



Research

The Effect of WT-1 Positivity on Survival Outcomes in Patients with High-grade Serous Epithelial Ovarian Cancer

High-grade Seröz Epitelyal Over Kanserli Hastalarda WT-1 Pozitifliğinin Sağkalım Sonuçlarına Etkisi

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ABSTRACT

Objective: This study investigated the significance WT-1 positivity on survival outcomes in patients with high-grade serous epithelial ovarian cancer (HGSOc).

Methods: The medical records of the women who underwent surgery due to HGSOc between January 2015 and January 2020 were recorded. The patients' basic characteristic, serum tumor markers, disease stage, chemotherapeutic response, overall survival (OS), and progression-free survival (PFS) were recorded. WT-1 immune-staining was performed for all tissue samples.

Results: The median age of the patients in the WT-1 (+) group was 59 (40-88) years, and it was 52 (41-78) in the WT-1 (-) group that was significantly higher in the WT-1 (+) group ($p=0.007$). The median Ca125 level was significantly higher in the WT-1 (+) group than the WT-1 (-) group [634 (7-12,580) vs. 274 (12-2,856), $p=0.03$]. The PFS [22 (8-54) vs. 12 (0-48), $p<0.001$] and OS [30 (8-56) vs. 16 (2-70), $p<0.001$] rates were significantly higher in the WT-1 (-) group than the WT-1 (+) group in Cox regression analysis, Ca125 was significantly associated with OS in univariate analysis (HR:1, 95% confidence interval:1-1.001, $p=0.005$).

Conclusion: The patients with WT-1 positivity was associated with significantly shorter PFS and OS. Besides the patients in the WT-1 (+) group was older and had higher Ca125 levels than the WT-1 (-) group. The number of postmenopausal patients was higher in the WT-1 (+) group.

Keywords: Ovarian cancer, prognosis, survival, tumor marker, WT-1

ÖZ

Amaç: Bu çalışma, high-grade seröz epitelyal over kanserli (HGSOc) hastalarda WT-1 pozitifliğinin sağkalım sonuçlarına etkisini araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Ocak 2015-Ocak 2020 tarihleri arasında HGSOc nedeniyle ameliyat olan hastaların tıbbi kayıtları değerlendirildi. Hastaların temel özellikleri, serum tümör belirteçleri, hastalık evresi, kemoterapötik yanıt, genel sağkalım (OS) ve progresyonsuz sağkalım (PFS) parametreleri analiz edildi. Tüm doku örnekleri için WT-1 immün boyama yapıldı.

Bulgular: WT-1 (+) grubundaki hastaların ortalama yaşı 59 (40-88) yıl olarak saptandı. WT-1 (-) grubunda ortalama yaş 52 (41-78) olup, WT-1'de anlamlı olarak daha yüksekti. Medyan Ca125 düzeyi, WT-1 (+) grubunda WT-1 (-) grubuna [634 (7-12.580) ve 274 (12-2.856), $p=0,03$] göre anlamlı derecede yüksekti. PFS [22 (8-54) vs 12 (0-48), $p<0,001$] ve OS [30 (8-56) vs 16 (2-70), $p<0,001$] oranları diğer grupta anlamlı olarak daha yüksekti. WT-1 (-) grubu, WT-1 (+) grubuna göre Cox regresyon analizinde, Ca125, tek değişkenli analizde OS ile anlamlı şekilde ilişkiliydi (HR:1, %95 güven aralığı:1-1,001, $p=0,005$).

Sonuç: WT-1 pozitifliği olan hastalar, önemli ölçüde daha kısa PFS ve OS ile ilişkilendirildi. Ayrıca WT-1 (+) grubundaki hastalar WT-1 (-) grubuna göre daha yaşlı ve Ca125 düzeyleri daha yüksekti. WT-1 (+) grubunda postmenapozal hasta sayısı daha fazlaydı.

Anahtar Kelimeler: Over kanseri, prognoz, sağkalım, tümör belirteci, WT-1

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INTRODUCTION

Ovarian cancer (OC) is with a high mortality rate and accounts for the most common gynecological malignancy. The increased mortality depends on the lack of symptoms in the early stage of the disease and insufficient diagnostic tools. The reported overall survival (OS) is quite low at 29%-51% in advanced-stage disease. The higher rates of recurrences, residual disease, and chemoresistance also reduce the expected OS rates (1,2).

The *Wilms' tumor-1 (WT-1)* gene is a well-known tumor suppressor gene mainly associated with WT development (3). The expression of the *WT-1* gene is found in the kidney, ovary, testis, spleen, endothelial cells, and mesothelium (3,4). Regarding OC, *WT-1* expression is used to differentiate serous phenotypes from endometrial type (5,6). A moderate-to-strong expression of *WT-1* was reported in more than 50% of the tumor cells in 94.7% of patients with ovarian serous carcinoma (5-7).

