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Impact of serum adipokines on tumor mitotic and apoptotic activity in endometrial cancer

Endometrial kanserde serum adipokinlerin tümör mitotik ve apoptotik aktiviteye etkisi

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Abstract

Aim: Endometrial cancer is the most common malignancy of female genital system. Obesity is one of the most important risk factors in endometrial cancer. Adipose tissue functions just like an endocrine organ by secreting many bioactive substances and contribute to tumor development. The aim of this study was to investigate the correlation between serum adipokine levels and tumor mitotic and apoptotic activity levels in endometrial cancer.

Methods: We designed a cross-sectional study. After obtaining the approval of Ethics Committee, 38 patients with endometrial cancer admitted between 2014 July-2014 December to Obstetrics and Gynecology outpatient clinic of Ondokuz Mayıs University, Faculty of Medicine were included in the study. Serum leptin and adiponectin levels were measured in 5 cc serum samples taken from the patients preoperatively. The pathology specimens of the patients were semi quantitatively evaluated with immunohistochemical study considering the percentage and intensity of staining for bcl-2 and the percentage of staining for Ki67. Moreover, preoperative estradiol levels, insulin resistance, body fat percentages and body mass index (BMI) values were determined. The Mann Whitney U test, Kruskal Wallis and Spearman's Correlation test were used statistically. The results were considered statistically significant for $P<0.05$.

Results: The patients included in the study were between 36-82 years of age and the mean age was 62.5 (10.4) years. The mean BMI value of the patients was 31.1 (4.8) kg/m2 (range: 19-38 kg/m^2). According to the FIGO 2010 staging system, the distribution of the patients were as follows: 30 patients (78%) Stage 1, 1 patient (2.6%) Stage 2 and 7 patients (18.4%) Stage 3. Of the patients, 6 (15.8%) had grade 1, 20 (52.6%) had grade 2 and 12 (31.6%) had grade 3. Twenty-seven patients were classified as endometrioid type and 11 patients were classified as nonendometrioid type. There was no statistically significant correlation between serum leptin and adiponectin levels and percentage of Ki-67 immunohistochemical staining in tumoral tissue and bcl-2 score ($P=0.05$). In the immunohistochemical examination of tumoral tissue, it was found that tumor grade statistically significantly increased as the staining percentage for Ki67 increased (r=0.571, $P<0.001$). There was no statistically significant correlation between Bcl-2 score and tumor grade or stage ($P=0.751$). It was found that serum leptin levels significantly increased as BMI increased (r=0.341, $P=0.036$). As HOMA-IR increased, adiponectin level statistically significantly increased (r=0.393, $P=0.015$). There was also no statistically significant difference between the endometrioid and non-endometrioid groups in terms of leptin, adiponectin and leptin/adiponectin levels ($P=0.554$, $P=0.652$). There was no statistically significant difference between the endometrioid and non-endometrioid groups in terms of median BCL-2 score and Ki-67 percentage ($P=0.05$).

Conclusion: There was no correlation between serum leptin and adiponectin levels and Ki-67 immunohistochemical staining percentage and bcl-2 score.

Keywords: Endometrial cancer, Adipokines, Apoptosis, Immunohistochemistry

Öz

Amaç: Endometrial kanser, kadın genital sisteminin en sık görülen malignitesidir. Obezite, endometrial kanserde en önemli risk faktörlerinden biridir. Adipoz doku, birçok biyoaktif madde salgılayarak tıpkı bir endokrin organı gibi işlev görür ve tümör gelişimine katkıda bulunur. Endometrium kanserinde serum adipokin seviyeleri ile tümör mitotik ve apopitotik aktivite düzeyleri arasındaki ilişkinin araştırılması amaçlanmıştır.

Yöntemler: Kesitsel bir çalışma tasarladık. Etik Kurul onayı alındıktan sonra 2014 Temmuz-2014 Aralık tarihleri arasında Ondokuz Mayıs Üniversitesi Tıp Fakültesi Kadın Hastalıkları ve Doğum polikliniğine endometriyal kanserli 38 hasta dahil edildi. Hastalardan preoperatif alınan 5 cc serum örneğinde serum leptin ve adiponektin seviyesi ölçüldü. Hastaların patoloji spesmenleri immünhistokimyasal çalışma ile bcl-2 için boyanma yüzdesi ve şiddeti ve Ki67 için boyanma yüzdesi gözönüne alınarak semikantitatif olarak değerlendirildi. Ayrıca hastaların preoperatif östradiol seviyesi, insülin direnci, vücut yağ oranları ve body mass index (BMI) değerleri belirlendi. İstatistiksel olarak Mann Whitney U Kruskal Wallis ve Spearman'ın Korelasyon testi kullanıldı. $P<0,05$ için sonuçlar istatistiksel olarak anlamlı kabul edildi.

