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CLINICAL STUDY

Adipocytokine levels of colon cancer patients before and after treatment

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Abstract: *Aims and background:* In the present study, we investigated the associations between pre- and post-treatment levels of adiponectin, ghrelin, resistin, visfatin and leptin levels in malign and benign groups
Methods and study design: 20 malign colon carcinoma groups and 20 benign groups were included in this study. Serum levels of leptin, adiponectin, resistin, ghrelin, and visfatin were measured by Elisa kits (Milipore Corporation, Billerica, MA, USA).
Results: In the malign group, serum ghrelin (71.90±23.7) levels significantly decreased ($p<0.05$) when compared to those in the benign (88.00±16.9) group. However, serum resistin (4.92±2.2, 3.39±1.1) levels increased statistically significantly ($p<0.05$). In the malign group, serum visfatin (0.85±0.6, 0.83±0.5), adiponektin (60.31±23.1, 56.39±25.9) and leptin (3.08±1.4, 3.74±1.3) levels were not statistically significantly different from those in the benign group. In the malign treatment group, serum adiponectin (102.64±50.3, 66.64±27.0) levels were increased significantly ($p<0.05$); however, serum visfatin (1.17±0.9, 0.68±0.3), ghrelin (85.52±29.5, 82.18±18.0), leptin (5.65±2.8, 3.16±1.1), and resistin (5.96±2.8, 5.65±1.7) levels did not change significantly ($p<0.05$) compared to those in the respective benign treatment group.
Conclusions: We showed that adipocytokines were involved in the carcinogenic process. The present results suggest that resistin and ghrelin may be important biomarkers of colon cancer. Furthermore, an *in vitro* study will also be necessary to evaluate the direct function of these adipocytokines in cancer cells. In addition, it will be appropriate to conduct new studies with a large number of patients at different stages *(Tab. 1, Fig. 2, Ref. 24)*. Full Text in PDF *www.elis.sk.*
Key words: colon cancer, adipocytokines, resistin, visfatin, leptin adiponectin, ghrelin.

During the last two decades, fat tissue has become increasingly viewed not only as an energy storage depot but also as an active endocrine organ that produces and secrets proteins acting as hormones. To this day, 20 adipohormones have been described (1). Adipocytokines such as TNF-α, IL-6, type-1-plasminogen activator inhibitor, hepatocyte growth factor, adiponectin, leptin, resistin, visfatin, and apelin, are cytokines secreted primarily by visceral adipose tissue. Many epidemiologic studies have shown a positive correlation between obesity and increased risk of cancer at various sites (e.g., breast, prostate gland, endometrium, colorectum) including gastric cancer (2–4). Several reports have indicated an association between low adiponectin levels and increased risk of breast, endometrium, and gastric cancers (5–7). Leptin plasma levels are reported to be lower in gastrointestinal, and pancreatic cancer patients (8). On the other hand, leptin may potentiate the growth and proliferation of cancer cells. The role of leptin in hormonally independent tumors still remains unknown. Similarly, the effect of resistin on cell proliferation and differentiation needs further investigations (Figs 1, 2). In addition, the role of these adipohormones in pre-cancerous states, especially in adenomatous colorectal polyps, has not been fully elucidated. Elevated ghrelin levels were recently reported in lung cancer cachexia (9) and in a cohort of male patients with mainly lung and prostate cancer (10). Interestingly, a recent study reported a short-term ghrelin infusion to increase the energy intake in cancer patients (11). Recent studies have shown that circulating visfatin levels are increased in the serum of gastric cancer patients (3), and that the proliferation of prostate cancer cells is increased by exogenous treatment with visfatin. These findings suggest that adipocytokines contribute to the induction of carcinogenesis and tumor progression. Therefore, we hypothesized that changes in the levels of adipocytokines may indicate carcinogenesis and progression of colorectal cancer and adenoma.

Taking these studies into account, the aim was 1) to compare serum levels of leptin, adiponectin, visfatin, ghrelin and resistin in colon carcinoma patients with those in healthy controls, 2) to find out the changes in serum levels of leptin, adiponectin, visfatin, ghrelin and resistin in patients treated radically.