Previous studies have evaluated the relation between the expression rate of *WT-1* and OS in various neoplasms (5,8). In a study by Miyoshi et al. (8), the high expression of *WT-1* was associated with a poor prognosis in patients with breast adenocarcinoma. However, to date, there is a lack of knowledge regarding the effect of *WT-1* positivity and survival rates in women with HGSOCS.

Therefore, this study evaluated the effect of preoperative *WT-1* positivity on the survival outcomes of the patients with HGSOCS.

METHODS

A retrospective cohort study was conducted with the medical records of 77 patients out of 205 serous OC patients who underwent surgery with *WT-1* immunostaining evaluation and confirmed diagnosis of HGSOCS between January 2015 and January 2020 in a tertiary referral hospital. After maintaining the Bakirkoy Dr. Sadi Konuk Training and Research Hospital Ethics Committee approval (decision no: 2021-03-02, date: 15.02.2021), Helsinki guidelines and ethical considerations on human studies were maintained. Informed consent was not obtained since the medical record of the women were used anonymously.

The data of women who had a pathological diagnosis of HGSOCS with a regular clinical follow-up and *WT-1* immunostaining results were included. Any women with an active infection, hematologic, liver or kidney neoplasms, and autoimmune disorders were excluded.

The basic characteristic such as age, body mass index (BMI), menopausal status, Ca125 levels, FIGO stage, the presence of residual disease, number of surgically removed pelvic and para-aortic lymph nodes, response to chemotherapy, time interval of follow-up, the length of hospitalization was recorded. The last recruited patient record was obtained in November 2020. The patients had a routine gynecological examination, computed tomography scan, and Ca-125 evaluation every 2-3 months in the first two years and then every 4-6 months. R0 was defined as no residual tumor on the main evaluation after the operation. Chemosensitivity was determined as the time interval between terminating the last chemotherapy dose and the presence of recurrence after more than 6 months. OS was defined as the time between treatment initiation till to death or the last follow-up. The term progression-free survival (PFS) was used for the treatment initiation time until the date when recurrence or progression consisted.

WT-1 Analysis

Formalin-fixed paraffin-embedded tissues were sliced at a thickness of 4 microns. Predilute ready-to-use (Cell Marque, 6F-H2, USA, Lot V0002192) primary antibodies were used, and immunohistochemical stainings were performed in a Ventana BenchMark XT (Roche, Mannheim, Germany) instrument following protocols proposed by the manufacturer (Figure 1-3).

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Descriptive data were presented as mean, standard deviation, median (minimum-maximum), number, and percentages. The Shapiro-Wilk test was used to evaluate the distribution of the continuous data. Student t-test or Mann-Whitney U tests were used to evaluate the quantitative data. The chi-square test or Fisher's Exact test was used to examine qualitative data.

Kaplan-Meier test was used to evaluate OS and PFS rates. Univariate and multivariate Cox regression model was used to define the prognostic factors that could affect OS. The potential factors that could affect OS, such as age, FIGO tumor stage, the presence of residual disease, chemotherapeutic response, Ca-125, *WT-1* positivity, menopausal status, were used in regression analysis. A p-value of <0.05 was considered statistically significant.

RESULTS

The median age of the patients in the *WT-1* (+) group was 59 (40-88) years, and it was 52 (41-78) in the *WT-1* (-) group that was significantly higher in the *WT-1* (+) group ($p=0.007$).

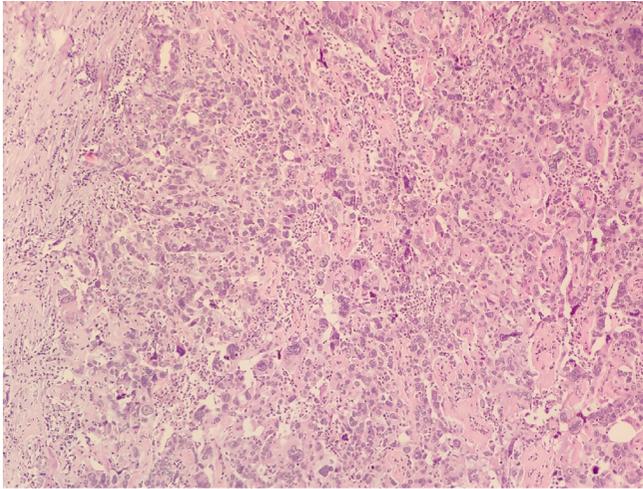


Figure 1. The picture shows atypical tumor cells with pleomorphic vesicular nuclei (H&Ex200)