Bulgular: Çalışmaya alınan hastalar 36-82 yaşlar arasında olup yaş ortalaması 62,5 (10,4) idi. Hastaların ortalama BMI değeri 31,1 (4,8) kg/m^2 idi (Aralık: 19-38 kg/m^2). FIGO 2010 evreleme sistemine göre 30 hasta (%78) Evre 1, 1 hasta (%2,6) Evre 2,7 hasta (%18,4) Evre 3 olarak dağılım göstermekte idi. Hastaların 6 tanesi (%15,8) grade 1, 20 tanesi (%52,6) grade 2 ve 12 tanesi (%31,6) grade 3 olarak dağılım göstermekte idi. 27 hasta endometrioid tip kalan 11 hasta nonendometrioid tip olarak sınıflandırıldı. Serum leptin ve adiponektin düzeyi ile tümöral dokuda Ki-67 immünhistokimyasal boyanma yüzdesi ve bcl-2 skoru arasında istatistiksel anlamlı korelasyon saptanmadı ($P=0,751$). Tümöral doku immünhistokimyasal incelemesinde Ki67 için boyanma yüzdesi arttıkça tümörün grade'i de istatistiksel anlamlı olarak arttığı belirlendi (r=0,571 $P<0,001$). Bcl-2 skoru ile tümör grade veya evresi arasında istatistiksel anlamlı korelasyon saptanmadı ($p>0.05$). BMI arttıkça serum leptin düzeylerinin istatistiksel anlamlı olarak arttığı saptandı (r=0,341, $P=0,036$). HOMA-IR arttıkça adiponektin düzeyi istatistiksel anlamlı olarak artmaktaydı (r=0,393, $P=0,015$). Endometrioid ve non-endometrioid gruplar arasında da; leptin, adiponektin ve leptin/adiponektin düzeyleri yönünden istatistiksel olarak anlamlı farklılık görülmedi ($P=0,554$, $P=0,652$). Endometrioid ve non-endometrioid grupları arasında medyan BCL-2 skoru ve Ki-67 yüzdesi yönünden istatistiksel olarak anlamlı farklılık görülmedi ($P=0,05$).

Sonuç: Serum leptin ve adiponektin düzeyi ile tümöral dokuda Ki-67 immünhistokimyasal boyanma yüzdesi ve bcl-2 skoru arasında istatistiksel anlamlı korelasyon yoktur.

Anahtar kelimeler: Endometrium kanseri, Adipokin, Apopitozis, İmmunohistokimya

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Introduction

Endometrial cancer is the most common malignancy of female genital system. Obesity is one of the most important risk factors in endometrial cancer. Therefore, the effect of numerous bioactive substances released from adipose tissue on tumor development has been investigated. Adiponectin and leptin are the most important adipokines released from adipose tissue. Today, serum L/A (leptin/adiponectin) ratio has been shown to increase in many cancers associated with obesity [1,2].

Leptin is the product of the Ob gene expressed by adipocytes in adipose tissue; it is similar to cytokines and is a protein hormone containing 167 amino acids. It is encoded by the ob/ob gene residing on the long arm of chromosome 7 (7q31). It has also been shown to be secreted by some placenta, gastric epithelium, skeletal muscle, pituitary and mammary gland. It circulates in free and protein-bound form in the blood. The free form is thought to be responsible for leptin activity. The level of serum leptin increases in proportion to the amount of adipose tissue in the body. It is higher in women than in men. After being released into the blood circulation, it binds to its receptors in the hypothalamus and prevents the development of obesity by establishing a balance between the body's energy requirement and weight gain with a negative feedback effect. About 6 different receptors of leptin have been identified in various tissues. It has also been found to play very important roles in metabolic regulation, sexual development, reproduction, hematopoiesis, immunity, regulation of gastrointestinal functions, sympathetic nervous system activation, angiogenesis and osteogenesis [3,4]. In the early secretory phase, expression of leptin receptors increases in the endometrium. Progesterone reduces the level of leptin receptor in the endometrium [5].

Leptin has been shown to play a role in the proliferative processes in breast, endometrium, prostate, colon and many other tissues [1,2].

