EJMA

DOI: 10.14744/ejma.2023.49368
EJMA 2022;2(4):173–180

Research Article

Relevancy of Serum SuPAR, PAI-1, MMP-9 Level, and Prognosis of Metastatic Colorectal Cancer During Diagnosis

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Abstract

Objectives: Serum suPAR, PAI-1, and MMP-9 biomarkers have been associated with the progression of the disease in many cancers, including the CRC. It aimed to determine the relation of serum suPAR, PAI-1, and MMP-9 levels with the prognosis of the disease in patients with mCRC.

Methods: Nonmetastatic CRC patients who had not yet been treated with the oncology polyclinic in İKCU and blood samples were taken from the patients with metastatic CRC before and after treatment, and the obtained serum was preserved. PAI-1, suPAR, and MMP-9 levels were studied by ELISA. The results were used to establish the relation with prognosis.

Results: When the metastatic CRC and non-metastatic CRC were compared before treatment for biomarkers; PAI-1(p-value:0.030), suPAR(p-value:0.009) and MMP-9(p-value:0.003) were found to be highly significant. PAI-1 (p-value:0.003), suPAR (p-value: 0.007), and MMP-9 (p-value:0.001) in the pretreatment metastatic group and the control group after the treatment were statistically significant. ROC was performed and cut-off values were determined as (pai:71.4 ng/ ml; supar:1.97 ng/ ml; mmp:812.5 ng/ ml). PFS and OS were evaluated to the cut-offs of markers using the Kaplan-Meier method. Cut off values were significant in terms of PFS (pai p-value, 0.008; supar p-value 0.019, mmp p-value 0.017) and OS (pai p-value, 0.03; supar p-value 0.059, mmp p-value 0.081).

Conclusion: Serum PAI-1, suPAR, and MMP-9 levels reveal the prognosis of patients with metastatic CRC.

Keywords: PAI-1, suPAR, MMP-9, metastatic colorectal cancer

Cite This Article: *Kalender ME, Demir L, Oflazoglu U, Kucukzeybek Y, Salman T, Varol U, et al. Relevancy of Serum SuPAR, PAI-1, MMP-9 Level, and Prognosis of Metastatic Colorectal Cancer During Diagnosis. EJMA 2022;2(4):173–180.*

Colon cancer is the uncontrolled proliferation of colon epithelium. Environmental and genetic factors control this proliferative state. Colorectal cancer (CRC) is the third most diagnosed malignancy and the second mortal cancer globally, with 1.9 million new cases and almost 1 million deaths were noted in 2020.[1] CRC is expected to increase by 60% to more than 2.2 million new cases and 1.1 million cancer deaths by 2030.[2]

Plasminogen is a freely circulating proenzyme that binds to the plasminogen receptor at the cell membrane.[3] The urokinase plasminogen activator (uPA), which plays an essential role in the plasminogen plasmin system, is secreted with a low intrinsic activity as a proenzyme,[4] binds to its receptor urokinase plasminogen activator receptor (uPAR) it is stabilized with the GPI anchor via an inner surface of the cell.[5] With the combination of two proen-

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Submitted Date: September 28, 2022 **Revision Date:** September 28, 2022 **Accepted Date:** January 03, 2023 **Available Online Date:** February 09, 2023

zymes, plasminogen and uPA, plasminogen transforms to plasmin. Activation of plasmin accelerates the conversion of pro-uPA to uPA via positive feedback, is resulting in an acceleration.[4,6] In addition, plasmin activating matrix metalloproteinases and latent growth hormones play a role in invasion and metastasis.[7] Plasminogen activators (uPA / tPA), which catalyzes plasminogen to plasmin, are inhibited by plasminogen activator inhibitors (PAI-1 and PAI-2), a2 antiplasmin. The uPA/ uPAR/ PAI-1 complex gets into the cell by LDLR-mediated endocytosis, and then subsequent cleavage of the uPAR gets out to the cell membrane.[3]

