RESEARCH ARTICLE

Parameters for Predicting Granulosa Cell Tumor of the Ovary: A Single Center Retrospective Comparative Study

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Abstract

Background: To evaluate factors for predicting the granulosa cell tumor of the ovary (GCTO) pre-operatively. Materials and Methods: This retrospective designed study was conducted on 34 women with GCTO as the study group and 76 women with benign ovarian cysts as the control group. Data were recorded from the hospital database and included age, body mass index (BMI), parity, serum estradiol (E_2) levels, diameter of the mass, ultrasonographic features, serum CA125 level, risk of malignancy index (RMI), duration of menopause, postoperative histopathology result, and the neutrophil/lymphocyte ratio (NLR). Results: The demographic parameters showed no statistically significant difference between the groups. Preoperative diameter of the mass, CA125, duration of menopause, and neutrophil/lymphocyte ratio were significantly different between the groups. ROC curve analysis demonstrated that diameter of the mass, serum estradiol and Ca125 levels, RMI and NLR may be discriminative factors in predicting GCTO preoperatively. Conclusions: In conclusion, we think that a careful preoperative workshop including diameter of the mass, serum estradiol (E_2) and Ca125 levels, RMI and NLR may predict GCTO and may prevent incomplete approaches.

Keywords: Granulosa cell tumor - ovary - risk factors - prediction - E2 and Ca125 - RMI and NLR

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Introduction

Granulosa cells are the somatic cells of the sex cords of the ovary. They are closely associated with the developing oocyte. The major functions of granulosa cells include the production of sex steroids and various peptides required for folliculogenesis and ovulation. Granulosa cell tumor of the ovary (GCTO) is a rare type of ovarian cancer with an incidence of 2-5% of ovarian neoplasms (Schumer et al., 2003). The diagnosis is generally made at early stages of the disease, because patients often have features of hyperestrogenism. GCTO is generally associated with a favorable prognosis, although they are much less chemosensitive, and tumor growth is slower (Bilici et al., 2014). There are two different clinical and histopathological types of GCTO including juvenile and adult type. The adult type forms more than 95% of all GCTO and is usually seen in perimenopausal and postmenopausal women with a peak incidence between 50-55 years (Kottarathil et al., 2013).

Previous studies reported various markers to predict the malignant potential of ovarian masses such as; BRCA1 protein immunohistochemical expression (Shawky et al., 2014), overexpression of TRPM7 (Wang et al., 2014), and ATAD2 (Wan et al., 2014). RMI was first defined by Jacobs et al. (1990) and defined as the multiplied score of "U" represents the ultrasonographic index; "M" is menopausal status, and serum CA125 levels. In a study RMI with a cut-off 150 was found to have a sensitivity of 84% and specificity of 97% in detecting ovarian cancer (Arun-Muthuvel et al., 2014). NLR is a new and easy calculated marker that has been used in determining various cancers (Acmaz et al., 2014; Guzel et al., 2014).

In this study we aimed to evaluate the discriminative factors of GCTO during preoperative period whether these factors may prevent incomplete surgical interventions where frozen section is not adequate.

Materials and Methods

Ethical approval for the entire study was obtained from the Ethics committee of Dr. Zekai Tahir Burak Women's Health Education and Research Hospital. This is a tertiary referral research and education hospital in Ankara, Turkey. Due to the retrospective design, informed consent was not obtained. The study included a total of 110 Turkish women with adnexal mass managed at our gynecology and gynecological oncology departments between 2007 and 2011. Of all women 34 had GCTO and 76 age matched women had benign ovarian mass. We excluded endometriomas from the benign masses. The data of the cases were collected from hospital database and patient

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files. The clinical characteristics evaluated were age, BMI, parity, serum E_2 levels, diameter of the mass (DOM), ultrasonographic features, CA125, risk of malignancy index (RMI), and duration of menopause, postoperative histopathology result the operation specimens, and neutrophil/lymphocyte ratio (NLR).

All of the patients after the initial evaluation that included a general and a gynecological and obstetrical history, vital signs were recorded. For all patients ultrasound scan was performed by expert radiologists of our institution [Aloka Prosound alpha 7, Aloka Prosound SSD 5500, Hitachi Avius and Toshiba Aplio 500, Tokyo, Japan, with abdominal probe (3, 5-5 MHz) and endovaginal probe (5-6, 5MHz)]. On ultrasound examination multilocularity, solid areas, bilaterality, presence of ascites and evidence of metastases were recorded for each patient. CA125 was measured using commercially available immunoassay kits (Immulite 2000 Immunoassay System by Siemens). RMI for all the patients was calculated, using the formula; RMI=(U)×(M)×(CA125). In the formula, "U" represents the ultrasonographic index; "M" is menopausal status, while CA125 is calculated directly into the equation. In our study menopause was accepted as 1 year amenorrhea for natural menopause and >50 year-old for surgical menopause. If premenopausal status is present 1 point was given and if postmenopausal status is present 3 points was given. The ultrasound scan result expressed as a score of 0, 1 or 3. If none of the ultrasound findings were detected 0 point was given, for one finding 1 point and two or more findings 3 points were given. All of the patients underwent laparotomy, and frozen section was performed in suspicious of malignancy. Histopathologic results of all patients were recorded postoperatively.