Adiponectin is a protein molecule with a weight of 30 kDa. Adiponectin is negatively correlated with obesity. Low adiponectin levels are associated with hyperinsulinemia and increased insulin resistance [6]. Two different adiponectin receptors have been identified in the tissue.

The L/A ratio is an indicator of insulin resistance in diabetic and nondiabetic patients [7,8]. Serum L/A ratio has been found to be high in breast, colon and endometrial cancer, and is more significant than increased leptin level alone [9,10]. In addition, high L/A ratios in endometrial cancer are associated with increased risk independent of diabetes and obesity. The presence of independent risk increase is explained by the fact that these adipokines contribute directly to the pathophysiologic mechanisms in tumor formation.

High leptin levels cause increased insulin resistance and hyperinsulinemia [11,12]. Moreover, leptin increases estrone formation from androstenedione by increasing the aromatase activity in peripheral adipose tissue [1]. In addition, by increasing (estrogen receptor) ER alfa stability, it increases the formation of estrogenic effects in the tissue. Adiponectin deficiency causes insulin resistance. It has been shown that serum adipokines also cause an increased risk in endometrial cancer independent of estrogen and insulin [13]. It has been shown that leptin increases the proliferation and invasiveness of tumor cells using the JAK/STAT and ACT pathways, while adiponectin decreases tumor proliferation by causing apoptosis [14,15].

Our study aims to investigate the effects of adipokines on tumor proliferation by comparing serum leptin and adiponectin levels with bcl-2 (anti-apoptotic protooncogen) and Ki67 (proliferation markers) levels in the tumoral tissue of patients with endometrial cancer. In addition, the correlation between serum estrogen level, body mass index (BMI), body fat percentage and insulin resistance (HOMA-IR) and serum leptin and adiponectin levels in endometrial cancer will be evaluated.

Materials and methods

Ethical committee approval was obtained (OMU-KAEK-2014/768-788). The informed consent was obtained from all participants.

Patient selection

The study included 38 patients admitted to the Obstetrics and Gynecology Outpatient Clinic of Ondokuz Mayıs University and diagnosed with endometrial cancer and scheduled for operation. We included all endometrial cancer patients between 2014 July-2014 December in this study. It is a cross-sectional study. While selecting the patients, the diagnosis of endometrial cancer was made by endometrial biopsy. Patients who were not scheduled for surgery because of systemic diseases or who refused surgery despite recommendation for surgery were excluded from the study.

Collection and storage of serums

Before the operation, serum samples of about 5-6 cc were taken from the antecubital region in the sitting position at room temperature (24 C) using the standard blood collection technique after the sterilization of the area with a alcohol infused cotton. The blood samples taken were transferred in vacuum biochemistry tubes with red cap that did not contain any anticoagulant agent. The tubes were centrifuged at 5000 rpm for 5 minutes and stored at -80 ° C until the time of supernatant study.

Study of serum leptin and adiponectin levels

The serum samples stored at -20°C were thawed at room temperature, and then studied using the leptin ELISA Kit (DRG, Germany) and adiponectin ELISA Kit (Assaypro, USA) microelisa kit in accordance with the manufacturer's recommendations in the Elisa research laboratory. During the study, a microplate washer (BIO-TEK, ELX-50 model, USA) was used, and for the results, a microplate reader (BIO-TEK ELX800 model, USA) was used. All parameters were read at a wavelength of 450 nanometers and the absorbance values were placed in their area in the calibration graph to obtain the results of the samples. The Human Leptin Elisa Kit Assay range was given as 1.25-80 ng/ml, the Human Adiponectin Elisa Kit Assay range as 0.7 ng/ml, and the intraassay and interassay as 4.3% and 7.2%. The results were interpreted.

Immunohistochemical staining and evaluation of the pathology specimens

All tissues of the patients that were sent to the pathology department after surgery were fixated in 10% neutral formalin solution and were blocked in paraffin. One block without

bleeding or necrosis representing the best histomorphology was selected from each case. Sections of 5 microns were taken from the selected blocks using microtome devices. Using immunohistochemical staining devices (Ventana, Benchmark, XT, USA) on the sections, immunohistochemical study was carried out with Ki67 (Anti Ki-67, Fremant CA 94538, Emego Europe, Netherlands) and bcl-2 (Bcl-2 oncoprotein, NCL-L-bcl-2, Newcastle Upon tyne NE128EW, United Kingdom) primary antibodies.