PAI-1 is a rapid and specific inhibitor of the plasminogen/ plasmin system and is an effective regulator of tumor growth in vivo. Elevated PAI-1 values are significant for poor prognosis in some solid tumors involving the CRC.[8] In addition, CRC can achieve high values of plasma PAI-1 correlated by liver metastasis. Besides the liver metastasis, tumor size, differentiation grade, serosa infiltration, Duke staging, and lymphatic metastasis were also significant with elevated plasma PAI-1 values. However, the proliferation, invasion, and migration were decreased at colorectal cancer cells which transfected via lentivirus producing PAI-1 siRNA, that called PAI knockdown cells, which belongs to experimental immunized mice, the detection of fewer metastatic nodules and smaller size tumoral kits in the liver was found to be significant in terms of PAI-1 levels in colorectal cancer patients.[9]

Plasma uPA levels may be unreliable because of the absence of uncorrelated conclusions of particular reasons between plasma uPA and PAI-1 levels in patients with liver metastases in colorectal cancer patients.[9]

uPA is a member of the plasminogen - plasmin system and is associated with cancer cells' growth, invasion, and metastasis.[10] It has an opposite function in cancer malignancy because of proteolytic and antiproteolytic effects. Understandably, uPA can break down extracellular matrix (ECM) proteins during cancer invasion; but PAI-1 associated tumor progression can not be explained by the direct inhibition of uPA by PAI-1. Studies have shown that the uPA and uPAR complex interact with vitronectin in integrin-associated cell adhesion mechanisms.[11] PAI-1 can separate the matrix-vitronectin (VN) bond on aVb3[12] and uPAR.[13] Thus, an increase in PAI-1 can reduce cell adhesion of ECM to increase cell invasion and migration;[14] since binding between uPA and uPAR enhances the binding affinity of uPAR to VN.[15]

Matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases. This group has been associated with ECM destruction, tissue repair, invasion, metastasis, and angiogenesis.[16] MMP-9, which (also known as gelatinase-B) is a member of the MMP family, is regulated in malignant tumors[17–19] and associated with invasion and metastasis of cancer cells.[16] There is evidence that MMP-9 expression is associated with Duke staging and distant metastasis in patients with CRC (mCRC).[20]

In some studies, MMP-9 has been used as a target for drug development about metastasis and invasion, although the relationship between PAI-1 and MMP-9 is still unclear. In some studies, a significant positive correlation was found in patients with CRC and liver metastases. At the same time, MMP-9 levels were simultaneously high in patients with high PAI-1 levels; lower levels of MMP-9 have been observed after silencing of PAI-1 in CRC cells, suggesting that there may be an inflammation-related association present.[9]

In studies conducted for about 30 years, uPA, uPAR, PAI-1 levels were tried to be found in the carcinogenesis, and the main effect was not apparent because of the proteolytic and antiproteolytic effects of uPA. However, many studies have shown that uPAR can be used as a biomarker because PAI-1 levels are significant in some metastatic cancers. There is a substantial correlation between PAI-1 and MMP-9 in studies performed. In the literature, there is no study showing the relation between adjuvant chemotherapy (CT) and plasma uPAR, PAI-1, MMP-9 levels in CRC patients during and after diagnosis. We aimed to understand the relationship of serum suPAR, PAI-1, and MMP-9 levels at the time of diagnosis to reveal the prognosis of the disease in patients with mCRC.

Methods

Patients

A total of 56 patients verified histologically as colon or rectum adenocarcinoma. After reviewing the medical records, general information was recorded, including age, sex, and biomarker levels. Patients were evaluated according to TNM staging as two groups: the metastatic (stage 4) and nonmetastatic (stage 1,2,3). Metastatic group (n=33) has 23 male and 10 female patients (age range 32-78 years) and non-metastatic group (n=23) has 16 male and 7 female patients (age range 34-80 years).

