Comparison of IGF-1, IGFBP-3, and Estradiol Levels in Cyst Fluid and Serums of Postmenopausal Women with Benign and Malignant Tumor

Benign ve Malign Over Tümörlü Postmenopozal Kadınlarda Kist Sıvısı ve Serumda IGF-1, IGFBP-3 ve Estradiol Düzeylerinin Karşılaştırılması

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Abstract
Purpose: Ovarian cancer is the most significant cause of morbidity and mortality among gynecologic cancers. Recently, effects of sex steroids and IGF/IGFBP system, which are the most important risk factors of ovarian cancer, has been investigated. In the present study, we compared the IGF-1, IGFBP-3, and estradiol levels in cyst fluid and serums of postmenopausal women with benign and malignant ovarian tumor.
Materials and Methods: In total, 28 patients,12 patients with malignant ovarian tumor and 16 patients with benign ovarian tumor, were included in the study. In order to minimize the hormonal changes taking place during menstrual cycle, only postmenopausal patients were included in the present study. IGF-1, IGFBP-3, and estradiol levels were measured in cyst fluid and serums.
Results: Estradiol level in cyst fluid of patients with malignant ovarian tumor, was significantly high. IGF-1 and IGFBP-3 levels measured in cyst fluid and serums, did not show a significant difference between patients with benign and malignant ovarian tumors.
Conclusion: The present study supported the hypothesis proposing an association between estradiol and ovarian carcinogenesis. Further studies are required to understand the importance of microenvironment during tumor growth.

Key Words: **Estradiol; Insulin-Like Growth Factor-1; Insulin-Like Growth Factor B-3; Ovarian Cancers.**

Özet
Amaç: Over kanseri jinekolojik kanserler arasında morbidite ve mortalitenin en önemli nedenidir. Over kanserinin en önemli risk faktörleri olan sex steroidler ve IGF/IGFBP sisteminin etkileri son yıllarda araştırılmaktadır. Bu çalışma da benign ve malign over tümörlü postmenapozal kadınlarda, serum ve kist sıvısında IGF-1, IGFBP-3 ve estradiol düzeyleri karşılaştırıldı.
Gereç ve Yöntemler: Çalışmaya malign over tümörlü 12 hasta ve benign over tümörlü olan 16 hasta olmak üzere toplam 28 hasta katıldı. Menstrüel siklus boyunca meydana gelen hormonal değişiklikleri en aza indirgemek için sadece postmenopozal hastalar çalışmaya dahil edildi. Serum ve kist sıvısında IGF-1, IGFBP-3 ve estradiol düzeyi ölçüldü.
Bulgular: Malign over tümörlü hastaların kist sıvısında estradiol düzeyi anlamlı olarak yüksekti. Serum ve kist sıvısı IGF-1 ve IGFBP-3 düzeyi benign ve malign over tümörlü hastalarda anlamlı fark göstermedi.
Sonuç: Bu çalışma ile estradiol ile ovarian karsinogenezis arasındaki ilişkiyi savunan hipotez desteklenmektedir. Tümör büyümesinde mikroçevrenin önemini anlamak için gelecekteki çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: **İnsulin benzeri büyüme faktörü-1; İnsulin benzeri büyüme faktörü bağlayan protein-3; Estradiol; Over Kanseri.**

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Introduction

When reproductive and hormonal elements were determined to be risk factors for ovarian cancer, studies focused particularly on this field. Most of the ovarian tumors contain estrogen receptors. Estradiol has a mitogenic affect on ovarian surface epithelial cells and stimulate ovarian cancer cell line in vitro. IGF-1/IGFBP-3 are believed to be the major underlying reasons for this influence (1,2). Karasik et al., found higher IGF-1 and estradiol levels in malignant ovarian cyst fluids compared to those of benign cysts (3). Detection of significantly high IGF-1 level in cyst fluid of malignant epithelial ovarian cancer, indicates a possible causal relation between IGF-1 system and ovarian cancer. While IGFBP-3 is known to suppress the mitogenic effect of IGF-1 over cell growth (4), the exact role of IGFBP-3 on ovarian cancer is not known yet. There are studies in literature which indicate that IGFBP-3 may increase cell growth (5,6). In the present study, considering that most accurate information on microenvironment of ovarian tumors may be obtained through cyst fluid, we investigated IGF-1, IGFBP-3, and estradiol levels of plasma and cyst fluids in benign and malignant ovarian tumors, and studied the relations among those. Because IGFBP-3 level has not been investigated previously, this study will be a first in the literature.

Materials and Methods

Twenty-eight patients presented to the Gynecology and Obstetrics Clinic in Erciyes University Medical School between March 2006-March 2007 and diagnosed with postmenopausal pelvic mass, were included in the study. Patients with a serum FSH level below 40mIU/mL who have not experienced menstruation at least for a year, were evaluated as menopause cases (7). Patients were divided into 2 groups according to the results of the pathological examination as follows: benign (n=16 Group 1) and malignant (n=12, Group 2). Three patients determined to have borderline ovarian tumor as a result of the pathological analysis and included in the malignant group. Patients using drugs which could change the levels of hormones or growth factors, were not included in the study.