The staining results were semi quantitatively evaluated by a pathologist under a Olympus Bx51 under light microscope (Olympus, USA, 1999) considering the percentage and intensity of staining for bcl-2 and the percentage of staining for Ki67 (Table 1, 2) (Figure 1, 2, 3).

Table 1: Immunohistochemical evaluation of Bcl-2

Parameter	Score
A - Staining intensity	(0 – 3)
Negative	0
Weak staining	1
Moderate staining	2
Strong staining	3
B - Staining percentage (percentage of stained cells)	(0 – 4)
No staining of tumor cells or staining in less than 5% of tumor cells	0
Staining in 5-25% of tumor cells	1
Staining in 25-50% of tumor cells	2
Staining in 50-75% of tumor cells	3
Staining in more than 75% of tumor cells	4
Total score (A + B)	(0 – 7)
Negative	0-1
Weak positive	2-3
Moderate positive	4-5
Strong positive	6-7

Table 2: Immunohistochemical evaluation of Ki67

	Staining percentage (percentage of stained cells)
0	0-10%
1	10-50%
2	50-75%
3	>75%

In the statistical evaluation, the data were evaluated according to the score for bcl-2 and the staining percentage for Ki67.

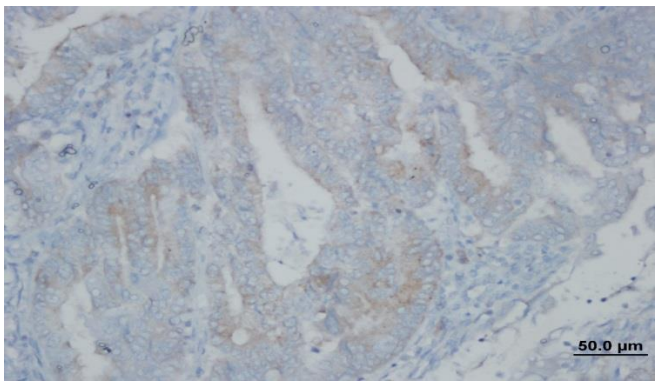

Figure 1: Endometrial cancer: Bcl-2, weak staining (DAB, X400)

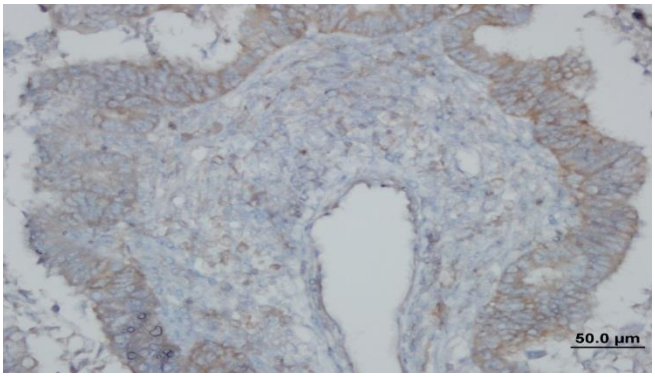

Figure 2: Endometrial cancer: Bcl-2; moderate staining (DAB, X400)

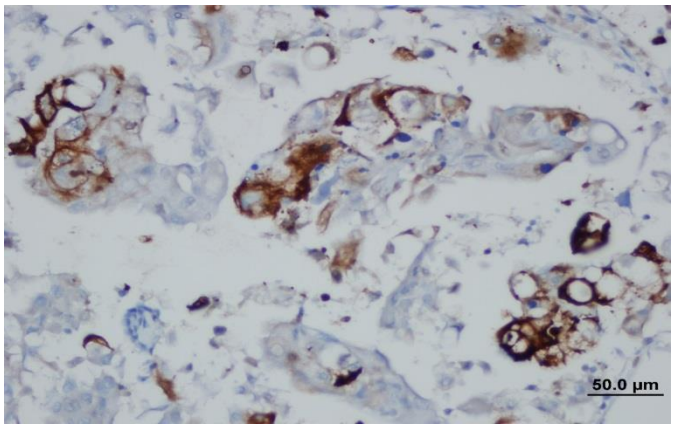

Figure 3: Endometrial cancer: Bcl-2; strong staining (DAB, X400)

Calculation of insulin resistance and preoperative estradiol level

Fasting glucose, insulin levels and estradiol levels were studied in 5 cc serum samples taken preoperatively after 8 hours of fasting.

The patients' insulin resistance was calculated using HOMA-IR (The Homeostatic Model Assessment of Insulin Resistance).