Sample Collection

A blood sample (5mL) for plasma analysis was taken in EDTA anticoagulant tubes at the time of diagnosis in the nonmetastatic group and on the day of chemotherapy in the metastatic group. After 30 minutes, samples were centrifuged at 3,000 rpm for 10 minutes at 48 ˚C. The plasma was collected after centrifugation and stored at -20 ˚C.

Enzyme-Linked Immunosorbent Assay

The plasma contents of PAI-1, SuPAR, and MMP-9 were detected by enzyme-linked immunosorbent assay (ELISA) kits (BosterBio Corp.; code: EK0536, EK0859, EK0465, Pleasanton, CA 94566, USA) according to the manufacturer's instructions. The optical density was measured, and the standard curve was delineated. The plasma contents of SuPAR, PAI-1, and MMP-9 were calculated according to the standard curve.

Ethics

Informed consent was obtained from each patient before the initiation of the study, and the Ethical Committee approved the study protocol of İzmir Katip Celebi University (IKCU) (approval number: 69, Date: 21.04.2016). The study was conducted according to the Principles of the Declaration of Helsinki.

Statistical Analysis

Data were expressed as mean values±standard deviation (SD). Statistical analysis was performed with SPSS version 20.0.

Power analysis could not be performed because it was a preliminary study.

It was evaluated by Independent Samples and Repeated Measures Analysis to assess the difference between metastatic and nonmetastatic in terms of PAI-1, SuPAR, and MMP-9, CA 19.9, CEA levels.

The cut-off value was found in 71,4 (ng/ml) for PAI-1, 1,97 (ng/ml) for SuPAR and 812,5 (ng/ml) for MMP-9. It' s evaluated by ROC curve, Figures 1-3.

Overall survival analysis was evaluated using the Kaplan-Meier method, Figure 4.

The relationship between the PAI-1, SuPAR, and MMP-9 was evaluated with the Pearson correlation value.

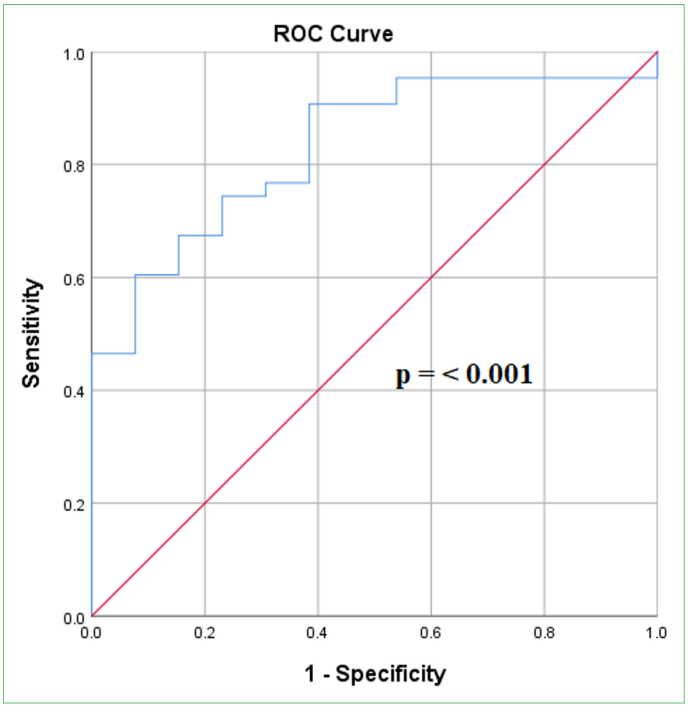

Figure 2. ROC curve, SUPAR cut off level 1,97 (ng/ml).