Sampling Process. Fasting serum samples were obtained on the morning of the operation day between 07.00-08.00. Cyst fluid was aspirated by a needle from the tumor which has been removed in sync with the oncological procedures. Following a centrifuge at 3000rpm in a nonheparinized tube for 15 minutes, stored in a refrigerator at -70°C. Cases with pelvic infection, non-ovarian malignity (tubal or endometrial cancer), and ovarian metastasis (colon cancer, thyroid cancer etc..) were not included in the study. Body mass indexes (BMI) of all the patients comprised in the present study, were measured. Mean age and BMI values of patients are shown in Table I.

IGF-1 and IGFBP-3 measurement. IGFBP-3 levels were measured in cyst fluid and serums by DSL-6600ACTIVE® IGFBP-3 Coated Tube IRMA kit (Diagnostics Systems Laboratories Inc., Webster, Texas) with 0.5ng/ml sensitivity, as described previously. First, IGF-1 was seperated from its binding proteins with acid-ethanol. Then, free IGF-1 was measured by DSL-5600ACTIVE® IGF IRMA (Diagnostics Systems Laboratories Inc., Webster, Texas) with 0.80ng/mL sensitivity as described previously.

Estradiol measurement. Estradiol concentration was measured by Automated Chemiluminescence System (ACS: 180, BAYER Corporation, NY, USA) (minimum measurable concentration: 10pg/mL [36.7pmol/L]).

Statistical Analysis. Measured parameters were stored in SPSS for Windows 13.0, a package computer program. Statistical analyses were carried out by this program, as well. The variables were tested for normal distribution. Kolmogorov-Smirnov method was employed for normalization of all the variables, and values were expressed as mean±standard deviation (S.D.) and median (minimum-maximum). Because the parameters didn't exhibit a normal distribution at the end of the study, Mann-Whitney U test was applied for comparison of differences between groups. $p<0.05$ was recognized as statistically significant.

Results

IGF-1 in serum and cyst fluid. No significant difference was determined between serum and cyst fluid IGF-1 levels in benign (Group 1) and malignant (Group 2) ovarian tumors. Whereas mean serum IGF-1 value was 71.76 ng/ml in Group 2, it was 104.19 ng/ml in Group 1 ($p>0.05$). Cyst fluid IGF-1 level was higher in Group 2 than Group 1 (Group 1: 25,21 ng/ml, Group 2 ; 58,41 ng/ml), however, the difference was not statistically significant ($p>0.05$).

IGFBP-3 levels in cyst fluid and serums. No significant difference was observed between the two groups regarding IGFBP-3 levels in cyst fluid and serums. However, serum

Table I. Histological types of ovarian tumors and median (min-max) values for the BMI and age of patients.

Histological Type	n	%	Age (Median) (Min-Max)	BMI (Median) (Min-Max)
Malignant Ovariant Tumors	12	42	58.00 (48-74)	32.00 (24-53)
Serous Carcinoma	4	14.3	53.50 (48-62)	32.50 (30-53)
Mucinous Carcinoma	2	7.1	62.00 (58-66)	31.50 (31-32)
Endometrioid Carcinoma	2	7.1	55.50 (54-57)	30.00 (24-36)
İndifferant Carcinoma	1	3.6	50	32
Borderline	3	10.7	73.00 (63-74)	31.00 (26-32)
Benign Ovarian Tumors	16	58	53.50 (47-68)	30.50 (25-40)
Serous Cystadenoma	10	35.7	52.50 (47-60)	28.00 (25-40)
Mucinous Cystadenoma	2	7.1	61.50 (55-68)	30.00 (28-32)
Cyst Adenofibroma	1	3.6	57	33
Functional Cyst	1	3.6	55	35
Sex Cord Stromal Tumors	2	7.1	53.00 (52-54)	35.50 (32-39)
TOTAL	28	100	54.50	32.00

IGFBP-3 was higher in Group 1 (3483 ng/ml) compared to that of Group 2 (2698ng/ml). IGFBP level in cyst fluid was found to be higher in Group 2 (1134ng/ml) than that of Group I (806ng/ml). However, no statistically significant difference was determined.

Estradiol in cyst fluid and serums. Cyst fluid estradiol level was found to be higher in malignant ovarian tumor (145.49 pg/ml) compared to that of benign Group (46.23pg/ml) ($p<0.01$). No significant difference was detected between the serum levels of the two groups (Group 1: 27.37pg/ml; Group 2: 27.47 pg/ml; $p<0.05$).

Mean, SD, median, and P values of IGF-1, IGFBP-3, and estradiol levels in cyst fluid and serums of benign and malignant ovarian tumor groups, are shown in Table II.

Table II. IGF-1, IGFBP-3, and estradiol levels in cyst fluid and serums of benign (group I) and malignant (Group II) ovarian tumors.