HOMA-IR: Fasting blood glucose (mg/dl).Fasting insulin microU/ml/405 (process constant value)

Calculation of body mass index and determination of body muscle-fat percentage of the patients

By measuring the heights and weights of the patients, BMI was calculated using body weight (in kg)/height squared (square meter) formula.

The body fat percentages of the patients were measured using Tanita-TBF 310 Body composition analyzer.

Statistical analysis

The analysis of the data was made on SPSS 11.5 software package for Windows. The Shapiro–Wilk test was used to analyze whether the continuous numerical variables are normally distributed, while Levene's test was used to analyze the homogeneity of variances. The descriptive statistics, the continuous numerical variables were expressed as mean (standard deviation), the ordinal variables as median (minimum-maximum), and the categorical variables as case number and (%). The significance of difference between the groups in terms of median values was analyzed by the Mann Whitney U test when the number of independent groups was two, while the significance of difference between more than two groups was analyzed by the Kruskal Wallis test. The Spearman's correlation test was used to determine whether there was a statistically significant correlation between variable pairs. The results were considered statistically significant for $P<0.05$.

Results

Clinicopathological and demographic characteristics of the patients

The patients included in the study were between 36-82 years of age and the mean age was 62.5 (10.4) years. The mean BMI value of the patients was 31.1 (4.8) kg/m^2 (range: 19-38 kg/m^2).

According to the FIGO 2010 staging system, the distribution of the patients were as follows: 30 patients (78%) Stage 1, 1 patient (2.6%) Stage 2 and 7 patients (18.4%) Stage 3.

Of the patients, 6 (15.8%) had grade 1, 20 (52.6%) had grade 2 and 12 (31.6%) had grade 3.

27 patients were classified as endometrioid type and 11 patients were classified as nonendometrioid type (Table 3).

Table 3: Demographic and clinical characteristics of the patients

Variables	n=38
Age (years)	62.5(10.4)
Age range (years)	36-82
Body mass index (kg/m^2)	31.1(4.8)
Fat mass	33.7 (14.3-47.2)
Type	
Endometrioid	27 (71.1%)
Non-endometrioid	11 (28.9%)
Stage	
I	30 (78.9%)
II	1 (2.6%)
III	7 (18.4%)
Grade	
I	6 (15.8%)
II	20 (52.6%)
III	12 (31.6%)

The correlation between serum leptin and adiponectin levels and Ki67 immunohistochemical staining percentage and Bcl-2 score

There was no statistically significant correlation between serum leptin level and percentage of Ki-67 immunohistochemical staining in tumoral tissue and bcl-2 score (P=0.554).

There was no statistically significant correlation between serum adiponectin level and percentage of Ki-67 immunohistochemical staining in tumoral tissue and bcl-2 score (P=0.652) (Table 4).

Table 4: Laboratory measurements of the patients

Variables	Mean	SD	Median	Minimum	Maximum
HOMA-IR	6.2	9.80	3.2	0.4	46.0
Leptin	6.5	5.59	5.1	0.0	21.9
Adiponectin	7.8	6.38	4.3	0.9	23.6
Leptin/Adiponectin	1.4	1.76	0.8	0.0	7.8
Estrogen	21.2	19.33	17.0	5.0	113.0

SD: Standard deviation

Immunohistochemical evaluation results for Bcl-2 and Ki-67

When the patients were immunohistochemically evaluated in terms of staining percentage for Ki67, it was found that there were 9 patients (23.7%) showing 0-10 percent staining, 16 patients (42.1%) showing 10-50 percent staining, and 5 patients (8.2%) showing 50-75 percent staining, and 8 patients (21.1%) showing >75 percent staining.

When the patients were immunohistochemically evaluated in terms of staining percentage for bcl-2, it was found that there were 13 patients (34.2%) with negative staining, 11 patients (28.9%) with 5-25 percent staining, 3 patients (7.9%) with 25-50 percent staining, 5 patients (13.2%) with 50-75 percent staining, and 6 patients (15.8%) with >75 percent staining.

When the patients were immunohistochemically evaluated in terms of staining intensity for bcl-2, it was found that the staining intensity was negative in 12 patients (31.6%), weak in 13 patients (34.2%), moderate in 11 patients (28.9%), and strong in 2 patients (5.3%) (Table 5).

Correlation between percentage of Ki67 staining in tumoral tissue and bcl-2 score and tumor grade and stage

In the immunohistochemical examination of tumoral tissue, it was found that tumor grade statistically significantly increased as the staining percentage for Ki67 increased (r=0.571 P<0.001).

