Plasma soluble thrombomodulin and soluble endothelial protein C receptor levels in colorectal cancer patients

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Aim: Thrombomodulin (TM) and endothelial protein C receptor (EPCR) are 2 transmembrane proteins that are thought to play an important role in cancer. We aimed to discover whether these 2 proteins are prognostic indicators in colorectal cancer.

Materials and methods: Plasma TM and EPCR levels were measured using the ELISA method in 50 patients in different tumor stages that had been recently diagnosed with colorectal cancer and in a healthy control group of 50 people.

Results: In colorectal cancer patients, higher plasma TM (21.3 ± 22.8 ng/mL, 13.2 ± 16.2 ng/mL, $P = 0.010$) and plasma EPCR levels (149.9 ± 79.6, 113.3 ± 49.3, $P = 0.007$) were determined compared to the control group. No statistically significant relationship was present between plasma TM, EPCR levels and tumor stage, tumor localization, tumor differentiation, lymphovascular invasion state, microvascular thrombus existence, and thrombosis progression ($P > 0.05$).

Conclusion: We think that these 2 proteins are released into plasma as an indicator of endothelial dysfunction and can play a role in pathogenesis and biology of colorectal cancer.

Key words: Thrombomodulin, endothelial protein C receptor, colorectal cancer

1. Introduction

Colorectal cancer is one of the most frequently diagnosed cancers in the world, and the biology of colorectal cancer is now known better because of ongoing studies. However, there is still much work to be done in defining new prognostic factors and guiding treatment decisions.

With recent studies, it is suggested that hemostatic system components play an important role in cancer progression. Thrombin and fibrin, which are the last products of the coagulation cascade, contribute to proliferation, migration, local invasion, angiogenesis, and metastatic spread of cancer cells (1,2).

Thrombin plays a key role in the operation of coagulation and anticoagulant systems. The most important components of the protein C pathway, which is classically known as the anticoagulant system, are thrombomodulin (TM) and endothelial protein C receptor (EPCR). TM activates protein C by forming a complex with thrombin. With the binding of protein C to in vivo EPCR, this activation increases 20 times more. When the activated protein C (APC) separates from

EPCR, it binds to an appropriate surface on protein S and together they decrease uncontrolled thrombosis formation by inactivating factor Va and factor VIIIa (3,4). The role of these 2 proteins' expression in tissues and the role of their soluble forms regarding malignancy are still not completely known. However, studies have shown that soluble TM increases in various cancer types (colorectal, pancreas, breast, leukemia) and plasma level increases further with progression of the disease (5). Although expression of EPCR in different tumor cell series is shown, there is no information about its role in malignancy, even though increased levels of its soluble form can be related with elevated thrombosis risk (6,7).

The aim of our study was to measure plasma-soluble TM and EPCR levels in colorectal cancer patients, and then compare these results with both a healthy control group and within the patient group stratified according to stage and metastatic status at the time of diagnosis. Additionally, the relation of soluble TM (sTM) and soluble EPCR (sEPCR) with tumor differentiation, lymphovascular invasion state, and microvascular thrombus existence was studied.

2. Materials and methods

Fifty patients who applied to the Karaelmas (now Bülent Ecevit) University Training and Research Hospital Medical Oncology Outpatient Clinic between January 2010 and December 2010, and who had recently been diagnosed with colorectal cancer and had not received any chemotherapy and radiotherapy, were enrolled in the study. The control group contained 50 sex- and age-matched healthy individuals free of inflammatory, neoplastic, atherosclerotic, or connective tissue diseases, who were recruited from hospital staff and patients who had come in for check-ups. Informed consent was obtained from every patient and control group member before enrollment. The study was approved by the Ethical Committee for Scientific Studies at Karaelmas University, Zonguldak, Turkey.

The following patients were not accepted into the study: those who received preoperative chemo/radiotherapy; those with systemic diseases such as renal insufficiency, chronic liver disease, and diabetes mellitus; those with cerebral infarct and multiple sclerosis history; those with sepsis and disseminated intravascular coagulation; those with a history of venous thromboembolism and coronary events; and those who were active smokers.

Five of the 50 patients who participated in the study were in the advanced stage (inoperable). Postoperative pathology records of the remaining 45 patients were reviewed and tumor differentiation (grade), lymphovascular invasion state, and microvascular thrombus existence were recorded. Lymphovascular invasion state and microvascular thrombus existence were determined with hematoxylin and eosin staining.

The seventh edition (2010) of the TNM classification system was used to stage patients. Staging was done by taking into account clinical, preoperative, radiological, intraoperative, and postoperative pathological findings.

The patients were followed in respect to thromboembolic events and general survival period as of their entry date into the study until 1 June 2010, when the study ended. From each participant (patients and controls), 5 mL of blood was taken for the study of plasma sEPCR and sTM levels, and the plasmas obtained after centrifuge were kept at –80 °C until the measurements were carried out.

