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RESEARCH ARTICLE

Investigation of the Differences Between the Different Angiogenic and Antiangiogenic Markers Before and After Treatment in Several Types of Cancer

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Abstract

Cancer is among the most common deaths in the world, and there are many studies on the increased invasion with angiogenesis. In this study, we aimed to compare angiogenic and antiangiogenic markers in various cancer patients before and after surgery in order to explain the mechanism of angiogenesis in more detail.

Methods: *Our study consists of 20 controls and 20 cases (before and after surgery). After all the blood was collected, our samples were stored at -80 ° C until the analysis period and the samples were analyzed by ELISA method.*

Results: *There was no significant difference in age, gender and weight of these groups ($p>0.05$). When the control group was compared with the pre-op group, we observed that the levels of TSP-1, ES, NF-κB in the pre-op group were high in each of the various types of cancer, while VEGF was low in all except in the breast. MMP-9 was found to be high in all cancer types. When pre-op and post-op were compared, there was an increase in TSP, ES, NF-κB, VEGF and MMP-9 levels in the colon and thyroid, while there was no statistically increase in the others ($p <0.05$).*

Conclusion:

In our study, we think that all markers are high in the preop period in all types of cancer, and that there is a statistical increase in the postop period, this is related to the duration of the treatment. We anticipate that this study should be performed by applying more cases and longer treatment.

Keywords: Cancer, NF-kB, VEGF, MMP-9, Endostatin ve Trompospondin -1

1 | INTRODUCTION

Cancer, smoking, poor nutrition, physical inactivity and reproductive changes (includ-

ing low birth and later age at first birth) are among the most common causes of death in the world, resulting in increased risk (1). Lung and breast cancer are most common in underdeveloped countries, while prostate and lung cancer are among the most common causes of death in more developed countries (2).

In 1971, she said by Judah Folkman that it was a new target in the treatment of cancer, defining the formation of new microvascular vessels from angiogenesis or neovascularization (3). Angiogenesis represents an essential step in tumor proliferation, expansion, and metastasis. Tumor cells may express both proangiogenic and/or antiangiogenic factors. Under normal circumstances, angiogenesis is controlled through the equilibrium of these factors. This balance is disrupted in malignancy, a shift in the equilibrium to a proangiogenic state occurs at an early to midstage in tumor development. This leads to activation of an "angiogenic switch" and, consequently, the formation of new vasculature (4). Angiogenesis result from the expression of angiogenic factors by tumor cells as a response to certain stimuli. Tumor hypoxia, oncogenes (such as ras, VHL, and bcl-2), cytokines, proangiogenic cell growth factors (such as bFGF and EGF), and hormones are important stimuli that cause the increased production of tumor Vascular Endothelial Growth Factor(VEGF). Among them, the most well-studied external stimulus is hypoxia, which actually is a key signal for the induction of angiogenesis (5).

VEGF is one of the most important modulators of angiogenesis (6). Some studies have shown that VEGF may be a potent factor for predicting tumor progression in hepatocellular carcinoma (HCC), gastric cancer and colorectal cancer (7). Inhibiting its interaction with the receptor via antagonistic peptides could present an effective anti-angiogenic therapy (6). Matrix Metalloproteinaz'lar (MMPs) are a family of enzymes that proteolytically degrade various components of the extracellular matrix (ECM). High expression levels of certain MMPs, either by the tumour cells themselves, by stromal fibroblasts, or by infiltrating inflammatory cells, are closely correlated with tumour invasive and metastatic potential. Furthermore, they participate in the degradation of the vascular basement membrane and remodelling of the ECM during angiogenesis (8). The 72 kDa. MMP-2 and 92 kDa. MMP-9 have been shown to play critical roles in the "angiogenic switch" and tumor cells could synthesise and secret large amounts of MMP-2 and MMP-9 in a paracrine and/or autocrine manner to stimulate angiogenesis and increase VEGF release (9).

ES produced by collagen cleavage weighing 20 kDa, inhibiting endothelial cell proliferation-migration, inducing apoptosis, and also inhibiting MMP-2 activity (10, 11,12) and TSP-1 angiogenesis inhibitors that function as a matricular glycoprotein that binds naturally occurring EC receptors in 1978 (13). It functions as a matricellular glycoprotein that binds EC receptors (14).

