ARAŞTIRMA / RESEARCH

Clinicopathological importance of survivin immunoexpression in reproductive age patients with serous borderline ovarian tumors

Seröz borderline over tümörleri olan reprodüktif çağdaki hastalarda survivin immünoekspresyonunun klinikopatolojik önemi

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Abstract

Purpose: The aim of the present study was to evaluate survivin immunoexpression and the clinicopathologic correlations in serous borderline ovarian tumors (BOT) patients ≤ 40 years old.

Materials and Methods: A total of 36 BOT cases were evaluated. After excluding the mucinous type and patients over 40 years of age, 20 serous BOT cases meeting the criteria were included. A similar number of benign ovarian cysts and 20 serous malign ovarian tumors were randomly included as the control group. The patients were then staged based on their surgical findings and 2014 FIGO criteria, and their histological tumor types obtained from pathological specimens were determined using WHO criteria. The survivin levels in the specimens were analyzed using immunohistochemical assays.

Results: Positive survivin expression was detected in 10% of benign tumors, 100% of serous BOTs, and 100% of serous malign ovarian tumors. Mean survivin immunoreactivity was statistically similar between groups and it was found to be significantly higher in both groups compared to the control group. Survivin expression was positively correlated with FIGO stage, tumor grade, microinvasion, and micropapillary pattern.

Conclusion: Survivin immunoexpression is correlated with the malignancy potential of serous BOTs, and that survivin immune expression may be a histopathological marker that will help in making a decision on fertility-sparing surgery and follow upin young patients.

Keywords: Serous borderline ovarian tumors, BOT, survivin, fertility-sparing surgery.

Öz

Amaç: Bu çalışmanın amacı, 40 yaş ve altındaki seröz borderline over tümörü (BOT) olan hastalarda survivin immünoekspresyonunu ve klinikopatolojik korelasyonları değerlendirmektir.

Gereç ve Yöntem: Toplam 36 BOT vakası değerlendirildi. Musinöz tip ve 40 yaş üzeri hastalar dışlandıktan sonra kriterleri karşılayan 20 seröz BOT vakası dahil edildi. 20 benign over kisti ile 20 seröz malign over tümörü kontrol grubu olarak rastgele dahil edildi. Hastalar daha sonra cerrahi bulguları ve 2014 FIGO kriterlerine göre evrelendirildi ve patolojik örneklerden elde edilen histolojik tümör tipleri WHO kriterlerine göre belirlendi. Örneklerdeki survivin seviyeleri, immünohistokimyasal testler kullanılarak analiz edildi.

Bulgular: Pozitif survivin ekspresyonu, iyi huylu tümörlerin %10'unda, seröz BOT'ların %100'ünde ve seröz malign over tümörlerinin %100'ünde tespit edildi. Ortalama survivin immünreaktivitesi açısından gruplar arasında istatistiksel olarak fark yoktu, her iki grupta da kontrol grubuna göre anlamlı derecede yüksek olarak saptandı. Survivin ekspresyonu, seröz BOT grubunda FIGO evresi, tümör derecesi, mikroinvazyon ve mikropapiller patern ile pozitif korelasyon gösterdi.

Sonuç: Survivin immün ekspresyonunun seröz BOT'ların malignite potansiyeli ile korele olduğunu ve survivin immün ekspresyonunun genç hastalarda fertilite koruyucu cerrahiye karar vermede ve takipte yardımcı olacak histopatolojik bir belirteç olabileceğini düşündürmektedir.

Anahtar kelimeler: Seröz borderline over tümörleri, BOT, survivin, fertilite koruyucu cerrahi

INTRODUCTION

Borderline ovarian tumors (BOTs) are a type of noninvasive neoplasm characterized by atypical epithelial proliferation, abnormal appearance of cell nuclei or an increased of mitotic activity less than invasive cancerous tumors. Destructive stromal invasion has not been observed with BOTs[1]. BOTs is responsible from about 20% of all epithelial ovarian cancers, which affect 1.8–4.8/100,000 women each year[2]. As with any invasive ovarian cancer, the standard criterion for deciding on the surgical treatment remains staging the tumor using specific categories. Because most BOTs are diagnosed in their early stages, the prognosis for these patients is favorable; however, even BOTs diagnosed in their advanced stages can have a positive patient prognosis[3]. Approximately one-third of those diagnosed with BOTs are <40 years old, which makes management of the condition more complex; clinicians treating these young women must consider approaches that would enable ovarian function and fertility[4]. Fertility-sparing approaches are important considerations for these young BOT patients because of their being at childbearing age and because early detection offers a favorable prognosis[5].

