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Original Research

Determination of IL-6, TNF-α and VEGF levels in the serums of patients with colorectal cancer

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Abstract: Cytokines are multifunctional polypeptides synthesized by different body cells. They have clinical significance in terms of disease diagnosis, treatment and prevention. Cytokines TNF-α and IL-6 play an important role in the growth and differentiation of cells.Vascular Endothelial Growth Factor (VEGF) is excessively produced in epithelial, mesenchymal, and particularly in tumor cells. Studies have shown that the increased serum concentrations of IL-6, TNF-α, VEGF are strongly associated with colorectal cancer and directly with the clinical stage of the disease. This can be used to diagnose cancer and to identify patients with a bad prognosis who can avail themselves of a more aggressive treatment. The present study investigated the role of cytokines in the development of cancer by comparing preoperative serum cytokine levels of patients suffering from colorectal cancer with those of the healthy control group. The prognostic significance of the data obtained has also been evaluated. For this purpose, IL-6, TNF-α and VEGF levels in 60 serums, 30 preoperatively taken from patients with colorectal cancer and 30 from a healthy control group at Çanakkale Onsekiz Mart University General Surgery Clinic, were determined by ELISA kits. The statistical analyses of the obtained data were evaluated on SPSS, a statistical package program. In this study, no significant difference was obtained between the mean scores concerning the IL-6 and VEGF serums of the colorectal cancer and healthy group ($p>.05$). But a statistically significant decrease was observed in the TNF-α serum level of the colorectal cancer group in comparison with the control group ($p= .016$; $p < .05$).

Key words: Colorectal Cancer; IL-6; TNF-α; VEGF.

Introduction

Colorectal cancer is the third most common type of cancer. It is the second after prostate cancer in men and the third after breast and cervical cancer in women (1,2).

Colorectal cancer is a multifunctional disease and there are numerous peripheral and genetic factors operative in the development thereof (2). Immune system is considered to exert an initiating effect in some of the subtypes of colon cancer, and it is known that cytokine and cytokine receptors in the circulatory system can be used as a biomarker for diagnostic and prognostic purposes when evaluating the biological properties of colon cancer (4,5)

Cytokines are referred to as low molecule weight proteins promoting cell-to-cell communication (6). They play a significant role in the development of cellular and humoral immune responses, the stimulation of inflammatory responses, the regulation of hematopoiesis, the surveillance of cellular proliferation and differentiation, and the initiation of wound recovery (7). They become operative by binding to their specific ligands in the target cells (8). They are secreted into blood and assorted cellular fluids by certain cells and bind to cellular receptors in other parts of the body (9).

Cytokines also play a crucial part in cancer pathogenesis. Among the best examples of this group is interleukin-6 (IL-6). It is an important cytokine which exhibits (IL-6) pleiotropic properties (a single cytokine's effect on more than one cell type) in colon cancer. It allows tumor cells to grow and differentiate (10). IL-6 has been shown to promote the formation of *in vitro* human colon carcinoma cells (11). It is a cytokine produced from lymphoid and non-lymphoid tissues and regulates immune reactions, acute phase response, inflammation, oncogenesis and hematopoiesis. It is produced in response to interleukin-1 (IL-1) and tumor necrosis factor (TNF) key to the emergence of immune and inflammatory issues (12,13). Increased serum IL-6 level is associated with numerous cancer types, particularly prostate, bladder, colon and breast cancer (14). A great many anti-inflammatory properties besides inflammatory property of IL-6 are well known. IL-6 was demonstrated to increase IL-1ra level but to decrease TNF level in the circulatory system (15). IL-6 suppresses TNF effect and induces TNF antagonists (16).

Tumor necrosis factor–α (TNF-α) is a cytokine secreted by most cells and allows the destruction of cancerous cells. They are produced by macrophages and some other cells (17). Previous studies have showed that TNF-α is the most important cytokine in acute inflammation and anti-tumor immunity (18).TNF-α is known to play an important role in tumor growth and progression. TNF-α is responsible for neoplastic tissue destruction and some tumor-induced local and systemic effects. However, it is still a hotly debated issue whether TNF-α plays an antiproliferative role in tumor (19, 20).