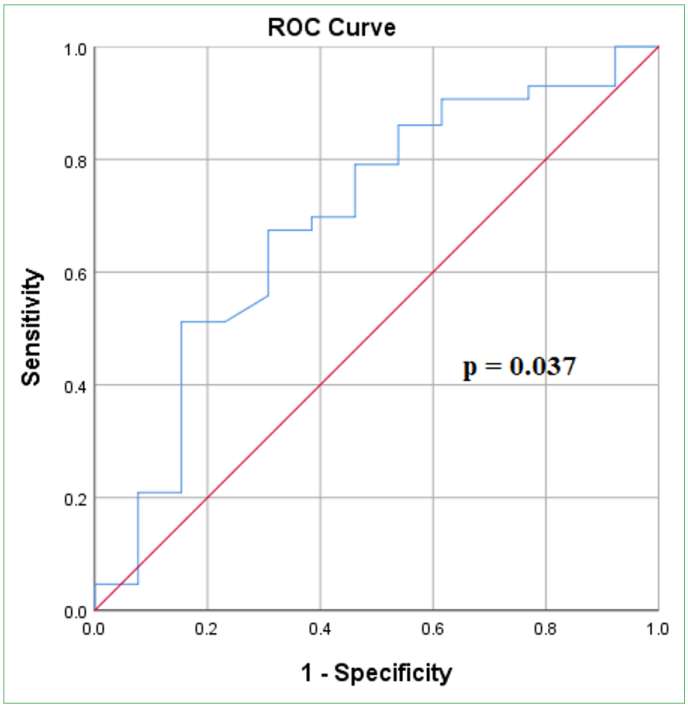

Figure 1. ROC curve, PAI-1 cut off level 71,4 (ng/ml).

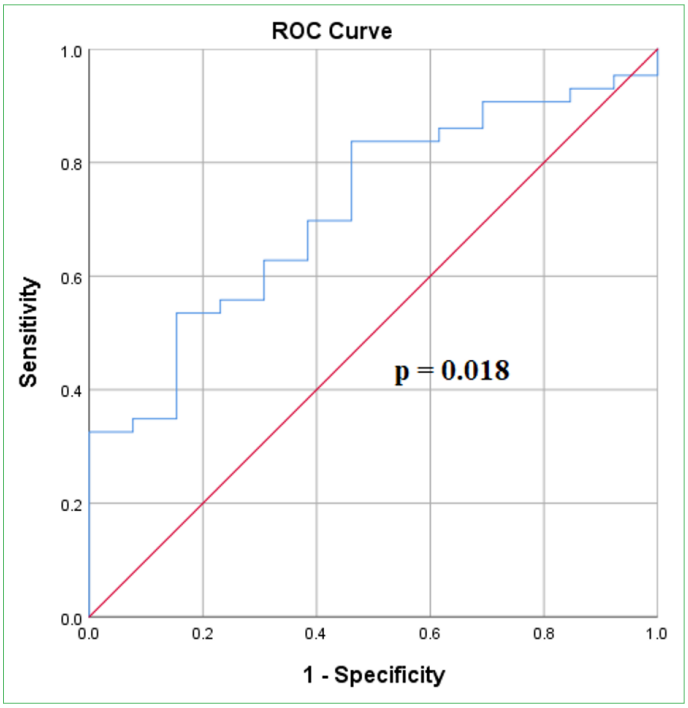

Figure 3. ROC curve, mmp-9 cut off level 812,5 (ng/ml).

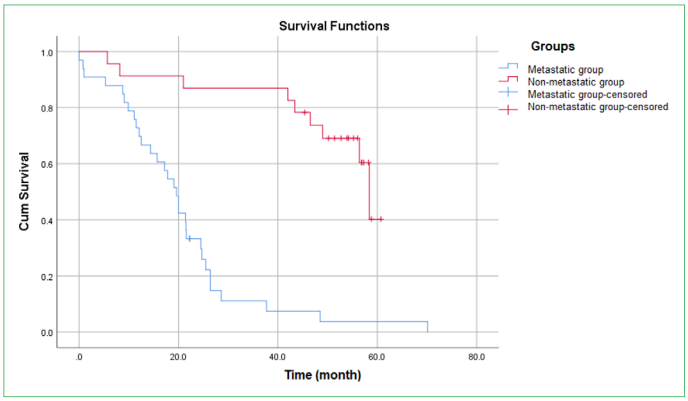

Figure 4. Kaplan-Meier curve, metastatic and nonmetastatic groups.