Survivin is a type of protein that inhibits caspases and apoptosis and is highly expressed in most cancers. Although survivin is undetectable in normal adult tissues, it is highly expressed in several types of cancer. Because it is an apoptosis inhibitor (IAP), survivin has a great potential for malignancy transformation and tumor growth[6]. Highly expressed survivin has been found in a large range of cancers and has been observed to be an indicator of a lower survival rate, especially in those with ovarian cancer[7]; therefore, this protein is taken into account a clinicopathological and prognostic marker for several malignant tumors. Fertility-sparing approaches are important considerations for young BOT patients. Hence, the aim of the present study was to evaluate the immunoexpression and clinical significances of survivin in serous BOT patient's ≤40 years old.

MATERIALS AND METHODS

The present study was conducted at a tertiary Medicine Faculty Hospital and ratified by the local Ethics Committees (approval no. 2019/108) and was conducted in accordance with the Declaration of Helsinki. The data used in this study were obtained from hospital database between January 2010 and January 2020. Demographic features of the patients, type of operation, histological subtype, and stages of cancer according to International Federation of Gynecology and Obstetrics (FIGO) were evaluated.

Study population

A total of 36 BOTs were evaluated between January 2010 and January 2020 in the initial analyses and patients with mucinous type and over 40 years of age were excluded, and 20 patients with serous patterns were included in the final analysis. Flow chart of patients was illustrated in Fig. 1. In the current study, we evaluated serous BOTs in young patients because it is the most common histological type in these women and has a higher malignancy potential than mucinous tumors. A similar number of benign ovarian cysts and 20 serous malign ovarian tumors were randomly included as the control group. The patients were then staged based on their surgical findings and 2014 FIGO criteria, and their histological tumor types obtained from pathological specimens that were assessed by two qualified pathologists were determined using WHO criteria. Micropapillary lesions were defined in the serous BOTs that showed complex micropapillary structures and demonstrated a filigree pattern. Microinvasion is stromal invasion that is restricted to an area ≤10 mm². The treatment, including the surgical protocols, was chosen according to the opinions of experienced gynecologic oncologists and the age of patients, extent of the tumor, desire for child, and time of identification. In the current study, we selected two types of surgery 1) fertility-sparing surgeries (for protection the uterus and one ovary), 2) radical surgery (peritoneal lavage for cytology, total abdominal hysterectomy and bilateral salpingooophorectomy with or without the excision of lymph nodes, some abdominal biopsies, and removal of the omentum). One of the following three fertility-sparing surgery modalities was performed: cystectomy, unilateral salpingo-oophorectomy, and unilateral salpingo-oophorectomy + contralateral cystectomy.

Immunohistochemistry

The patient's biopsy specimens were retrieved from the pathology archives. The patients' demographic data and histological tumor characteristics were retrieved from the original pathology reports. The samples were reevaluated by the same experienced pathologist for confirmation the pathological

diagnosis of the specimens. Ten percent buffered formalin was utilized to fix the tissues, which was then embedded in paraffin. One specimen block tissue that was embedded in paraffin was usedfor each patient. These block tissues were cut into 4-μmsectionsand purified from the paraffin, rehydrated and observed usingtarget-retrieval solution. Endogenous peroxidase activity was inhibited using3% H_2O_2, and 10% goat serum was used to block nonspecific immunoglobulin binding in phosphate-buffered saline (PBS). Primary rabbit polyclonal anti-survivin antibody at a ratio of 1:300