There was no statistically significant correlation between Bcl-2 score and tumor grade or stage (P=0.751 and P=0.622, respectively).

There was no statistically significant correlation between Bcl-2 score and BMI, HOMA-IR and estrogen level (P=0.275, P=0.135 and P=0.569, respectively).

There was no statistically significant correlation between percentage of Ki67 staining and BMI, HOMA-IR, FIGO stage and estrogen level (P=0.540, P=0.365, P=0.130 and P=0.855, respectively) (Table 6).

Table 5: Descriptive statistics for BCL-2 and Ki-67 staining status

Variables	n=38
BCL-2 Intensity	
Negative	12 (31.6%)
Weak	13 (34.2%)
Moderate	11 (28.9%)
Strong	2 (5.3%)
BCL-2 percentage	
Negative or <5%	13 (34.2%)
5-25%	11 (28.9%)
25-50%	3 (7.9%)
50-75%	5 (13.2%)
>75%	6 (15.8%)
BCL-2 Score	2.5 (0-6)
Ki-67 staining percentage	
0-10%	9 (23.7%)
10-50%	16 (42.1%)
50-75%	5 (13.2%)
>75%	8 (21.1%)

Table 6: Correlation coefficients and significance levels between BCL-2 score and Ki-67 percentage and other clinical and laboratory measurements

	BCL-2 Score		Ki-67	
	r	P-value †	r	P-value †
BMI	-0.182	0.275	0.103	0.540
Stage	0.083	0.622	0.250	0.130
Grade	-0.053	0.751	0.571	<0.001
HOMA-IR	0.247	0.135	0.151	0.365
Estrogen	0.095	0.569	-0.031	0.855

r: Correlation coefficient, † Spearman's correlation test

Determination of the correlation between serum adipokine levels and BMI, body fat percentage, serum estrogen level and insulin resistance (HOMA-IR):

There was no statistically significant correlation between serum adipokine levels and tumor grade and stage (P=0.225, P=0.915, P=0.304 and P=0.418, respectively).There was no statistically significant correlation between serum leptin levels and serum estrogen level, body fat percentage and HOMA-IR (P=0.784, P=0.059 and P=0.975, respectively). On the other hand, in our study group of patients with endometrial cancer, it was found that serum leptin levels statistically significantly increased as BMI increased (r=0.341, P=0.036).

There was no statistically significant correlation between serum adiponectin level and serum estrogen level, BMI, body fat percentage (P=0.115, P=0.125 and P=0.082, respectively). On the other hand, as HOMA-IR increased, adiponectin level statistically significantly increased (r=0.393, P=0.015) (Table 7).

Table 7: Correlation coefficients and significance levels between Leptin, Adiponectin and Leptin/Adiponectin levels and other demographic, clinical and laboratory measurements

	Leptin		Adiponectin		Leptin/Adiponectin	
	r	P-value †	r	P-value †	r	P-value †
Age	0.131	0.432	0.070	0.676	-0.039	0.814
BMI	0.341	0.036	0.087	0.603	0.254	0.125
Stage	0.202	0.225	-0.029	0.864	0.171	0.304
Grade	-0.018	0.915	-0.055	0.742	0.135	0.418
HOMA-IR	-0.005	0.975	-0.393	0.015	0.260	0.115
Fat mass	0.309	0.059	-0.010	0.951	0.286	0.082
Estrogen	0.046	0.784	-0.286	0.081	0.260	0.115

r: Correlation coefficient, † Spearman's correlation test

Comparison of the endometrioid and nonendometrioid groups

There was no statistically significant difference between the endometrioid and non-endometrioid groups in terms of leptin, adiponectin and leptin/adiponectin levels, respectively. There was no statistically significant difference between the endometrioid and non-endometrioid groups in terms of median BCL-2 score and Ki-67 percentage.