Vascular endothelial growth factor (VEGF) is one of the most powerful stimuli in angiogenesis (the formation of new blood vessels play a significant role in cancer

and metastasis). VEGF is a multifunctional glycoprotein which has an angiogenic, mitogenic and vascular permeability-increasing effect on vascular endothelium in particular. VEGF is overexpressed in epithelial cells, mesenchymal cells and especially tumor cells (including breast, non-small cell lung and colorectal cancer) (21). VEGF can exert several effects on tumor growth such as endothelial cell proliferation and survival, the stimulation of neovascularization, and the suppression of host immune response (22-24). VEGF could have a crucial role in the risk, diagnosis and survival (25).

There is exiguous research on what kind of a preoperative effect cytokines as markers have on the treatment of patients suffering from colorectal cancer. Therefore, the present study is intended to associate the serum levels of cytokines (IL-6, TNF-α) and VEGF (particularly in the metastatic stage), which are considered to provide guidance in the prognosis and treatment of the disease, with regular checks in preoperative patients with colorectal cancer.

Materials and Methods

Venous blood samples were taken from preoperative 30 patients (19 males and 11 females) at 42-82 years old who came to the General Surgery Clinic of Çanakkale Onsekiz Mart University and were diagnosed with colorectal cancer by colonoscopic examination and 30 patients (16 males and 14 females) at 44-88 years old who came to the same clinic but only scanned patients without clinical complaints.Serum isolated from blood samples which were centrifuged at 3000 rpm for 10 min were put in eppendorf tubes and stored at -80 C till the biochemical analysis.

Measurement of the cytokines

The serum samples stored at -80°C till the measurement day were let to warm up to room temperature and then IL-6, TNF-α and VEGF serum concentrations of the cancer and control group were determined by ELISA (Enzyme-linked Immunusorbent Assay) The analysis results obtained by human ELISA kit (Diaclone) were elicited on ELISA reader (ELISA reader BioTek ELx800) by measurements at 450 nm. The IL-6 and TNF-α concentrations and VEGF concentrations in the studied samples were expressed in pg/ml and ng/ml, respectively.

Statistical evaluation

The test of normality conducted using Shapiro Wilk test with the data obtained from the patients with colorectal cancer yielded a non-normal distribution. Paired comparisons were performed on the non-parametric Mann-Whitney U test. The differences between two groups were evaluated by the Mann-Whitney U test. The means of the standard deviations of the obtained results were calculated and the values below .05 were considered statistically significant ($p<.05$). The statistical analyses were carried out on SPSS 20.0 (Statistical Package for Social Science 20.0).

Results

The IL-6, TNF-α and VEGF serum concentrations of 30 patients (19 males,11 females; average age: 63.97) with colorectal cancer were compared with those of 30 healthy patients (16 males,14 females; average age: 63.80). The patients' characteristics are presented in Table 1. The elicited means and standard deviations

Table 1. Patient characteristics (n=30).

No	Gender	Age	Tumor size	TNM stage	Location
1	M	52	4cmx4cmx1 cm	T3	Sigmoid
2	F	67	4x5x4 cm	T3	Rectum
3	M	61	4 cm	T3	Column
4	F	58	6 cm	T4	Column
5	M	46	5 cm	T3	Column
6	F	60	5,5x4,5x1,7 cm	T3	Column
7	M	76	2,5x1,5x0,5cm	T2	Sigmoid
8	M	78	0,6x0,4x0,4 cm	T3	Rectum
9	M	60	0,3x0,3x0,2 cm	T3	Rectum
10	F	79	4,5x2,5x1,5 cm	T3	Column
11	M	78	2X3X4	T2	Column
12	F	45	4,5x2,5x1 cm	T3	Column
13	F	70	5 cm	T3	Column
14	F	82	8x6x5 cm	T3	Column
15	M	68	4 cm	T3	Column
16	M	54	3 cm	T2	Column
17	F	66	4 cm	T3	Column
18	M	72	6 cm	T 4	Column
19	M	42	5 cm	T2	Column
20	M	61	2,5 cm	T3	Column
21	F	62	5 cm	T1	Column
22	M	62	5cm	T4	Column
23	M	67	8x7cm	T3	Rectum
24	M	55	5 cm	T3	Column
25	M	78	5 cm	T2	Column
26	F	62	3 cm	T2	Column
27	F	56	6 cm	T3	Rectum
28	M	64	2x1,5x1 cm	T3	Column
29	M	59	5 cm	T2	Rectum
30	M	79	4,5 cm	T2	Rectum

Table 2. Mean and standart deviations values of patients and control groups. (%CV=SD/mean x100).