Results

Pre-treatment marker levels were examined and a statistically significant difference was found between the metastatic and nonmetastatic group; PAI-1 (p=0.030), SuPAR (p=0.009) and MMP-9 (p=0.003) (Table 1).

Metastatic group, pre- and post-treatment marker levels were compared and it was observed that marker levels decreased with treatment; PAI-1 (p=0.003), SuPAR (p=0.007), MMP-9 (p=0.001) (Table 2). Eleven patients between the two groups were because of did not receive adequate treatment.

The diagnostic ability of the markers evaluated to determine whether the patients are metastatic or non-metastatic was evaluated with the ROC curve. PAI-1, SuPAR and MMP-9 levels and cut off values were determined; (PAI-1: 71.4 ng/ml; SuPAR: 1.97 ng/ml; MMP-9: 812.5 ng/ml). Cut off values were found to be statistically significant in terms of PFS (PAI-1 p-value, 0.008; SuPAR p-value 0.019, MMP-9 p-value 0.017) and OS (PAI-1 p-value, 0.03; SuPAR p-value 0.059, MMP-9 p-value 0.081) (Table 3).

Overall survival (OS) was evaluated according to the cut-offs of markers in metastatic and nonmetastatic groups using the Kaplan-Meier method (Table 4).

Relationship was evaluated with Pearson correlation between PAI-1, SuPAR and MMP-9 and the relationship between PAI-1 - SuPAR (r=0.232, p=0.085) and between MMP-9 - SuPAR (r=0.228, p=0.090) was found (α=0,10).

In Repeated measures and independent samples test analyzes, CEA and CA 19-9 were positively correlated (p=0.000); SuPAR has correlated both CA 19-9 (p=0.000) and CEA (p=0.044). PAI-1 had a positive correlation with MMP-9 (p=0.053).

There are not enough relapses to understand the relationship between serum markers and PFS of nonmetastatic patients.

Discussion

It pioneers other studies in comparing the post-treatment values of PAI-1, suPAR, and MMP-9 together for the first time in metastatic patients with colorectal cancer. When further studies in the literature are evaluated, there are predictions that it can be used for follow-up or prognosis to show tumor burden. In our research, it was statistically significant that the biomarker levels of PAI-1, SuPAR, and MMP-9 were higher in metastatic patients than in nonmetastatic patients, and these levels decreased with chemotherapy. ROC analysis was performed using pre-treatment values of all three biomarkers to reveal the relationship between PAI-1, suPAR, and MMP-9 levels in the blood samples we received

Table 1. Comparison of the metastatic group and the nonmetastatic group before chemotherapy serum marker levels

	Metastatic group (n=33) Mean (ng/ml)±SD	Non-metastatic group (n=23) Mean (ng/ml)±SD	p
PAI-1 (ng/ml)	76.46±11.41	69.39±12.10	.03
SUPAR (ng/ml)	3.90±3.41	1.93±0.66	.009
MMP-9 (ng/ml)	2455.97±2879.93	803.23±504.99	.003

Table 2. Comparison of serum marker levels in the metastatic group before and after treatment

	Before Treatment (n=33) Mean (ng/ml)±SD	After Treatment (n=22) Mean (ng/ml)±SD	p
PAI-1 (ng/ml)	76.46±11.41	66.56±12.23	.003
SUPAR (ng/ml)	3.90±3.41	1.83±0.63	.007
MMP-9 (ng/ml)	2455.97±2879.93	624.50±526.62	.001

Table 3. Cut off values associated PFS and OS

	Cut off value (ng/ml)	PFS Median (month)	p	OS Median (month)	p
PAI-1 (ng/ml)	>71.4	11.80	0.008	21.6	0.033
	<71.4	28.60		49	
SUPAR (ng/ml)	>1.97	14.10	0.019	24.5	0.059
	<1.97	56.4		56.4	
MMP-9 (ng/ml)	>812.5	11.8	0.017	24.5	0.081
	<812.5	28		28.6	

Table 4. Number of death in metastatic and nonmetastatic groups

	Total Patient	Number of Death
Metastatic group	33	31
Non-metastatic group	23	9
Overall	56	40

before treatment in the metastatic and nonmetastatic groups cut off values were determined. The Kaplan- Meier method was used, and the results were significant in PFS and OS.