Plasma sEPCR and sTM levels were studied in the Zonguldak Karaelmas University Medical Faculty Hospital Immunology Laboratory. sEPCR and sTM concentrations from plasma samples were studied in microplate well and SIRIOS devices by Asserachrom EPCR and Asserachrom TM kits (Diagnostica Stago, France) with the enzyme-linked immunosorbent assay (ELISA) method.

2.1. Statistical analyses

Statistical evaluations were done using SPSS 13.0. Descriptive statistics were expressed as mean ± standard deviation for numeric data and as number and percent for categorical data. For categorical variants, the relations between intergroup differences and variants were tested using the chi-square test. The significance test of the difference between 2 means was used for the comparison of 2 groups in respect to numeric variants, which shows normal distribution. The Mann–Whitney U test was used for numeric variants that did not show normal distribution. The difference between TM and EPCR levels according to stages was tested with Kruskal–Wallis variance analysis. Results were evaluated to be within the confidence interval of 95%, and $P < 0.05$ was accepted as significant.

3. Results

The median follow-up period was 11.5 (2–17) months. In both groups, there were 25 female (50%) and 25 male (50%) cases. The mean age of the patients was 60.8 ± 11.7 years and the mean age of the control group was 57.6 ± 6.4. No significant difference in respect to age and sex between control and patient groups was seen. In the patient group, the median general survival period was 11.5 (2–17) months, and the general survival rate was 78% during the follow-up period. The death rate was 22%.

Plasma sTM and sEPCR levels in the control group and the colorectal cancer group are shown in Table 1. The plasma sTM levels in colorectal cancer patients were found to be significantly higher than in the control group (21.3 ± 22.8 ng/mL, 13.2 ± 16.2 ng/mL, P = 0.010). In addition, plasma sEPCR levels in colorectal cancer patients were found to be significantly higher than in the control group (149.9 ± 79.6, 113.3 ± 49.3, P = 0.007). There was no significant difference between the groups for sTM and sEPCR according to sex (Table 2).

Table 1. Average plasma sTM and sEPCR levels in colorectal cancer patients and control groups.

	Patient n = 50	Control n = 50	P
sTM (ng/mL)	21.3 ± 22.8	13.2 ± 16.2	0.010
sEPCR (ng/mL)	149.9 ± 79.6	113.3 ± 49.3	0.007

Table 2. Average plasma sTM and sEPCR levels in patient and control group according to sex.

	Patient			Control		
	Female n = 25	Male n = 25	P	Female n = 25	Male n = 25	P
sTM (ng/mL)	19.0 ± 18.8	23.6 ± 26.4	0.477	12.7 ± 15.5	13.7 ± 17.1	0.398
sEPCR (ng/mL)	136.5 ± 75.7	163.3 ± 82.7	0.239	104.1 ± 39.9	122.5 ± 56.5	0.190

According to the tumor localization, metastases, lymphovascular invasion, microvascular thrombus, tumor grade, and tumor stage, there were no statistically significant differences for plasma sTM and plasma sEPCR levels between the patient group and control group (Table 3).

From the beginning of the study to 1 June 2010, when the study ended, the patients were followed up with for thromboembolic events, both in polyclinic examinations and at home via telephone. No thromboembolic event (deep vein thrombosis, pulmonary embolism, cerebral embolism, etc.) occurred in any patient within the follow-up period

Table 3. Average plasma sTM and sEPCR levels in patient and control group according to pathology.

	Colon n = 35		Rectum n = 15		P
sTM (ng/mL)	23.6 ± 25.6		16.1 ± 13.8		0.296
sEPCR (ng/mL)	156.3 ± 76.0		135.0 ± 88.3		0.391
	With metastasis n = 12		Without metastasis n = 38		P
sTM (ng/mL)	20.0 ± 15.0		21.7 ± 24.9		0.496
sEPCR (ng/mL)	131.5 ± 63.8		155.7 ± 83.9		0.496
	Grade 1 n = 14		Grade 2–3 n = 31		P
sTM (ng/mL)	27.1 ± 31.5		19.2 ± 19.2		0.304
sEPCR (ng/mL)	165.0 ± 86.2		150.7 ± 78.4		0.584
	Microvascular thrombus (+) n = 6		Microvascular thrombus (–) n = 39		P
sTM (ng/mL)	27.9 ± 25.0		20.7 ± 23.6		0.676
sEPCR (ng/mL)	199.9 ± 94.6		148.3 ± 76.8		0.144
	Stage 1 n = 4	Stage 2 n = 20	Stage 3 n = 14	Stage 4 n = 12	P
sTM (ng/mL)	17.0 ± 16.6	28.7 ± 31.2	13.1 ± 10.8	20.0 ± 15.0	0.514
sEPCR(ng/mL)	108.7 ± 32.1	168.2 ± 81.8	151.3 ± 95.2	131.5 ± 63.8	0.364

4. Discussion

TM is mainly a transmembrane glycoprotein expressed in microvascular endothelium. It has 3 important functions: coagulation, inflammation, and cell adhesion. The role of TM in malignancy is still not completely known. It may affect tumor growth and metastasis with its natural anticoagulant function (8). Since TM expression is shown in many different tumors including breast cancer, esophageal squamous cell cancer, lung cancer, melanoma, and ovary cancer, it is thought that this protein can play a role in the control of tumor growth and metastasis (9–14). TM expression tends to be lower in metastatic lesions compared to primary samples. In tumors, the decreased TM can induce differentiation loss and can improve metastatic character. In vitro studies showed that TM decreases tumor cell proliferation and invasion (15,16).