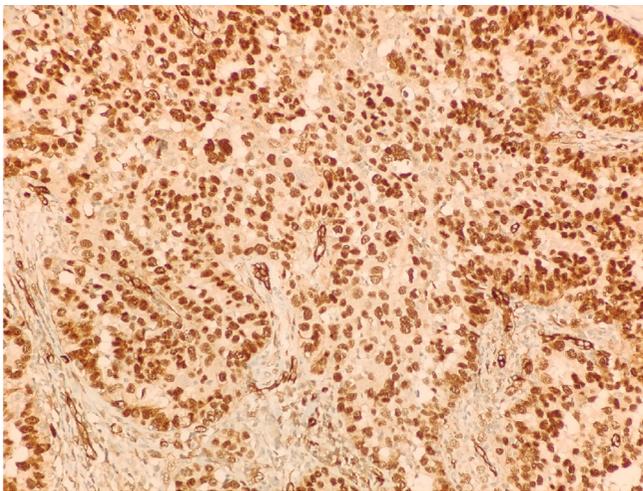


Figure 2. High nuclear positive staining with WT-1 in tumor cells (WT-1 immune-staining x200)
WT: Wilms' tumor

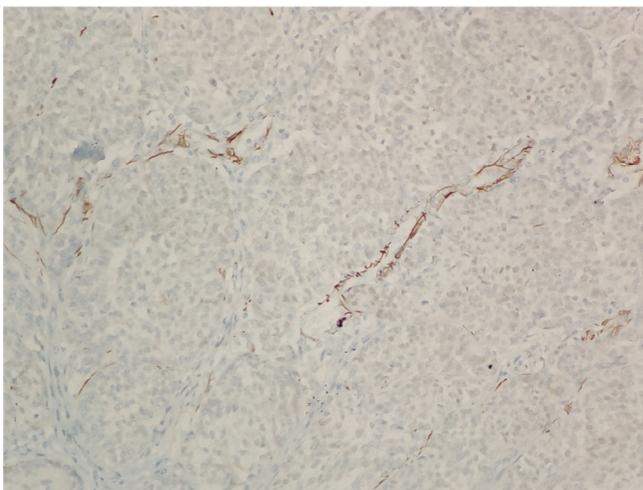


Figure 3. Negative staining with WT-1 in tumor cells (WT-1 immune-staining x200)
WT: Wilms' tumor

No significant differences were observed between the study groups regarding BMI, number of pelvic and para-aortic lymph nodes, and hospital stay length ($p > 0.05$, for all comparisons). The median Ca125 level was significantly higher in the WT-1 (+) group than the WT-1 (-) group [634 (7-12,580) vs. 274 (12-2,856), $p = 0.03$]. The PFS [22 (8-54) vs. 12 (0-48), $p < 0.001$] and OS [30 (8-56) vs. 16 (2-70), $p < 0.001$] rates were significantly higher in the WT-1 (-) group than the WT-1 (+) group (Table 1).

There was no significant difference between the study groups regarding FIGO stage, chemosensitivity, the presence of metastatic pelvic para-aortic lymph nodes, recurrence, residual disease ($p > 0.05$, for all comparisons). The number of postmenopausal patients was significantly higher in the WT-1 (+) group than the WT-1 (-) group [43 (89.6%) vs. 18 (62.1%), $p = 0.004$] (Table 2). There were six deaths in the WT-1 (+) group, and no death was observed in the WT-1 (-) group. In Cox regression analysis, Ca125 was significantly related to univariate analysis (HR:1, 95% confidence interval:1-1.001, $p = 0.005$). However, no significance was observed in multivariate analysis for the Ca125 level. The HR was 71.6 for WT-1 positivity considering OS. However, this was not reached statistical significance. Besides, no significance was observed regarding age, FIGO stage, residual disease, chemotherapy response, menopausal status in univariate Cox regression analysis (Table 3).

DISCUSSION

The patients with WT-1 positivity were associated with significantly shorter PFS and OS. The patients in the WT-1 (+) group were older and had higher Ca125 levels than the WT-1 (-) group. The number of postmenopausal patients was higher in the WT-1 (+) group.

Since OC remains one of the prevalent gynecological malignancies with higher mortality rates, recent studies have focused on a noninvasive marker to aid early cancer diagnosis (9). In this context, it was reported that WT-1 expression could be characteristic for the differential diagnosis of serous OCs. Its expression is much lesser in other OC subtypes (9). Al-Hussaini et al. (10) reported a 94.7% of WT-1 expression in serous OCs. They concluded that WT-1 positivity could be associated with poorly differentiated ovarian neoplasm morphology.