In this study, we aimed to compare angiogenic and antiangiogenic markers in various cancer patients before and after surgery in order to explain the mechanism of angiogenesis in more detail

2 | MATERIAL AND METHODS

This is a sex-matched case-control study, conducted at the Department of General Surgery of Ankara Dr. Abdurrahman Yurtaslan Oncology Hospital and the Department of Biochemistry, Manisa Celal Bayar University Faculty of Health Science, Manisa. The study was approved by the Medical Faculty Health Sciences Ethics Committee. All patients and volunteers involved in the study gave their informed consent.

Patients

Recruitment of study participants was performed at the Department of General Surgery, Ankara Dr. Abdurrahman Yurtaslan Oncology Hospital, by trained physicians. Our study was composed of 20 patients

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with control, 20 pre and postop breast, thyroid, stomach and colon cancer. In our preoperative group will be selected from the group who applied to the Dr. Abdurrahman Yurtaslan oncology hospital and who make biopsy and want to participate in the study. Other exclusion criteria included diabetes mellitus, hypertension, hormonal therapy such as oral contraceptives or estrogen replacement therapy, treatment with thyroxin derivates, and coexistence of active infectious and chronic diseases. All patients had normal complete blood counts and C-reactive protein (CRP) values before surgery. Our blood was taken from preoperative day 1 and postoperative day 30 from each group

Assay

Nuclear Factor-kB (NF-κB), VEGF, MMP-9, ES and TSP-1 were evaluated in serum samples isolated from peripheral blood and banked at -80 °C. All samples from each patient were run in the same assay. NF-κB, VEGF, MMP-9, ES and TSP-1 were analyzed from the collected blood by Elisa method. The values were measured in triplicate and the mean concentrations were determined from the standards provided.

Statistical analysis

The software SPSS for Windows, version 16.0 was used in the statistical analysis of the data. Associations between continuous variables were assessed by the Kruskal-Wallis test. In all analyses, a p value of 0.05 was used as the cutoff for significance.

3 | RESULTS

In this study we have analyzed NF-κB, VEGF, MMP-9, ES and TSP-1 levels in pre and post-op. cancer patient.

We created this study from a control and case group (before and after treatment). There was no significant difference in age, gender and weight of these groups ($p>0.05$).Figures 1, 2, 3 and 4Table 1

The comparisons between the control group and case groups (Table-1) were performed by Mann Whitney-U test. When the control group meets the preop group, TSP-1, ES, NF-κB levels are high in different cancer groups, while other than breast cancer, VEGF decreased and MMP-9 increased in all types of cancer. Compared to pre and postop, TSP-1, ES, NF-κB, VEGF and MMP-9 levels increased in the colon and thyroid, while no statistically significant increase was found in other cancer types ($p <0.05$).

4 | DISCUSSION (1--34)

In 1971, Judah Folkman described the formation of new micro-vessels from the vascular network as angiogenesis and showed it as a target for cancer treatment (3). They suggested that blood sources must be present in order for tumors to develop (15). They have shown that one of the most important factors in angiogenesis is VEGF (16,17). The process of angiogenesis is governed by a delicate balance between multiple endogenous pro- and antiangiogenic factors. An imbalance in these factors leads to the development or progression of pathological conditions. The angiogenic process involves interactions among multiple cell types including: endothelial cells (EC) and circulating endothelial progenitor cells, pericytes, vascular smooth muscle cells, stromal cells, including stem cells, and parenchymal cells. These interactions occur through secreted factors such as VEGF, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and angiopoietins, as well as through cell- extracellular matrix (ECM) interactions (18–20). Angiogenesis is crucial to the progression of cancer and particularly in metastasis (21). Vascular endothelial growth factor (VEGF) is one of the most important modulators of angiogenesis. Inhibiting its interaction with the receptor via antagonistic peptides could present an effective anti-angiogenic therapy. Studying the binding interaction of VEGF8–109 with VEGF receptor-1 (VEGFR1), one of the VEGF-A binding partners, resulted in the identification of critical residues responsible for binding (21). An editorial devoted to angiogenesis inhibitors suggested that “Angiogenesis inhibitors, particularly polypeptides or endogenous peptides, may become the safest and least toxic therapy for diseases associated with abnormal angiogenesis” (22).