EPCR binds to protein C, and thereby increases the activation of protein C by the thrombin–TM complex (17). Under normal conditions, there is sEPCR in plasma in a measurable quantity. Although the physiologic importance of sEPCR is not known, it is known to increase in inflammatory conditions such as sepsis and lupus (18). It is known that EPCR is expressed from a few cell series in different tumors (7); however, there is not any information about plasma level of EPCR in colorectal cancer.

In the studies carried out up until now, the tissue expressions of TM and EPCR in colorectal cancer have been studied, but the soluble levels of these 2 proteins in plasma have not been studied yet. However, in 1993, Lindahl et al. measured plasma TM levels with ELISA in 97 patients, including 35 colorectal cancer patients and patients with different cancer types. It was stated that plasma TM level increases in cancer patients compared to the healthy control group; however, this increase was at different rates according to each cancer type (5). In our study, both plasma TM and EPCR levels increased in colorectal cancer patients compared to the healthy control group, and this result was statistically significant.

Plasma level of TM increases depending on the endothelial damage seen in different pathological situations. Therefore, it is accepted as an indicator of endothelial cell damage (15). Zhou et al. measured plasma TM levels in cancer patients who received radiotherapy and stated that there is a positive correlation between radiation dose and plasma TM levels, and that plasma TM is a useful marker for early determination of the radiation damage to the endothelial cells in patients exposed to radiotherapy (19). Suehiro et al. evaluated TM expression in patients with hepatocellular carcinoma (HCC), and they suggested that TM can inhibit adhesion of portal vein tumor cells due to anticoagulant activity and thereby it can prevent intrahepatic metastasis (8). When Salmaggi et al. compared plasma TM levels of patients with glioblastoma

multiforme with a healthy control group, they saw that it increased significantly (20). As seen in many studies, the plasma sTM level is higher in cancer patients compared to healthy individuals.

When we extensively reviewed the studies conducted to date, we saw that the relation between tumor differentiation, lymphovascular invasion state, microvascular thrombus existence in colorectal cancer, and plasma sTM and sEPCR levels was not studied. In our study, we compared these variants with plasma sTM and sEPCR levels. We could not find a statistically significant difference between tumor differentiation and lymphovascular invasion and plasma sTM and sEPCR levels.

In the study conducted by Xu et al., plasma TM level and tissue TM expression were measured in 188 cancer patients (including lung, pancreas, stomach, colon, nasopharyngeal, and laryngeal cancer patients), and they stated that TM expression was low and plasma TM levels were high in those patients who were metastatic. This shows that the increase of plasma TM levels in cancer patients is related to metastasis (21). In addition, low or no TM expression in cancer cells of lung, oral, and breast cancer is related to a poor prognosis (8,10,12,14). As is seen, TM levels can be used as a sensitive indicator to evaluate the metastasis and progression of cancers. In our study, we could not find a statistically significant difference when we compared plasma sTM and sEPCR levels of patients who were in the advanced stage and had metastasis with those who were in early stage and did not have metastasis at the time of diagnosis.

There is limited information about EPCR expression in cancer tissues. Tsuneyoshi et al. reviewed EPCR expression in tissue samples with invasive ductal breast carcinoma and strong EPCR expression was determined. Therefore, it is suggested that EPCR can play a role in the pathogenesis and progression of some cancer types (7). In another study, although the sTM level was higher in chronic liver patients with HCC compared to chronic liver patients without HCC, no difference between sEPCR levels in the 2 groups was found. However, it was shown that high sEPCR level is related to worse prognosis and death in HCC (22). In some studies, the effect of the APC/EPCR pathway on tumor migration and metastasis in melanoma cell series was reviewed, and it was shown that liver metastasis decreases 50% and lung metastasis decreases 92% in mice showing EPCR overexpression. With these findings, it is suggested that the APC/EPCR pathway decreases tumor metastasis by inhibiting tumor adhesion and migration (23,24). In our study, EPCR level was determined to be statistically significantly higher in colorectal cancer patients compared to the healthy control group. However, no relationship was found between plasma sEPCR level and disease stage, metastatic state, tumor differentiation, lymphovascular

invasion state, and microvascular thrombus existence. This study is the first to show the increase of plasma sEPCR level in colorectal cancer patients.

Consequently, these results show that the protein C pathway, including TM and EPCR, can play roles in the pathogenesis and biology of colorectal cancer.

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