Discussion

Endometrial cancer is the most common malignancy of female genital system. Obesity is one of the most important risk factors in endometrial cancer. Adipose tissue functions just like an endocrine organ by secreting many bioactive substances, and contributes to tumor development. Leptin and adiponectin, called serum adipokines, are cytokines which are released from adipose tissue and contribution of which to tumor development has been frequently investigated. In the study by Yu Ma et al. [16] investigating 206 patients with endometrial cancer and 310 healthy controls, it was found that serum leptin levels were significantly increased in the endometrial cancer group than in the control group. It was found that adiponectin levels of the endometrial cancer patients were borderline statistically significantly lower than that of the control group. In the study by Friedenreich et al. [17] included 541 endometrial cancer patients and the control group of 961 individuals, the correlation between insulin resistance markers (leptin, adiponectin, A:L, HOMA-IR) and the risk of endometrial cancer was investigated, and it was found that as insulin and HOMA-IR increased, the risk of endometrial cancer increased, and as the level of adiponectin increased, the risk of endometrial cancer decreased. There was no correlation between fasting blood glucose, leptin and A:L ratio and endometrial cancer. In the study by Cymbaluk et al. [18] comparing 40 patients diagnosed with endometrial cancer and 46 patients diagnosed with endometrial hyperplasia and the control group of 46 individuals with normal endometrium, a positive correlation between serum leptin levels and BMI was found. The mean serum leptin concentration was significantly higher in the endometrial cancer and endometrial hyperplasia groups than in the control group. Three groups were formed by considering BMI, and it was found that leptin levels of patients with endometrial pathology were significantly higher in each BMI group than in the control group. In the study by Mihu et al. [19] enrolled 44 endometrial cancer patients and the control group of 44 healthy subjects, the amount of abdominal fat was measured using (dual X-ray absorptiometry) DXA, and it was demonstrated that the amount of abdominal fat increased directly proportional to leptin and inversely proportional to adiponectin. It was also found that abdominal fat and serum leptin levels were significantly higher and adiponectin levels were significantly lower in patients with endometrial cancer than in the control group. In the study by Ashizawa et al. [13] comparing 146 patients diagnosed with postmenopausal endometrial cancer and the control group of 150 individuals, it was found that serum leptin levels and L/A ratios were significantly higher in patients with endometrial cancer than in the control group. Adiponectin levels of patients with endometrial cancer were found to be significantly lower than that of the control group. In the endometrial cancer group, a significant correlation was found between serum adipokine levels and BMI. There was a significant correlation between leptin level and L/A ratio and HOMA-IR. In patients with endometrial cancer, depending on the levels of leptin or adiponectin alone, L/A ratio was found to be more significant in determining the risk of endometrial cancer, and it was stated that L/A ratio was significant in determining the risk of endometrial cancer independent of obesity, hypertension and diabetes. There was no statistically significant correlation between serum adipokines or L/A ratio and tumor grade or FIGO stage. In our study, serum leptin level statistically significantly increased as BMI increased in the patients with endometrial cancer. However, there was no statistically significant correlation between leptin and HOMA-IR, fat mass and preoperative estrogen levels, respectively. In accordance with the literature, there was no significant correlation between tumor stage and grade and leptin, adiponectin or L/A. There was no statistically significant correlation between adiponectin and BMI, fat mass and estrogen levels, respectively. On the other hand, unlike the literature, adiponectin level statistically significantly increased as HOMA-IR level increased. There was no statistically significant correlation between L/A ratio and BMI, body fat percentage, HOMA-IR and estrogen levels. The fact that the correlation between adipokine levels and endometrial cancer risk was found to be significant independent of obesity in the studies conducted suggested that adipokines may play a direct role in tumor proliferation independent of estrogen and insulin [13,16]. It has been shown that leptin increases the proliferation and invasiveness of tumor cells using the JAK/STAT and ACT pathways, while adiponectin decreases tumor proliferation by causing apoptosis [14,15]. In the study by Sharma et al. [20], it was shown that leptin increased the invasiveness and proliferation of tumor using JAK / STAT and ACT pathways in endometrial cancer cells. In numerous studies, bcl-2 has been evaluated as an anti-apoptotic oncoprotein and Ki67 as a proliferation marker for endometrial cancer. In the study by Henderson et al. [21] on endometrial tissues of proliferative, hyperplastic, atypical hyperplastic and endometrial cancer, it was found that bcl-2 expression (bcl-2 score, defined as 0-4) was higher in proliferative (n:11, score: 3.59) and hyperplastic (n:18, score: 3.47) endometrium than in atypical hyperplasia (n:11, score: 0.82) and adenocarcinoma (n:34, score: 0.86) ($p < 0.001$). Moreover, it was also shown that there was no statistically significant correlation between bcl-2 expression and stage and grade in endometrial cancer group. In the study by Vaskivuo et al. [22], grade 1 (n:16), grade 2 (n:6), grade 3 (n:6) endometrial cancer specimens were analyzed and it was found that BCL-2 staining intensity (0: negative, 1: limited, 2: moderate, 3: strong staining) decreased inversely proportional to grade ($P<0.05$). They found that BCL-2 expression was high in normal proliferative endometrium and decreased in line with the severity of aggression in endometrial hyperplasia and cancer. In the study by Zheng et al. [23] comparing 21 endometrioid and 21 uterine papillary serous carcinomas, it was shown that endometrioid endometrial cancers had a higher immunohistochemical staining for bcl-2 than that of uterine papillary serous carcinoma ($P=0.002$). In the study by Canlorbe et al. [24] on 69 patients