Endostatin weighs 20 kDa derived from the C-terminus of collagen XVIII with the activity of var-

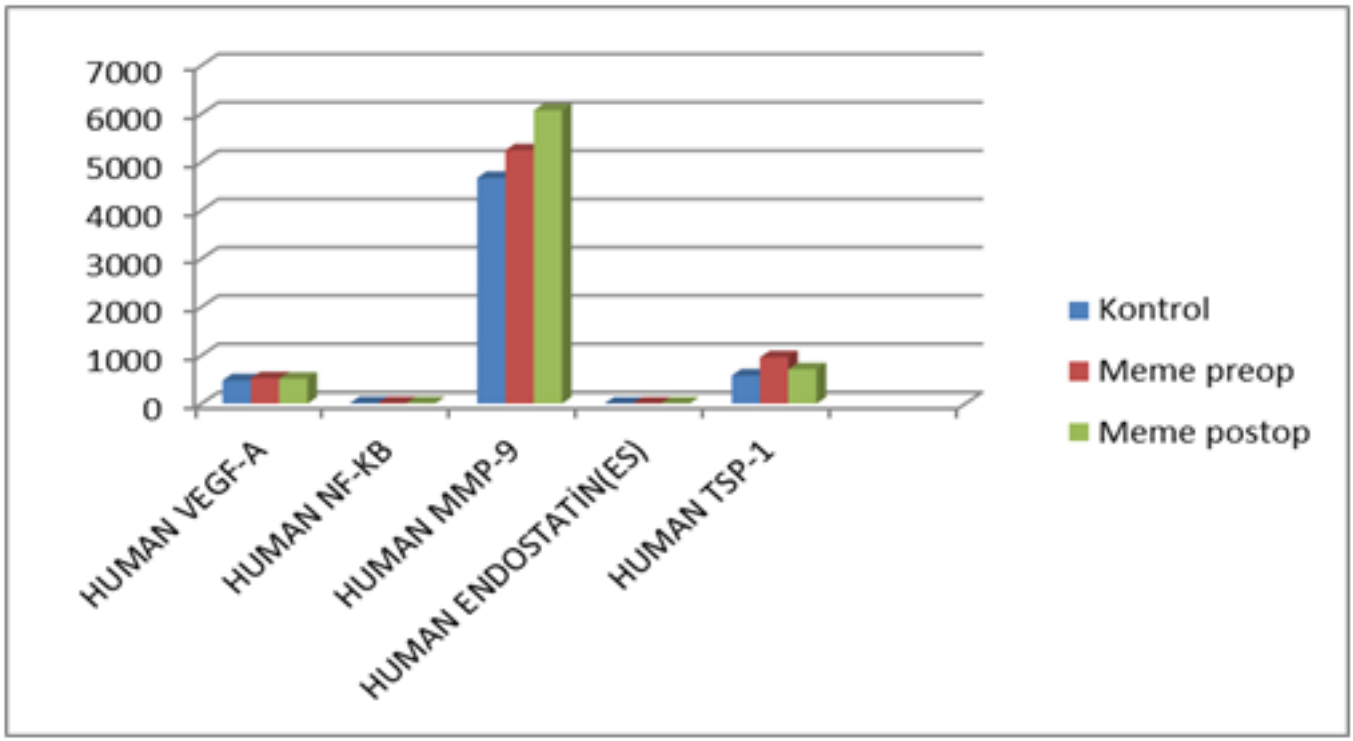

FIGURE 1: NF-kB, VEGF, MMP-9, ESand TSP-1 levels in breast cancer.