was used to incubate the sections, after which the slides were washed with PBS and incubated with secondary antibodies and 3,3'-diaminobenzidine (DAB) (ROCHE ultra-View Universal DAB Detection Kit Catalog Number:760-500). The sections were counter stained with hematoxylin. Each sample was determined independently by one pathologist using polarized light microscopy. For analyses, the sections with the highest rate of stained tumor cells, scored on a scale of 0–3 (i.e., 0 being weakest; 3 being strongest), were used.
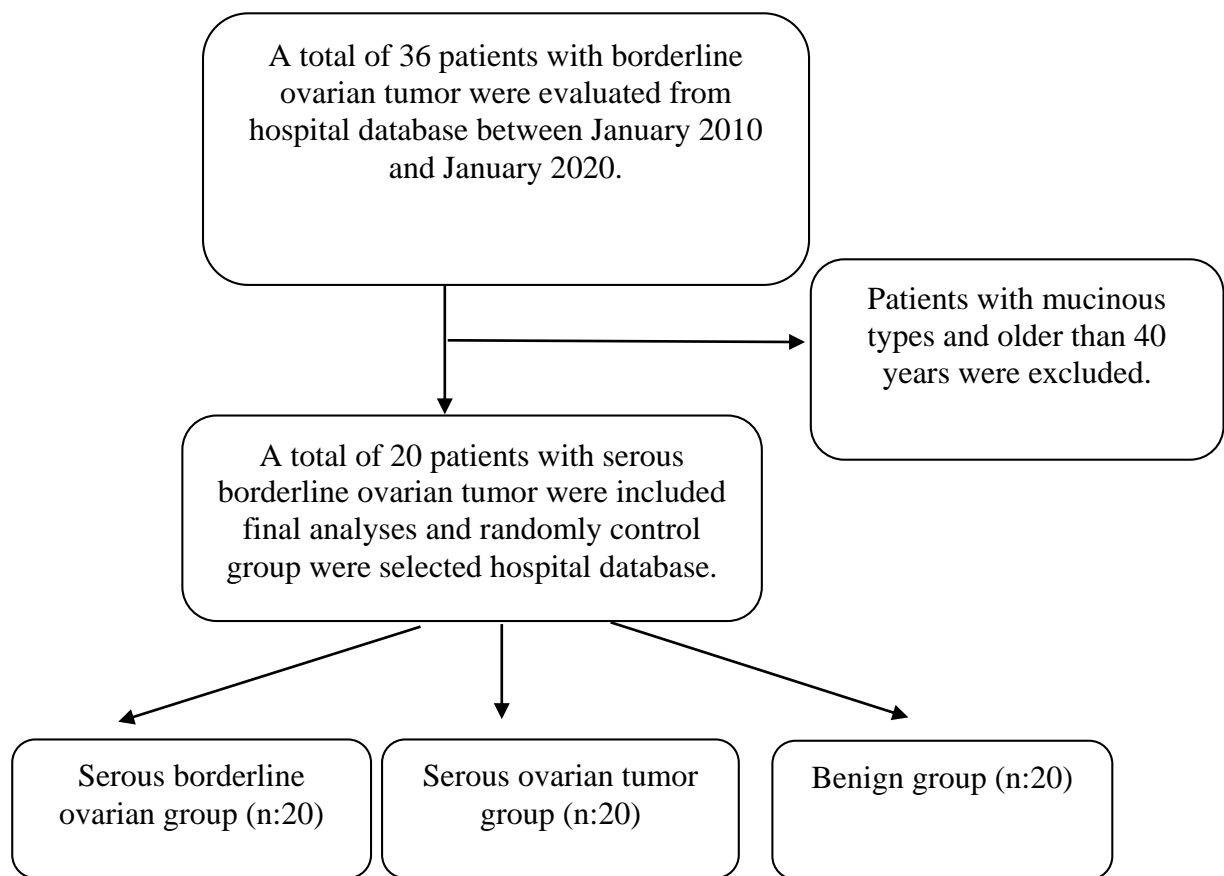

Figure 1. Patients flow chart.