Materials and methods

This consecutive prospective study was conducted at the Department of Medical Biochemistry and Department of General

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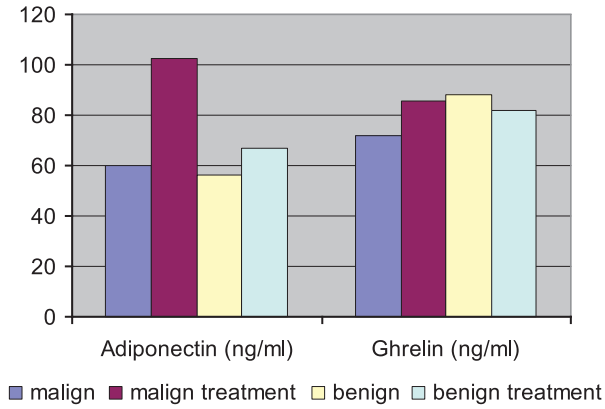

Fig. 1. Adiponectin and ghrelin levels in groups.

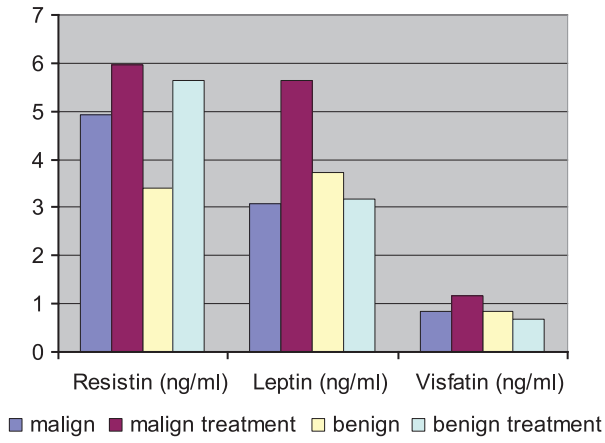

Fig. 2. Resistin, leptin and visfatin levels in groups.

Surgery of Celal Bayar University Medical Faculty, Manisa, Turkey. The study was approved by the hospital ethical committee. All patients and volunteers involved in the study gave their informed consents.

Patients

Trained physicians performed recruitment of subjects by convenience sampling at the outpatient Department of General Surgery of Celal Bayar University Medical Faculty, Turkey. Twenty consecutive patients with colon carcinoma (13 patients with stage III and 7 patients with stage II) and 20 benign with anal disease (11 females and 9 males) were included in this study. There were 8 female and 12 male patients with carcinoma of the colon or rectum. All patients underwent radical resection of their tumor and had chemotherapy. If necessary, they came to a control visit after six months of treatment. Blood samples were taken from all patients on their admission and six months after surgery. Malign and benign patients with diabetes mellitus, hypertension, and those taking hormonal therapy such as oral contraceptives, thyroxin derivates, estrogen replacement therapy, and medications for chronic diseases were excluded. We lost seven patients in the malign group after chemotherapy and radiotherapy treatment.

Group 1; Malign (before treatment) patients group (n=20)
Group 2; Malign (after chemotherapy and radiotherapy treatment) patients group (n=13)
Group 3; Benign (before treatment) patients group (n=20)
Group 4; Benign (after operation) patients group (n=20)

Assay

Blood samples were kept at –80 °C until analysis. All samples from each patient were run in the same assay. Serum leptin, adiponectin, resistin, ghrelin and visfatin were measured by Elisa kits (Millipore Corporation, Billerica, MA, USA).

Statistics

Nonparametric methods were performed in the cross-sectional analysis of biomedical data (Mann-Whitney U test and Wilcoxon test). Two-tailed probability (p) values were calculated and statistical significance was defined as $p<0.05$. All analyses were performed by statistical software SPSS 15.00.

Results

In the malign group, serum ghrelin levels were significantly decreased ($p<0.05$) when compared to those in the benign groups. However, serum resistin levels were statistically increased ($p<0.05$) (Tab. 1).

The examination of the assessments of the malign group and the malign treatment group revealed that whereas visfatin, resistin, leptin and ghrelin levels were not statistically different (for all $p>0.05$), adiponectin levels was found to increase statistically significantly ($p<0.05$) (Tab. 1).

In the benign group, serum resistin levels were significantly increased ($p<0.05$) when compared to those in the benign treatment group (Tab. 1).

In the malign treatment group, serum adiponectin levels were significantly increased ($p<0.05$), however serum visfatin, ghrelin leptin, and resistin levels were not significantly changed ($p<0.05$) compared to those in the respective benign treatment group (Tab. 1).

Tab. 1. Adipocytokine values in groups.

