed with colon cancer. Regarding the stage at diagnosis, 1 patient was Stage-1, 12 were Stage-II, and 25 were Stage-III. The patient group's mean age was 60.6, which was 52.8 for the control group. The mean weight of the former was 69.2 kg and BMI was 25.4, which were 76.1 kg and 26.1 for the latter. The patients' demographic characteristics are given in Table 1.

In the follow-up period, 43 patients presented metastasis at the onset or during the follow-up, while 24 patients presented no metastasis or progression (tumor-free patient group). Endocan and VEGF levels of metastatic patients were 10.43 ± 2.59pg/mL and 304.2 ± 314.07pg/ml respectively. No significant difference was find between the patient and control groups in terms of height, weight, age or BMI levels. The examinations showed no significant difference between the groups except the VEGF level. A comparison of two groups with respect to VEGF levels revealed a significant difference (p: 0.040). No significant difference was observed between the groups in terms of Endocan levels. Table 2 presents the serum VEGF, Endocan levels, body composition and anthropometric measurements of patients and the control group.

The correlations of VEGF, Endocan and overall survival rates were observed in the patient group. The correlation analysis presented no significant difference. Although a negative correlation was detected between VEGF levels and overall survival, it was not significant (Table 3).

Characteristics	Patient Group (n:67) n%
Age (mean ± SD)	60.57 ± 13.14
Height (mean ± SD) cm	165.31 ± 10.7
Weight (mean ± SD) kg	69.21 ± 14.9
BMI	
Sex	
Male	45 (67%)
Female	22 (33%)
Rectum/Colon	
Rectum	26 (39%)
Colon	41 (61%)
Ras Mutation (32 patients)	
Panras Wild	2 (6%)
Wild	15 (47%)
Mutant	15 (47%)
Stage at Diagnosis (SAT)	
I	1 (1%)
II	12 (18%)
III	25 (38%)
IV	29 (43%)
Neo-AdjuvantCT	14 (19%)
AdjuvantCT	37 (55%)
1st LineCT	40 (60%)
2nd LineCT	16 (23%)
Recent Condition	
Alive	40 (60%)
Exitus	27 (40%)

Table 1: Demographic characteristics of patients with colorectal cancer.

	Metastatic	Tumor-Free Colorectal	Control	p
Endocan	10.43	10.17	11.19	0.49
VEGF	304.26	186.65	142.14	0.040
Age	60.93	59.92	52.81	0.118
Weight (kg)	69.54	68.62	76.18	0.249
BMI	25.66	24.9	26.17	0.696
Fat-Free Body Mass (FFM)	51.19	54.47		0.122
Fat Mass (FM)	18.34	14.14		0.105
Muscle Mass (MM)	48.63	51.73		0.15
Total Body Water (TBW)	35.86	37.57		0.163
Basal Metabolism Rate (BMR)	6259.58	6582.29		0.078

Table 2: Age, weight, BMI, serum VEGF, ESM-1, body composition anthropometric comparisons between groups.

Table 4 presents a comparison of parameters between groups in order to examine intergroup differences of VEGF levels. No significant difference was found between the control group and tumor-free colorectal cancer group in terms of VEGF levels. However, VEGF levels in metastatic colorectal cancer cases were significantly higher than that of the tumor-free colorectal cancer cases (p: 0.005, p:0.038, respectively).

Discussion

In this study, we found no significant difference in terms of Endocan levels between the groups. Moreover, there was no correlation between Endocan levels and VEGF levels.

Endocan is a proteoglycan that plays a role in many pathophysiological processes such as inflammatory diseases, adhesion,

Variables	ESM-1		VEGF		Overall Survival	
	R	P	R	P	R	P
Endocan	1		0.18	0.146	0.005	0.967
VEGF	0.18	0.146	1		-0.223	0.069
Overall Survival	0.005	0.967	-0.223	0.069	1	
Weight	-0.155	0.21	-0.058	0.641	0.138	0.266
FM	-0.151	0.222	0.041	0.74	0.13	0.296
FFM	-0.086	0.49	-0.127	0.307	0.08	0.519
MM	-0.084	0.503	-0.124	0.32	0.078	0.535
BMI	-0.236	0.055	-0.077	0.537	0.102	0.134

Table 3: Correlation of variables in the patient group by Pearson's Test.

	N	Mean	SD	p
Tumor-Free	24	186.6571	138.2103	0.317
Control	16	142.1482	110.2186	
Metastatic	43	304.2698	314.0766	0.005
Control	16	142.1482	110.2186	
Tumor-Free	24	186.6571	138.2103	0.038
Metastatic	43	304.2698	314.0766	

Table 4: Evaluation of VEGF levels by groups.

angiogenesis and tumor progression. However, Endocan is reported to be expressed at lower rates in colorectal cancers [12,13,17].

A study conducted by vant Weer et al. on 78 patients with breast cancers shows that Endocan expression is associated with reduced 5 years survival and increased risk of metastasis [18]. Likewise, previous studies demonstrated that increased tissue-level expression of Endocan levels was associated with poor prognosis and metastasis in breast cancer, renal-cell carcinoma and lung cancer [18,19].

Zou et al. showed that Endocan expression was higher in healthy subjects and well- and moderately-differentiated colorectal cancer cells, whereas it was low in poor-differentiated colorectal cancer [20]. In another study by Jiang et al., Endocan expression was detected significantly higher in patients with colorectal cancer than healthy subjects. The same study also demonstrated a correlation between increased tumor stage, lymph node positivity, increased histological tumor grade, and Endocan levels [21].

Although high Endocan levels are associated with poor prognosis in many other types of cancer, it was examined at the tissue level and no positive correlation was observed with stage, unlike other cancers [22].

VEGF is a lymphangiogenic marker that is typically expressed by cancer cells to a high degree than normal cells. In a study conducted on 121 patients, Cascinu et al. showed that VEGF expression was higher in metastatic patients than non-metastatic patients. The tissue-level VEGF expression was evaluated in patients with Stage-II colorectal cancer; 5 years disease-free survival was 90% in patients without VEGF expression than those with VEGF expression, which remained at 50% for the latter group. Therefore, it is suggested that high VEGF levels may be associated with advanced stage and worse prognosis [22]. In a meta-analysis by Des Guets et al. that included 27 studies examining the relationship between VEGF and colorectal cancer, high VEGF expression was observed to have a marked correlation with reduced overall survival [23]. If one generally considers the findings reported by other studies, colorectal cancer cells seem to be directly or indirectly related to the high expression of neovascularization-associated molecules.

Conclusion

In this study we found pre-treatment serum VEGF levels in the metastatic patient group significantly higher than both the tumor-free patient group and the control group. A comparison of tumor-free colorectal cancer cases with the control group showed no significant difference in terms of VEGF levels. These findings support the idea that high VEGF levels could be associated with poor prognosis. An assessment for a cut-off value to indicate poor prognosis revealed no threshold VEGF level to anticipate prognosis. In the present study, although a negative correlation was observed between VEGF levels and overall survival, the difference was not significant.

The limitations of the present study include a small sample size and short follow-up period. Only 27 patients passed away throughout the period of study, which could account for the non-significance of overall survival findings. There is a limited number of studies on Endocan levels in colorectal cancer, and the findings are contradictory when compared with the findings reported by previous studies investigating other types of cancer. This study is the most current study on Endocan levels in colorectal cancer. In conclusion, this study showed that there was no significant relationship between pretreatment Endocan levels with prognosis and VEGF levels. Further studies with larger samples are required to clarify this issue.

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