Statistical analysis

All study statistics were done with SPSS ver. 22 (IBM Corp., Armonk, NY, USA). To test the assumption of homogeneity of variance, the Levene's test was performed. To determine the normality of the data,

the Kruskal Wallis-H Test was performed. Because the present study has three groups, One-Way ANOVA test (Tukey's post-hoc test) was used to compare the study groups. P value <0.05 was considered statistically significant. The values are expressed as the mean ± standard deviation or n (%).

Pearson correlation test was used to analyze the correlation between Survivin expression levels and clinicopathologic paramaters.

RESULTS

After the final analysis, 20 patients with benign, 20 with serous BOTs, and 20 with serous malign ovarian tumors were enrolled in the study. The demographic characteristics of the patients with serous BOTs are provided in Table 1.

The distribution of survivin immunoreactivity according to staining among the groups is provided in Table 2. The immunohistochemical survivin staining is illustrated in Fig. 2. Positive survivin expression was detected in 10% (2/20) of benign tumors, 100% (20/20) of serous BOTs, and 100% (20/20) of serous malign ovarian tumors (P<0.001). Mean survivin immunoreactivity was +3 (2-3) in serous BOTs and +3 (3-3) in serous malign ovarian tumors and statistically similar between groups (p=0.780).

Table 1. Demographic characteristics and surgery modalities of patients with serous borderline ovarian tumors (BOTs).

Patient	20
Age (year)	31.3±4,8
Nulliparity (n%)	7 (35)
Body mass index (kg/m²)	27.5±3,8
Etnicity (Caucasian)	20 (100)
Previous cesarean history	6 (30)
Smoking	8 (40)
Surgery modalities	
Fertility-sparing surgery	17 (85)
cystectomy	3 (17,6)
unilateral salpingo-oophorectomy	11 (64,6)
unilateral salpingo-oophorectomy + contralateral cystectomy	3 (17,6)
Radical surgery	3 (15)
Pathological characteristics	
FIGOstage IA	14 (70)
Micropapillary lesions	8 (40)
Microinvasion lesions	2 (10)
Unilateral BOTs	11 (55)
Pre-operative CA125 >35 UI/I	17 (85)

Note: Values are presented as the mean ± SD, or n (%).

Table 2. Distribution of survivin immunoreactivity according to staining and specimen number among the groups.

Survivin Reactivity	Benign (n = 20)	Serous BorderlineTumor (n = 20)	MalignOvarian Tumor (n = 20)	P value*
Intensity				
0	18	0	0	
+1	2	2	2	
+2	0	4	3	
+3	0	14	15	
Mean immunoreactivity	0 (0-1)^A	3 (2-3)^B	3 (3-3)^B	<0.001