The increase in PAI-1 level has been confirmed in many solid tumors and has been associated with poor prognosis in some types of cancer, including CRC.[8,21–24] The PAI-1-VN complex has been shown to inhibit integrin-mediated cell adhesion.[25] PAI-1-associated angiogenesis has been demonstrated in mouse studies[26] and in metastatic patients treated with angiostatin, circulating antiangiogenetic factor, and angiostatin in different studies.[27] Excessive use of expression can lead to cell migration and tissue remodeling over PAI-1, which cannot bind to VN.[28] The uPAR molecule is involved in integrin-associated cell adhesion over VN.[11]

In a study by Chen et al., Plasma PAI-1 levels are elevated in patients with CRC with liver metastasis and correlated with tumor size, differentiation degree, serosa infiltration, lymph metastasis, and Duke staging. It has been shown that the proliferation, invasion, and migration ability of CRC cells decreases by inhibiting the expression of PAI-1 siRNA and PAI-1 knockdown cells in mice, and there are fewer and smaller metastatic nodules in the liver. In this study, after using PAI-1 siRNA, the serum level of MMP-9 was low, like PAI-1.[9] The current situation is consistent with the positive correlation of PAI-1 with MMP-9 in our study.

In some studies, it has been shown that in patients with pre-op high plasma SuPAR levels in CRC, overall survival decreases, and increased suPAR levels correlate with the risk of mortality. Multivariate analysis revealed elevated plasma suPAR levels in survival significantly showed prognosis independent of Duke staging and serum CEA levels. [29–31] In some large-scale studies, including CRC patients, uPA, uPAR, and PAI-1 levels at the tumor tissue level were associated with short survival.[32–34]

Fernebro et al. and Sier et al. showed that high suPAR levels in patients with CRC are related to shortened survival.[35,36] Nørgaard-Pedersen B and Nielsen HJ showed in their study that serum Tetranectin and plasma SuPAR levels have an independent prognostic value in the survival of patients. This study showed that the risk increase of patients with low serum Tetranectin and high plasma SuPAR levels compared to patients with median Tetranectin and SuPAR levels was statistically significant.[37]

In a study conducted by Nijziel et al., No statistically significant difference was shown in plasma PAI-1 and uPA levels in the metastatic, nonmetastatic, and control groups. There was no correlation between uPA, uPAR, and PAI-1 in patients with metastases. There was also no correlation between suPAR levels and overall survival; However, previous studies have studied uPAR levels in tumoral tissue. It has been shown that disease-free survival and overall survival are shortened in patients with postmenopausal breast cancer whose node is positive.[34,38]

High serum MMP-9 level is associated with susceptibility to metastasis and shortened survival.[39] It is thought that the levels of MMP in the circulation may be helpful in cancer follow-up.[40] Rašić et al. Showed that the serum MMP-9 level was higher than the control group and that the serum value towards metastatic disease increased in correlation with disease progression.[41] Biasi et al. MMP-9 serum level was significantly higher in patients with stage 2-3 CRC.[42] Hurst et al. and Wilson et al. showed that MMP-9 level is elevated in patients with CRC.[43,44] Jonsson et al. compared plasma and serum levels of MMP-9 and showed that serum levels are higher than plasma levels.[45]