The presence of WT-1 was investigated to differentiate it from the tumors with adenocarcinoma morphology arising at other sites (5-7). Şakirahmet Şen et al. (11) conducted a study to evaluate the benefits of WT-1 antigen. The authors concluded that the ovarian adenocarcinoma slides were positively stained with WT-1 in 82.5% of the patients

(11). In another study, Hylander et al. (12) reported that WT-1 could significantly predict the prognosis of ovarian adenocarcinoma. Moreover, they concluded that WT-1 expression could be correlated with tumor grade and tumor stage, but they found no relationship with the survival (12).

In previous studies WT-1 was significantly associated serous OCs, especially in high-grade cancers sensitivity varying

between 64-96% (13-17). Moreover, it was reported that serous borderline ovarian tumors related to early-stage OCs were negative or weak expression of WT-1 (10). In our study, shorter PFS and OS rates were observed in patients WT-1 positivity. We may speculate that WT-1 could be presented in high-grade tumors due to increased affinity to endothelial tissue since positive staining of WT-1 was noted in endothelial cells (5).

Table 1. Comparison of the study groups regarding basic characteristic and survival rates

	WT-1 negative (n=29)	WT-1 positive (n=48)	p-value
Age (years)	52 (41-78)	59 (40-88)	0.007
BMI (kg/m ²)	30.7 (16-49)	32.5 (23-53)	0.63
Menopause	No	5 (10.4%)	0.004
	Yes	43 (89.6%)	
Preoperative Ca125 U/mL	274 (12-2,856)	634 (7-12,580)	0.03
Number of pelvic lymph node	17 (0-35)	14 (0-38)	0.65
Number of paraaortic lymph nodes	17 (0-35)	16 (0-46)	0.27
PFS (months)	22 (8-54)	12 (0-48)	<0.001
OS (months)	30 (8-56)	16 (2-70)	<0.001
Mean follow-up (months)	24 (8-56)	16 (2-70)	0.001
Hospitalization (days)	10 (7-18)	10.5 (3-30)	0.18

BMI: Body mass index, PFS: Progression-free survival, OS: Overall survival, WT: Wilms' tumor

Table 2. Comparison of the study groups regarding surgical outcomes and response to the treatment

	WT-1 negative (n=29)	WT-1 positive (n=48)	p-value
FIGO stage	I	6 (20.7%)	0.17
	II	3 (10.3%)	
	III	19 (65.5%)	
	IV	1 (3.4%)	
Chemotherapeutic response	Sensitive	29 (100%)	0.08
	Resistant	0 (0)	
The presence of metastatic pelvic lymph node	No	20 (69%)	0.71
	Yes	9 (31%)	
The presence of metastatic paraaortic lymph node	No	19 (65.5%)	0.71
	Yes	10 (34.5%)	
Recurrence	No	18 (62.1%)	0.68
	Yes	11 (37.9%)	
Residual disease	No	24 (82.8%)	0.39
	Yes	5 (17.2%)	
Mortality	No	29 (100%)	0.052
	Yes	0 (0)	

WT: Wilms' tumor

Table 3. Cox regression analysis considering the variables that could affect on OS

	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age (years)	1.02	0.94-1.09	0.69	-	-	-
FIGO stage	2.6	0.45-14.83	0.28	-	-	-
WT-1 positivity	71.6	0.09-533	0.21	-	-	-
Residual disease	0.03	0.1-62	0.51	-	-	-
Chemotherapeutic response	2.7	0.31-23.21	0.36	-	-	-
Menopause	-	-	-	-	-	-
Preoperative Ca125 U/mL	1	1-1.001	0.005	1	1-1.001	0.13

OS: Overall survival, HR: Hazard ratio, CI: Confidence interval, WT: Wilms' tumor

Study Limitations

The limitations of our study can be considered its retrospective design and the small number of patients. Besides, we could not report the expression percentage of WT-1. Our study presented the results of a single oncology center, and thus further multicenter studies could confirm our results. However, this is the first study that has evaluated the relation between WT-1 positivity and survival rates in patients with HGSOc.

CONCLUSION

In conclusion, the patients with WT-1 positivity were with significantly shorter PFS and OS. Besides, the patients in the WT-1 (+) group were older and had higher Ca125 levels. In regression analysis only Ca125 was associated with OS.

ETHICS

Ethics Committee Approval: Approval for this study was obtained from the Clinical Research Ethics Committee of Bakırköy Dr. Sadi Konuk Training and Research Hospital (decision no: 2021-03-02, date: 15.02.2021).

Informed Consent: Informed consent was not obtained since the medical record of the women were used anonymously.

Authorship Contributions

Surgical and Medical Practices: S.K., S.A., Concept: S.K., M.E., Design: S.K., Data Collection or Processing: S.A., Analysis or Interpretation: S.A., Literature Search: A.F.T., G.Ö.Y., Writing: S.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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