	Control groups (n=30) Mean ±SD	Patient groups (n=30) Mean ±SD	p
IL-6	42.99±12.73	33.78±10.12	0,652
% CV	28.6	29.9	
TNF-alpha	70.33±33.8	14.92±2.76	0,016
% CV	48.06	18.5	
VEGF	891.96±137.83	859.11±88.03	0,569
% CV	14.45	10.25	

(±SD) are shown in Table 2. No significant difference was obtained between the mean scores concerning the IL-6 and VEGF serums of the colorectal cancer and healthy group (p>.05). But a statistically significant decrease was observed in the TNF-α serum level of the colorectal cancer group in comparison with the control group (p= .016; p < .05).

Discussion

Although the pathogenesis of colorectal cancer has not been understood yet, it has been proven through various studies that cytokines play an important role in the development of colorectal cancer (26). Biological evaluation of colon cancer indicates that circulating cytokine levels may be used as a biomarker for diagnostic and prognostic purposes in cancer patients. The development of colorectal cancer is associated with changes in cytokine production (27).

Pro-inflammatory cytokines (IL-6 and TNF-α) play a central role in inflammatory reactions. Many studies in the field of colorectal cancer suggest that TNF-α supports cancer development. The recently obtained data demonstrate that IL-6 has a strong role in colon cancer. Previous studies have shown that IL-6 levels in sera of patients with colorectal cancer are 2-10 fold higher than controls and this increase is even greater in metastatic patients. Many studies have reported that IL-6 is associated with metastasis (28,29). Askisawa et al. investigated the diagnostic significance of IL-6 in lymph node metastasis and hepatic metastasis in patients with colorectal cancer and suggested that the serum IL-6 levels exhibited a statistically significant correlation in such cases. Another research states that cytokine regulation in human colorectal cancer could not be clarified thoroughly (30-33).

It has been claimed in a study that TNF-α can play a negative role in the development of colorectal cancer by promoting neovascularization and increasing tumor metastasis and in another study that TNF-α's role in the local regulation of tumor development is unclear(34-35). It is also stressed in a different study that TNF-α is produced by malignant cells of late-stage cancer and considered to be a bad prognostic factor (36).

The relationship between the serum VEGF levels in cancer patients and the prognosis has been investigated for a long time. There are studies evidencing and refuting the relationship between the serum VEGF levels in cancer patients and its stage and prognosis (37,38). A study on this matter found a correlation between tumor and VEGF expression in the early stage cancer (39,40). De Vita et al. mentioned that VEGF level was increased in patients suffering from cancer in comparison with regular checks (and especially pre-operative serum VEGF level in patients with lymph node metastasis was significantly increased) and that the VEGF expression was associated with tumor growth and metastasis (41,42). Today the role of VEGF levels in the prognosis of colorectal cancer is still a matter of debate.

In our study, no significant difference was observed between IL-6 and VEGF levels in the serum of patients with colorectal cancer and the control group. Further, serum TNF-α levels of the control group decreased compared with cancer patients. It can be considered that TNF-α may circulate only periodically in cancer patients. We believe that periodic sampling is needed to obtain more meaningful results.

This study relates the absence of significance concerning the preoperative IL-6 and TNF-α serum levels of the patients with colorectal cancer in comparison with the healthy cases to the small number of the sampling and to the fact that the tumor stage generally covers patients in the middle stage. With larger numbers of patient groups, the study may help to better assess the clinical relevance of cytokines.The fact that the measured parameters did not have a prognostic value according to the obtained data may have resulted from different factors. Such factors as the biological and clinical table of the disease and lymph node metastasis affect clinical evaluation. We recommend that we conduct more extensive investigations to contribute to the effects of cytokines on colorectal cancer.

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