		GROUP 1 (n=16)		**GROUP 2 (n=12)**		
		Median (Min-Max)	$\overline{X} \pm sd$	Median (Min-Max)	$\overline{X} \pm sd$	P
Serum	IGF-1 (ng/ml)	92.9 (0,1-270)	104.1±86.1	41.3 (0.1-381)	71.7±103.4	>0.05
	IGFBP-3 (ng/ml)	3625 (1064-5202)	3483.7±1167.6	2017.5 (1402-4787)	2698.5±1261.1	>0.05
	Estradiol (pg/ml)	21.3 (0.0-99.3)	27.3±27.3	17.1 (0.0-72.2)	27.4±26.1	>0.05
Cyst Fluid	IGF-1 (ng/ml)	7.9 (0.01-126.8)	25.2±39.2	15.6 (0.1-396.7)	58.4±110.9	>0.05
	IGFBP- 3 (ng/ml)	557.5 (27-3122)	806.8±988.2	609.5 (47-3102)	1134.7±1071.6	>0.05
	Estradiol (pg/ml)	5.4 (0.0-432)	46.2±108.5	172.9 (0.0-256.8)	145.4±100.1	<0.05

Discussion

Studies investigating IGF/IGFBP system in ovary, have shown IGF and IGFBPs as playing a regulatory role on ovarian follicle develoment by their endocrine/paracrine effects (8). In IGF system ovarian carcinogenesis, two mechanisms, either IGF-1 receptor-related or estrogen receptor-related, may be responsible. When those cells are stimulated, they create the appropriate cancer microenvironment by exhibiting a mitogenic and anti-apoptotic influence over normal and neoplastic cells (9). IGF-1 has a high affinity for IGF receptor and estrogen receptor. Binding of IGF-1 to its receptor, is inhibited by IGFBPs, particularly IGFBP-3. IGF-1 is a strong negative regulator of SHBG which increases the bioavailability of estrogen in the environment (10,11).

IGFBP-3 is a spesific IGF-binding protein. It does not only regulate the IGF activity, but supresses the IGF-free cell proliferation and induces apoptosis. While high levels of IGFBP-3 in ovarian tumor cases, is associated with good prognosis, high levels of IGFBP-3 in breast cancer patients is associated with poor prognosis (12). These findings suggest that IGFBP-3 exhibits different effects on different cancer types.

IGFBP-3 has been reported to bind with IGFs and increase the bioavailability of IGFs by protecting peptide hormones from destruction in the literature. Serum IGFBP-3 level has been found to be lower in patients with ovarian cancer compared to those of healthy women (3,13). Although the suppressive effect of IGFBP-3 over mitogenic influence of IGF-1on cell growth is well known, the exact role of IGFBP-3 in ovarian cancer appears to be unclear. Several investigators have obtained results suggesting that IGFBP-3 might elevate cell growth (5,6).

Estradiol has been shown to boost the production and influence of IGF-1 in breast cancer (14,15). Similarly, estrogen has been shown to have a mitogenic effect on ovarian surface epithelium alongside increasing number of ovarian cells in vitro (16).

Majority of the ovarian tumors include estrogen receptor and most of them respond against estrogen by increasing the synthesis of progesterone receptors. Antiestrogens, remove those effects in normal and malignant cells and improve prognosis of ovarian cancer patients (17).

Karasik et al. found a higher estradiol level in malignant ovarian cancer cyst fluid, compared to that of benign group (3). Our study supported this result, as well. A study conducted by Jeppson et al. exhibited higher ovarian vein estradiol concentration in patients with ovarian cancer compared to that of benign group. In those patients, estradiol concentration in peripheral blood did not manifest a correlation with the cyst fluid (18). In our study, while estradiol level in cyst fluid was significantly high, serum estradiol level did not show a difference. Our study revealed the highest estradiol level in sex cord stromal tumor cyst fluid. Ovarian stroma is responsible of steroid synthesis, and in malignant tumors, due to tumor compression and elevated blood circulation, steroid synthesis increases (19). High IGF-1 levels in ovarian cyst fluids containing estradiol, suggest that estrogen might be showing its effects on those cancer cells via IGF/IGFBP. Same study conducted by Karasik et al. found IGF-1 level in cyst fluid of malignant ovarian tumor, significantly higher than that of benign tumor (3).In the present study, serum IGF-1 level was not compared. Studies of Shah et al. (20) and Waksmanski et al. (21), showed significantly lower IGF-1 levels in women with malignant ovarian tumor compared with those of the control group. A drop has been determined in IGF-1 level along with the progression of the disease. In the literature, serum IGFBP-3 level has been found to be low in patients with ovarian tumor, however, IGFBP-3 level in cyst level has not been studied. In our study, IGF-1 level in cyst fluid of malignant group was higher than that of benign group, but it was not statistically significant. IGFBP-3 levels did not exhibit a significant difference in ovarian tumors between serum and cyst fluid results. Histological differences between groups and the low number of test subjects, may be the underlying reason for that. Moreover, there were differences between the materials and methods used in the studies.

Because the participation of fat tissue in steroid synthesis by androgen aromatization in postmenopausal women is well known, BMI values were compared between the groups. In the present study, BMI values of the pateints did not show a difference level which could affect our results.

In conclusion, the proposition that estradiol could play a role in tumor growth, is supported. However, more comprehensive studies further investigating the IGF/IGFBP system, are required.

Kaynaklar

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