Notes: The general staining intensities were used in the calculations (0: negative; 1: slight dyeing; 2: moderate dyeing; 3: strong dyeing).Values were expressed as median and percentiles (25-75).
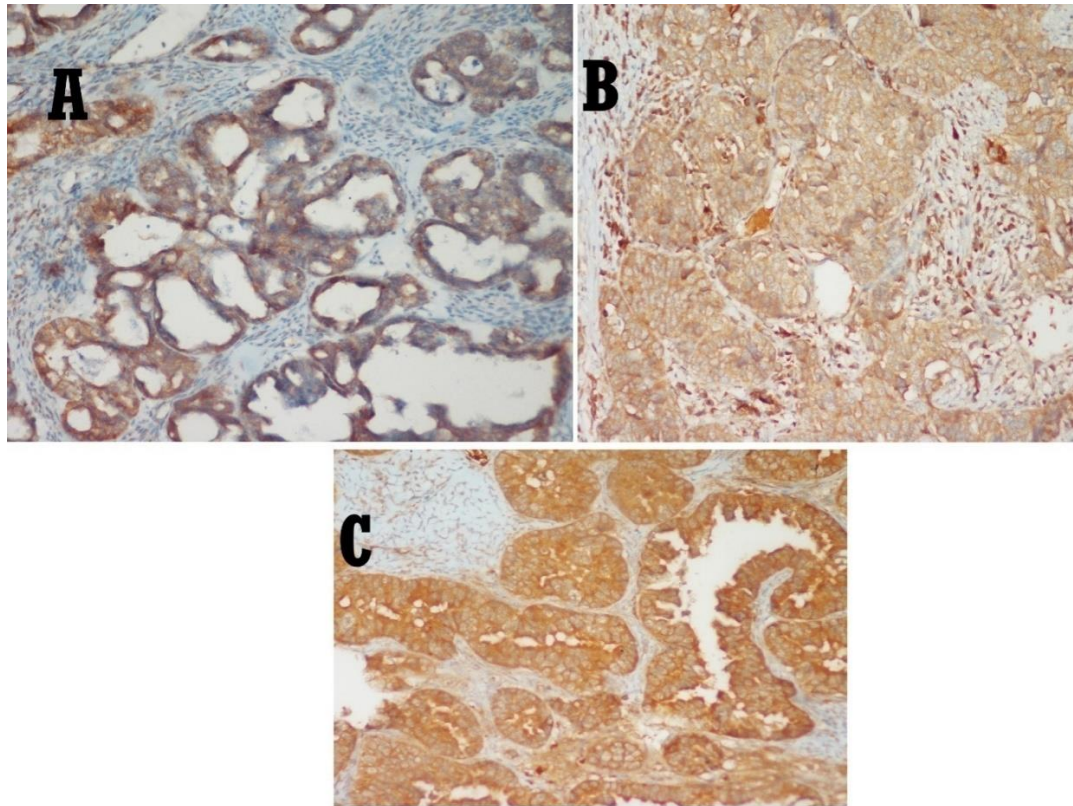* Statistical analyses was made by ANOVA test. ^A vs ^B - significant

Figure 2. Survivin immunostaining A) slight B) medium C) strong immunostaining in serous ovarian cancer

Among the cases, 17 (85%) had undergone fertility-sparing surgery, comprising 3 cystectomies, 11 unilateral salpingo-oophorectomies, and 3 unilateral salpingo-oophorectomies + contralateral cystectomies, while 3 (15%) receiving radical surgeries. Radical surgery was performed at the request of patients who had no desire for fertility, completed the number of children, and were found to have bilateral serous BOT. The majority (88.2%, 15/17) in this group had a stage I FIGO score, which included 93.3% (14/15) in stage IA. Very few cases (11.8%, 2/17) were in stage II. Among these patients, 40% (8/20) had micropapillary lesions and 8% (2/20) had microinvasion lesions. Notably, unilateral BOTs were more common (55% [11] than bilateral BOTs 45% [9]). The level of pre-operative cancer antigen (CA125) >35 UI/I was observed in 17/20 (85%) patients with BOTs. Survivin expression was positively correlated with FIGO stage (r=0.42, P=0.010), tumor grade (r=0.48, P=0.013), microinvasion (r=0.52, P<0.001), and micropapillary pattern (r=0.48, P<0.001) but not with pre-operative CA125 levels (r=0.21, P=0.540).

DISCUSSION

Fertility-sparing approaches are important considerations for young BOT patients. Young women frequently have BOT; however, these are usually related to a favorable prognosis. According to the recent studies, the 5 year survival rates of BOT patients were found as 95% and the 10 year survival rates was 93%; however, BOT patients had about 8% recurrence rate and about 2% these advance to ovarian cancer [8,9]. Highly expressed survivin has been found in a large range of cancers and has been observed to be an indicator of a lower survival rate, especially in those with ovarian cancer[7]; therefore, this protein is taken into account a clinicopathological and prognostic marker for several malignant tumors. In the present study we aimed was to evaluate the immunoexpression and clinical

significances of survivin in serous BOT patient's ≤ 40 years old.