with the diagnosis of endometrial cancer, it was found that immunohistochemical staining for ki-67 was higher in patients with grade 3 endometrial cancer than in those with grade 1 and 2 (P<0.001). Pinheiro et al. [25] divided 515 patients undergone hysteroscopic polypectomy into two groups as obese and nonobese, examined the specimens and found no significant difference between obese and nonobese groups in terms of Ki67 expression. Whereas, they found that Bcl-2 expression was higher in obese patients than in nonobese patients. In the study by Villavicencio et al. [26], 31 patients with benign endometrial tissue were divided into three groups based on their body mass index and 10 patients with type1 endometrial cancer were enrolled as the control group. In their study, a positive correlation was found between Ki67 percentage in endometrial tissues and serum leptin, insulin, estrogen levels and BMI of patients with benign endometrial tissue (P<0.05). In our study, there was no statistically significant correlation between serum adipokines and Ki67 percentage in tumoral tissue and BCL-2 score, and there was also no significant correlation between L/A ratio and Ki67 percentage and BCL-2 score. In our study, Ki67 staining percentage of the tumor statistically significantly increased as tumor grade increased. There was no statistically significant correlation between tumor stage and Ki-67 and Bcl-2 scores. There was no statistically significant correlation between tumor grade and BCL-2 score. There was no statistically significant correlation between BMI, HOMA-IR and estrogen levels and Ki67 percentage or Bcl-2 score. There was no statistically significant difference between the endometrioid and non-endometrioid groups in terms of median BCL-2 score and Ki-67 percentage.

Yuan et al. [27] investigated 80 cases of cervical cancer and found a statistically significant correlation between tumoral tissue leptin levels and tumor grade and Ki67 expression, and a positive correlation between tissue leptin level and bcl-2 expression. This study demonstrates the correlation between tumoral tissue leptin levels and tumor proliferative markers. However, serum adipokine levels were not investigated in this study. Although many experimental studies have shown the cell proliferation-promoting and apoptosis suppressive effects of leptin in cancer cells) [20,28], and although a significant correlation has been found between local leptin levels and tumor Ki67 and bcl-2 levels in many tumors [27] the absence of a study comparing serum adipokines with tumoral tissue proliferation markers was indicated as the most important weakness in these studies [28]. Because the studies have shown that serum adipokine levels are not a reflection of tissue adipokine levels [29]. Although serum leptin level increased in direct proportion to BMI in the study by Jeong et al. [28], they showed that adipokine levels in tumoral tissue were not correlated with BMI, and argued that local adipocyte tissue in tumoral tissue secretes adipokine locally and its participation in peripheral circulation is low. The results of our study support this hypothesis.

After adipokines are released from adipose tissue, they exhibit endocrine (being effective in all peripheral organs by participating in circulation), autocrine (being effective in the cell it is secreted) and paracrine (causing local effect in adjacent tissue) effects. For example, while it regulates energy metabolism with its endocrine effects, its paracrine effect is involved in wound healing. Our results support these mechanisms of action. Regulation of body weight is an endocrine function of leptin, in other words, the level of free-circulating leptin in serum determines this, and in line with this, serum leptin level increased as BMI increased in our study. However, there was no correlation between tumor Ki67 and bcl-2 level and serum adipokine levels, since tumor development may be related to local leptin level with paracrine effect. The most important weakness of our study is that we could not study serum adipokine levels and tissue adipokine levels simultaneously. However, our study is quite important in terms of being the first study in the literature investigating the correlation between serum adipokines and tumor mitotic and apoptotic markers in endometrial cancer to the best of our knowledge. Further studies investigating the relevant serum and tissue adipokines simultaneously in a larger sample size are needed.

Conclusion

Our study found that Ki67 expression determined by immunohistochemical examinations in patients with the diagnosis of endometrial cancer increased as tumor grade increased; however, there was no correlation between Ki67 expression and serum adipokine levels.

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