G Groups	Adiponectin (ng/ml)	Ghrelin (ng/ml)	Resistin (ng/ml)	Leptin (ng/ml)	Visfatin (ng/ml)
Malign	60.31±23.1[a]	71.90±23.7[c]	4.92±2.2[d]	3.08±1.4	0.85±0.6
Malign after treatment	102.64±50.3[a]	85.52±29.5	5.96±2.8	5.65±2.8	1.17±0.9
Bening	56.39±25.9	88.00±16.9[c]	3.39±1.1[b,d]	3.74±1.3	0.83±0.5
Bening after treatment	66.64±27.0	82.18±18.0	5.65±1.7[b]	3.16±1.1	0.68±0.3

a,b: $p<0.05$ according to Wilcoxon
c,d: $p<0.05$ according to Mann–Whitney U

Discussion

Colon cancer ranks third among the most common types of cancer (12). Cancer cachexia is a complex metabolic disorder which develops due to decreased food intake, anorexia, decrease in the skeletal muscle mass, and reduction in adipose tissue caused by lipolysis (12, 13). Adipocytokines produced by adipose tissue have been investigated as new risk factors for cancer and metabolic syndromes (14). In our study, we investigated the relationship between the pre- and post-treatment levels of adiponectin, visfatin, ghrelin, leptin and resistin in patients having malignant or benign colon cancer.

Adipocytokines such as TNF-α, IL-6, hepatocyte growth factors, adiponectin, leptin, resistin, visfatin and apelin are cytokines secreted by visceral adipose tissue. In several epidemiological studies, a positive correlation has been observed between increased risk of cancer in various tissues (breast, prostate, endometrium, colorectum) and obesity. It has been suggested that adipocytokines contribute to tumor progression and the induction of carcinogenesis (3).

Resistin is produced mostly by monocytes and macrophages of peripheral blood (1). There are studies on correlation between resistin and cancer. Lehrke M et al demostrated that in human macrophages, an inflammatory cascade with secretion of cytokines including TNFα and IL-6 is sufficient and necessary for the induction of resistin (15). Kumor et al (1) reported that the resistin levels in colorectal cancer patients are higher than those in controls and that the resistin levels in colorectal adenoma patients and controls were also significantly different. In their study, Nakajima et al (14) also observed higher serum concentrations of resistin in patients with colorectal carcinoma independently of BMI, compared to the control group. In our study, we too found the resistin levels in the malign group to be statistically significantly higher than those in the benign group ($p<0.05$). These results are consistent with the ones in literature. We found no statistically significant difference in the post-treatment scores of the malign group whereas we observed a statistically significant increase in the post-treatment scores of the benign group. We normally expected resistin levels to decrease after the treatment. However, since it remained high, resistin seems unlikely to be used as a marker during the postoperative period. Nevertheless, we consider that it may be used as a marker during the preoperative period. We also consider that this increase in resistin levels might be due to the stress of surgery and/or anesthesia. Therefore, we consider it necessary to investigate further whether or not surgery and/or anesthesia have an effect on resistin increase by checking the resistin levels in patients who have had surgery or anesthesia.

The hormone ghrelin is a 28 amino-acid peptide, unique for esterification of its third serine residue by n-octanoic acid. The major source of ghrelin is the stomach, where it is synthesized in distinct endocrine cell type known as the X/A-like cells. The peptide is a powerful inducer of growth hormone (GH) release, acting at the pituitary and hypothalamic levels (16). Ghrelin participates directly in hypothalamic regulation of nutrition, it causes weight gain by reducing the food utilization, increasing the food intake, and inhibiting the leptin-induced feeding reduction (17). Recently, increased levels of ghrelin have been reported in lung cancer and prostate cancer in males and in cachexia caused by lung cancer (18). In their study, Kemik et al (18) determined that ghrelin levels decreased in patients with colon cancer compared to those in the control group. Kemik et al investigated C-reactive protein (CRP), albumin, IL-1a, IL-1β, IL-6, IL-8, IL-10, TNF-a, VEGF-A, VEGF-C , leptin, adiponectin, and ghrelin serum levels in 126 patients with colon cancer and 36 healthy subjects. They determined statistically significant differences and correlation between the two groups. We found that ghrelin levels were statistically significantly lower in the malign group than those in the benign group ($p<0.05$). It is known that ghrelin is a hormone secreted by the oxyntic cells of the stomach. Several studies indicate that this hormone increases the release (secretion) of growth hormone. Low ghrelin levels determined in our study may be due to the damage of the oxyntic cells of the stomach in patients with colon cancer and as a result of this, the secretion of ghrelin may have been reduced. Although the difference was not statistically significant, we observed an increase in the post-treatment scores of the malign patients, which indicated that the treatment had no effect on ghrelin. Although the treatment did not cause a statistical difference on the level of ghrelin, this increase in the ghrelin level due to the treatment dose suggested that this increase might vary depending on the duration and stage of the disease.