Survivin is not detectable in normal adult tissues, while highly expressed in some types of cancer, and has the potential to be involved in malignancy transformation and tumor growth[6]. The importance of survivin immunoexpression and its correlation with clinicopathological variables in malignant ovarian tumors are well documented in the literature[10-12]. Turan et al.[10] have reported a significant and linear correlation between the potential malignancy of a tumor and survivin expression levels, and observed that expression of survivin is positively correlated with advanced tumor grade and stage. According to recent studies, it has a potential role in the progression of serous ovarian malign neoplasms and is significantly correlated with tumor grade in some genital malignancy[11]. In their recent meta-analysis, Xiaoyan et al.[12] have reported that survivin over expression is closely associated with FIGO stage in ovarian carcinoma and tumor grade but is not significantly related to lymphatic metastasis or ascites. Survivin immunoexpression and clinicopathologic correlations in malignant ovarian tumors, but the role of survivin and its clinical significance in young patients with BOTs have not been fully investigated. In the present study we evaluated serous BOTs because of it is the most common histological type and has a higher malignant potential than mucinous tumors in young patients. In the current study, opposite to the literature, we found that survivin immunoreactivity in BOT group as higher as serous malign ovarian tumors group. The rate of borderline ovarian tumors staining positively with survivin is around 60% in the literature, but we found 100% positive staining with survivin in the serous BOT group (stage 1A tumors constitute the majority of group) in this study. This finding indicates that serous BOT's have a high malignancy potential. It is known that histological type (i.e., serous), the existence of invasive implants, improved FIGO stage, the existence of papillary lesions, residual disorder, the existence of bilateral tumors, and the existence of stromal invasion have typically been related to a adverse prognosis in BOT patients. Several studies have presented that advanced FIGO stages are usually accompanied by increased cancer recurrence rates. In addition, the 5-year survival of BOT patients in stage I is about 97%, while that in stages II–III was only 65–87%[13]. Other studies have reported that micropapillary pattern is an independent prognostic factor for patients with BOTs[14,15]. In the present

study, we observed that survivin immunoexpression was positively correlated with FIGO stage, tumor grade, microinvasion, and micropapillary pattern but not with pre-operative CA125 levels. We can explain our results using the role of survivin as an apoptosis inhibitor. Some studies have demonstrated that survivin has a fundamental role in specifically inhibiting caspases 3 and 7, the terminal effector proteases in the apoptotic pathway[16,17].

Our study had some clinical strengths and limitations. Plett et al.[18] have reported that fertility-sparing surgery for patients in stage I is a safe procedure, and that delivery rates after the surgery are high; however, they suggest that more advanced FIGO stages should be discussed with each patient and the rates of recurrence should be weighed against the decision for fertility-sparing surgery. Additionally, it is well documented that clinicopathological features, was an independent predictor for recurrence of the disease [19-21]. In the present study consistent to literature, we observed that survivin expression was correlated with clinicopathological features in serous BOT patients.

Secondly, Bendifallah et al.[21] have described a nomogram, which is aimed at predicting postoperative recurrences in BOT patients. In that study, a multivariate logistic regression analysis of selected prognostic features was conducted, and a nomogram was constructed to estimate disease recurrence. The constructed nomogram was confirmed using FIGO stage, histologic subtype, and age at diagnosis, type of surgery, each of whichis related to an increased risk of recurrence and is included in the nomogram. These nomograms can create individualized predictions; therefore, they can be used to plan treatments and provide more complete information to patients about their therapeutic options. Thus, patients can be more involved in the therapeutic decision-making process, which could develop their compliance with any treatment. Additional studies can add survivin immunoexpression to the nomograms, which would provide histopathological informative on tumor aggressiveness.

Some of the study limitations were, first, the small sample size and its retrospective nature, which cannot exclude bias. Second, any staging modality modifications and modifications of surgical techniques (fertility-sparing surgery or laparoscopy) that happened during the data-collection period could also result in bias. Third, because of the high

rate of late recurrences, a follow up period of >5 years is crucial to appropriately appraise the patients. In the present study, the 5-year follow up period was not completed for ~30% of the patients with serous BOTs for whom fertility-sparing surgery was preferred, which limited the determination of any 5-year recurrence.

Our results suggest that survivin immunoexpression is correlated with the malignancy potential of serous BOTs, and that survivin immunoexpression may be a histopathological marker that will help in making a decision on fertility-sparing surgery and follow up in young patients.

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