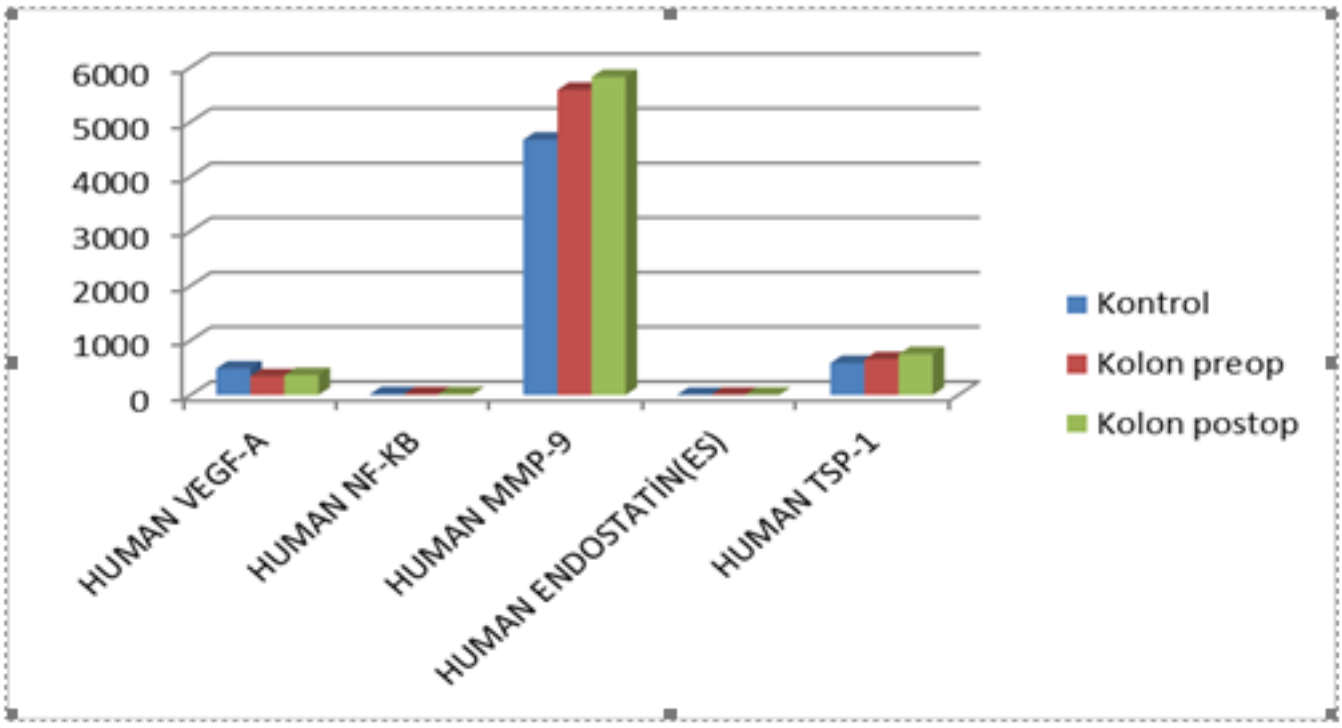

FIGURE 2: NF-kB, VEGF, MMP-9, ESand TSP-1 levels in colon cancer.