Adiponectin (also known as ACRP30) is a member of a protein group secreted by adipocytes known as adipocytokine (19, 20). Several studies indicated that there is an association between low levels of adiponectin and an increased risk of stomach, endometriosis and breast cancer (5, 7). The mechanism regulating adiponectin levels has not been clarified yet. Kumor et al aimed to investigate resistin, adiponectin and leptin concentrations in patients (1). Their study included 36 patients with colorectal cancer and 37 patients with colorectal adenomas. They observed higher serum adiponectin concentrations in patients in the control group, compared to patients with colorectal adenoma or carcinoma. In our study, there were no differences between serum adiponectin levels of the patients with colon cancer and those of benign patients. To date, the effects of treatment on adipocytokines in malign patients have never been studied anywhere else. However, in our study we investigated the effects of treatment on adipocytokines too. We observed that the levels of adiponectin increased statistically significantly in malign patients after the treatment. This increase may indicate a possible association between adiponectin levels and the effectiveness of the treatment, which suggests that adiponectin might be an important marker in the monitoring of the treatment

Visfatin is also a new adipocytokine identical to a previously known pre-B cell colony enhancing factor expressed by lymphocytes. Visfatin was originally identified as a growth factor for immature B cells, and recently demonstrated to bind insulin receptor and increase the proliferation of cancer cells. Although clinical correlation between visfatin and cancer has been reported rarely, it may be a new and promising marker of colorectal cancer as is resistin (14). In one of their studies, Nakajima et al (14) reported that resistin and visfatin levels were higher in gastric cancer patients than in control subjects. In our study, there was no statistically significant difference between the visfatin levels of patients with benign colon disease and those of patients with colon carcinoma.

However, although not statistically significant, we observed an increase in visfatin levels. In various studies, a correlation between visfatin and resistin has been reported. Our study results also suggest that there might be a correlation between visfatin and resistin.

Leptin is another member of the adipocytokine family (18). Leptin, adiponectin, and resistin are protein-like products secreted by white adipose tissue (21, 22). Although there are quite many studies on the invasion, migration and stimulation of cancer cells by leptin, the effects of the other members of adipocytokine family on cancer are not clear (23). Leptin's primary function is to control body's fat stores but it also plays a role in the progression of cellular proliferation, inhibition of cellular apoptosis and angiogenesis. The presence of leptin receptors in normal human colon mucosa, adenoma and cancer suggests that leptin may have a direct effect on carcinogenesis. Kumor et al observed low leptin concentrations in patients with colorectal adenoma (1). In their *in vivo* and *in vitro* studies, Hardwick JC et al observed an increase in the formation of colon cancer cell lines after having them stimulated with leptin (24). Nakajima et al (14) investigated the association between adipocytokine and several cancer levels. They measured the blood levels of adiponectin, leptin, resistin, visfatin, and C-peptide in 115 patients with colorectal cancer and in 115 control subjects of the same age and gender. They repeated the same analysis in 72 patients with colorectal adenomas and in 72 control subjects. They found no significant difference between leptin levels in patients with colorectal adenoma or other cancer types and those in control subjects. In our study, when we compared the group with benign colon disease and that with colon cancer, we found no statistically significant difference in their leptin levels either ($p>0.05$). In several different studies on leptin, it can be seen that very different results were obtained. However, in order to eliminate this difference, we consider that conducting a research on a large number of very homogeneous patients would help to eliminate this difference.

As a result, the studies on adipocytokines revealed that adipocytokines played a part in the carcinogenic process to some extent. These roles of the majority, if not all of the adipocytokines have been demonstrated in patients with cancer *in vitro* and *in vivo* studies. In the light of this information and our findings, it can be proposed that resistin and ghrelin, two members of adipocytokine family, may have a promising role as markers in the diagnosis of colon carcinoma. The effects of treatment on adipocytokines in malign patients have never been studied anywhere else, whereas in our study, we investigated these patients too. These results suggested that adipocytokines could be appropriate markers in monitoring the cancer treatment. We consider that it would be appropriate to carry out studies for each of these cytokines on a larger number of patients at different stages of the disease in order to clarify the issue.

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