In many studies in the literature conducted with the biomarkers uPA, uPAR, SuPAR, PAI-1 that are evaluated freely in tumoral tissue and circulating plasma, correlation can sometimes be shown in survival analysis, and sometimes opposite results can be displayed. The fact that PAI-1 uses VN to stabilize,[46] uPA uses uPAR as a receptor. The excess uPA expression increases the binding of uPAR to VN[15] after the formation of the uPA-uPAR complex, membrane-bound uPAR The presence of PAI-1 affinity at the subendothelial matrix level due to the construction of the SuPAR concept with the inclusion of the GPI hook into the circulation and the decrease in the uPAR concentration at the subendothelial matrix level,[47] the existence of an ongoing competition between PAI-1 and uPA at the subendothelial matrix level,[25] The involvement of uPA in the activation of the MMP cascade,[48] the ability of MMP-9 and PAI-1 to play a role together in some processes,[27,49] and the destruction of type IV collagen MMP-9 via VEGF[50] and via different pathways. Many reasons such as angiogenesis examination,[40] the presence of MMP-9 and PAI-1 levels in conditions that correlate with each other, and many reasons such as metastatic and nonmetastatic cancer types measured in tissue, serum, and plasma levels of PAI-1, suPAR, and MMP-9. Relative and survival differences; can explain the different results of the local and systemic activities of diseases. Although there are studies on plasma and locally in the literature, serum studies of the patients were used in our study due to the lack of large-scale studies explaining the relationship between serum, plasma, and tissue.

It is essential that the staging of the patients before the treatment was done perfectly, as it affects the treatment and consequently the survival of the patients in terms of the differentiation of metastatic-nonmetastatic patients. In the staging process, imaging and pathological surgical

evaluation are mainly used; However, the demonstration of metastatic status or increased tumor burden is essential in the follow-up of the patient and the treatment regimen to be taken. PAI-1, suPAR, and MMP-9 biomarkers are promising in distinguishing between metastatic and non-metastatic disease in cases that progressed to metastatic disease in the follow-up or in patients where the treatment efficacy can be evaluated only with the treatment a sample taken from the serum, which is metastatic.

Serum and local expression of the biomarkers or genetic expression can be examined in a study involving larger and homogeneous groups to show significance about PSF and OS to have more accurately healthier results. Still, high-cost conditions and the difficulty of forming homogeneous groups were the main limiting reasons.

In conclusion, PAI-1, suPAR, MMP-9 levels in mCRC patients are high and decrease with chemotherapy. These biomarkers levels in CRC patients can give us information about PFS and OS. However, studies to be conducted to show the progression of biomarkers need to determine certain cut-off levels by evaluating more patients inhomogeneous groups without comorbid diseases, sepsis, and severe systemic inflammatory conditions. The relationship between uPA and PAI-1 in patients with mCRC is not known. SuPAR, VN, and PAI-1 play a vital role in the known factors affecting this relationship, and the local positive or negative effect of SuPAR on the tumoral mass while entering the systemic circulation is not known yet. The positive correlation between PAI-1 and MMP-9 levels in our study and the correlation similar to other studies is unknown. A more detailed evaluation of coagulation and inflammation responses in the microenvironment where invasion, angiogenesis, and migration steps take place to illuminate tumoral processes may help us reach statistically more significant results in terms of biomarker levels. Studies in the literature are based on plasma, serum, and tissue expression; Since the relationship between them could not be demonstrated in large-scale studies, evaluating the biomarkers of systemic, tissue, or genetic terms together may be a more accurate option.

Disclosures

Ethics Committee Approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Izmir Katip Celebi University Ataturk Education and Research Hospital (approval number:69, Date: 21.04.2016).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Funding: This work was supported by Izmir Katip Celebi University Ataturk Education and Research Hospital, grant number is 2016-TDU-TIPF-0025.

Authorship Contributions: Concept – M.E.K.; Design – T.S., H.T.; Supervision – Y.K., A.A.; Materials – Y.Y.; Data collection and/or processing – L.D.; Analysis and/or interpretation – U.O.; Literature search – U.V.; Writing – M.E.K.; Critical review – Z.S.G.

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