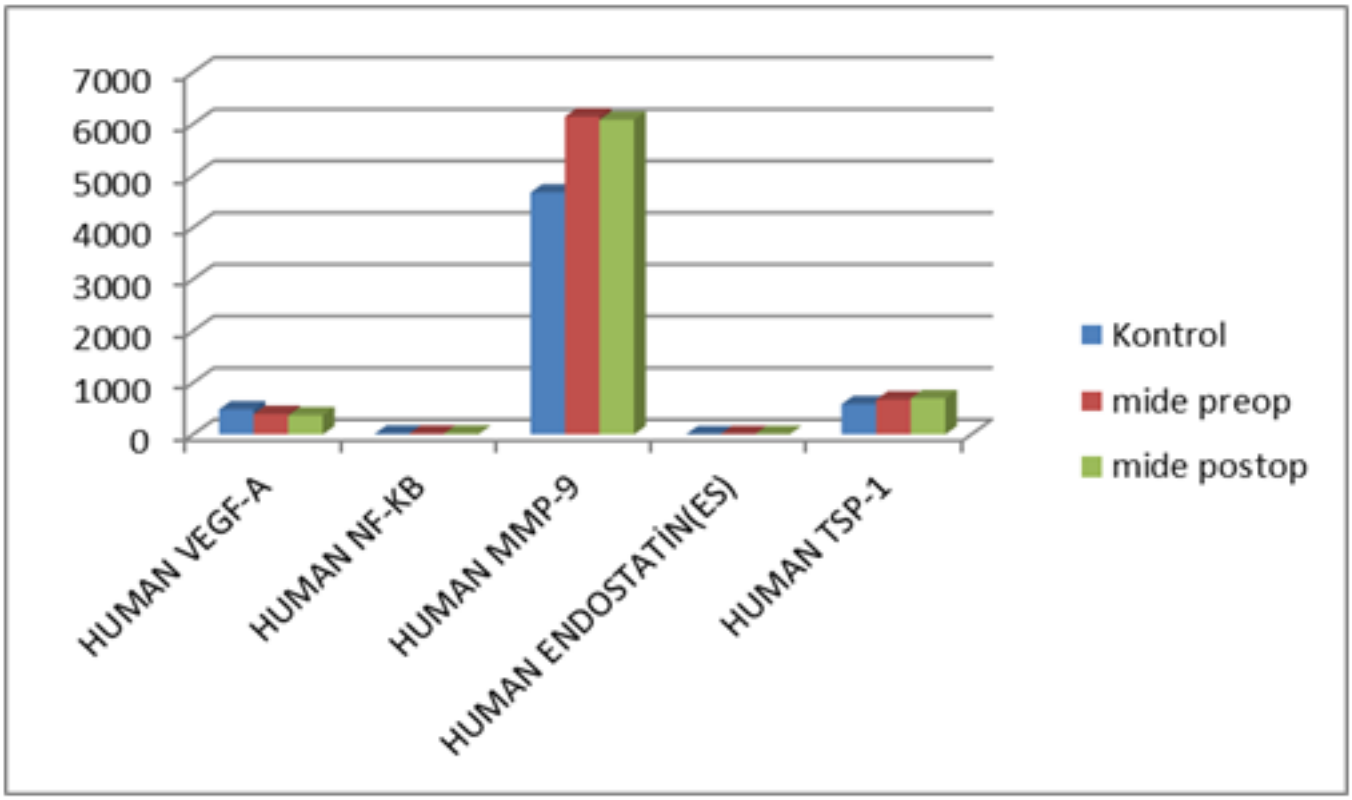

FIGURE 3: NF-kB, VEGF, MMP-9, ESand TSP-1 levels in gastric cancer.

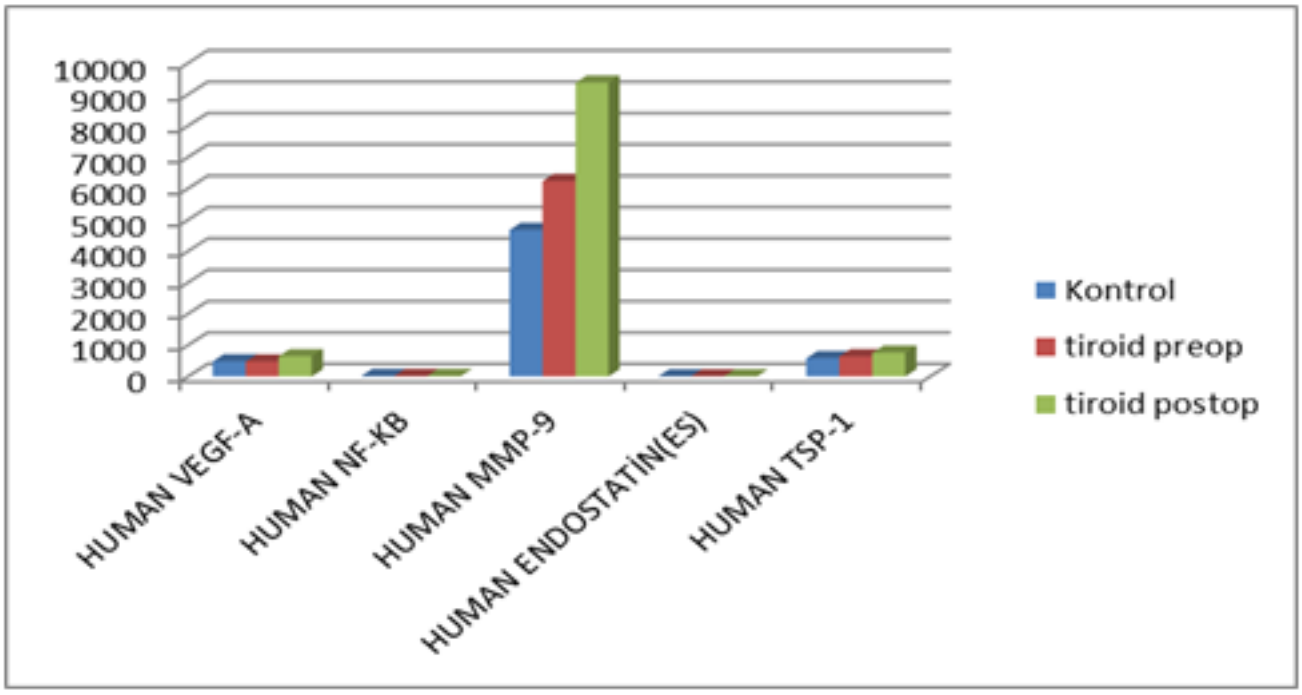

FIGURE 4: NF-kB, VEGF, MMP-9, ESand TSP-1 levels in thyroid cancer.

TABLE 1: NF-kB, VEGF, MMP-9, ES and TSP-1 levels in various cancers.

GROUP	VEGF-A (pg/mL)	NF- κB (ng/mL)	MMP-9 (pg/mL)	ES (ng/mL)	TSP-1 (ng/mL)
Control	483,9249	13,81926	4674,483	2,282038	584,4931
Thyroid pre-op.	476,642	15,11609	6237,624	5,513462	643,5864
Thyroid post-op.	646,0319	17,28186	9374,411	7,30751	759,1636

ious proteinases, such as MMP9 (23). ES induces apoptosis while inhibiting endothelial cell proliferation and migration. Although the mechanism of endostatin is not entirely clear, it has been shown that it can suppress VEGF expression and induce expression of antiangiogenic pigment epithelial derivatives (24-26); it can also upregulate the antiangiogenic VEGF165b isoform by inhibition of specificity protein 1 (27). ES binds to VEGFR-2 (VEGF receptor KDR / Flk-1) on endothelial cells, inhibiting VEGF-induced activation of p38 MAPK (28). It also reduces TNF α and vascular cell adhesion molecule-1 (VCAM-1) (25).

In animal models of ulcerative colitis, increase in VEGF in the colonic tissue was paralleled by a concomitant increase in ES as a defense mechanism; the larger the colonic lesion, the greater the increase in VEGF and therefore ES (29). Induction of ulcerative colitis in MMP9-deficient mice resulted in less ES than in wild-type mice pointing to the in vivo role of MMP-9 in generating endostatin from collagen XVIII in lesions. The levels of VEGF and ES are often linked, and although the molecular mechanism is not completely understood, VEGF might positively influence ES levels through the activation of MMP-9 (30). As a result, its effects on a large number of genes that are regulated up or down by ES and are effective in angiogenesis are still being investigated (31).

TSPs are an antiangiogenic activity protein consisting of five cartilage matrix proteins (32). TSP was the first identified endogenous inhibitor of angiogenesis (33). In the presence of TSP-1 and TSP-2, CD36 receptor, TSP-1, Src family kinase p59fyn, caspase-3-like proteases and p38 mitogen-activated protein kinase (MAPK) - Fas ligand (FasL) (34,35) or FasL tumor necrosis factor (TNF) -receptor 1 and shows antiangiogenic activity through the signal mediated by TNFα. TSP-1 also inhibits lymphangiogenesis, which facilitates the formation of metastases in lymph nodes and distant organs in cancer (36,37, 38).

The nuclear factor kB (NF-kB) pathway has become increasingly appreciated for its involvement in carcinogenesis, as studies continue to uncover its roles in primary tumor growth, angiogenesis, and metastasis (39). The role of NF-kB signaling in these cells during tumorigenesis is controversial and seems to be organ and/or context dependent. Some cancer models show that blocking NF-kB signaling in myeloid cells elicits a protective, anti-tumorigenic response (40).

In a previous study by Kosovo et al demonstrated that CAPE inhibits proliferation of cultured gastric cancer cells; the effects of CAPE on these cells originate from interactions among angiogenic factors including VEGF. VEGF may be regulated by ES and TSP and matrix proteins such as laminin and collagen, which may by organized by MMP (41).

As a result; We think that the fact that all markers are high in the preop period and that the markers are statistically higher in the postoperative period in different types of cancer will depend on the duration of treatment and increase ES and TSP-1. We think that this study should be tried in more case groups and longer treatment groups. We anticipate that this study should be performed by applying more cases and longer treatment.

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