Statistics

Means and standard deviations (SD) were calculated for continuous variables. Subject characteristics and demographics were analyzed descriptively. The normal distribution of the variables was analyzed by the Kolmogorov-Smirnov test. The Chi-square (X^2) test and the Student's t test were used to evaluate associations between the categorical and continuous variables. ROC curve analysis was used to assess the discriminative role of diameter of the mass, serum estradiol (E_2) and Ca125 levels, RMI and NLR. All variables were included in the backward stepwise procedure. Two-sided p values were considered statistically significant at $p<0.05$. Statistical analyses were carried out using the statistical package SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

In current study a total of 110 women with ovarian masses were included to the study. 34 women with GCTO were classified as the study group and 76 as the control group. Table 1 depicted the demographic and clinical parameters between the groups. The mean age of the study group was 49.8±15.5 years old and 47.8±7.3 years old in the control group. The age of the patients was normally distributed in the GCTO group and ranged between 20 to 82 with a median 48.5 versus 46.5 (22 to 71) in the control group. There was no statistically significant difference between the groups in terms of age (p=0.344). BMI, parity, menopause status, duration of the menopause and serum E_2 levels were also similar parameters between the groups ($p>0.05$). Bilaterality was also different factor between the groups but was more common in the study group. Serum Ca125 levels, DOM, RMI and NLR was higher in the study group showed statistically significant difference between the groups.

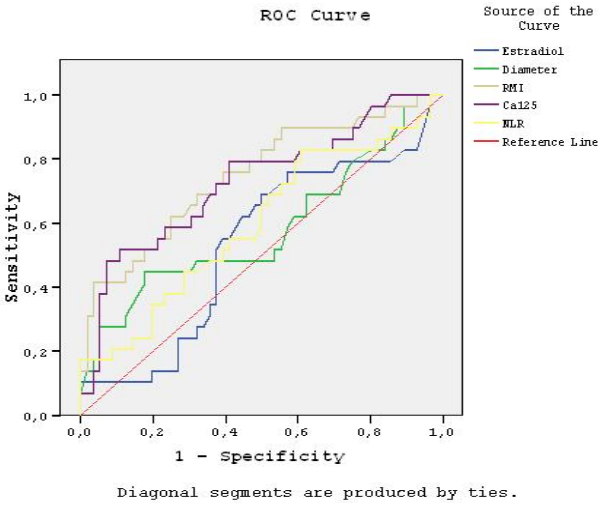

Figure 1. ROC Curve Analysis Showing the AUC of Discriminative Parameters

Table 1. Comparision of Demographic, Clinical and Laboratory Parameters Between Study and Control Groups.

Variables	GCTO (n:34)	Non-GTCO ovarian mass (n:76)	p value
Age (years)	49.8±15.5	47.8±7.3	0.344
BMI (kg/m^2)	29.6±4.8	27.9±3.2	0.212
Parity			
Nullipar	5 (15)	8 (11)	0.53
Multipar	29 (85)	68 (89)	
E_2 levels (pg/ml)	86.0±97.1	73.2±59.5	0.344
Menopause status			
Menopausal	14 (41)	23 (30)	0.201
Non-menopausal	20 (59)	53 (70)	
Menopause duration (years)	11.8±7.8	7.5±7.9	0.085
Bilaterality			
Right	24 (71)	34 (45)	
Left	10 (29)	38 (50)	0.029
Bilateral	0	4 (5)	
Ca125 levels (IU/ml)	64.5±130.3	23.2±29.9	0.002
Diameter of the mass (mm)	92.2±36.7	65.3±32.7	0.048
RMI	285.6±677.6	40.1±65.4	0.001
NLR	2.4±1.4	1.9±0.5	0.024

*BMI: Body Mass Index; RMI: Risk of Malignancy Index; NLR: Neutrophil/Lymphocyte Ratio; E_2: Estradiol; $p<0.05$ is considered statistically significant

Table 2. Area Under Curve and 95% CI of the Discriminative Parameters in Study Group

	AUC	95 % CI
RMI	0.748	0.635-0.861
Serum CA125 levels	0.729	0.612-0.846
NLR	0.598	0.468-0.729
DOM	0.581	0.443-0.718
Serum estradiol levels	0.533	0.401-0.664

*RMI: Risk of Malignancy Index; NLR: Neutrophil/Lymphocyte Ratio; DOM: Diameter of the Mass; $p<0.05$ is considered statistically significant

Histopathologic results were as follow; 33 (44%) serous cyst, 9 (12%) mucinous cyst, 10 (13%) mature cystic teratoma, 10 (13%) hemorrhagic cyst, 10 (13%) follicle cyst and 4 (5%) fibroma in the control group, 33 (97%) had adult type GCTO and 1 (3%) juvenile type GCTO in the study group. The half of GCTO cases was at stage 1a (17 patients), twelve of them were at stage 1c, 4 were at stage 3c and one had distant metastases. Abnormal uterine bleeding was the most common presenting symptom among the patients.

ROC curve analysis demonstrated that RMI, serum CA125 levels, NLR, DOM and serum estradiol levels was discriminative factors with an AUC (95% CI) of; 0.748 (0.635-0.861), 0.729 (0.612-0.846), 0.598 (0.468-0.729), 0.581(0.443-0.718), 0.533 (0.401-0.665); respectively.

Discussion

In this study, we designed a retrospective analysis of women with GCTO who was managed at our clinic. We classified our patients into two groups as the GTCO group and the benign cyst group. We aimed to assess discriminative risk factors in order to predict the GCTO preoperatively and found that RMI, serum CA125 levels, NLR, DOM and serum estradiol levels was discriminative factors to predict GTCO preoperatively.

GCTO is a rare type of the ovarian cancer with an incidence of 1.5-3% of all ovarian tumors (Murkey et al., 2011). These tumors differ from other ovarian cancers with its hormonal behavior and low grade nature. GCTO grow relatively slowly and can recur 10 to 15 years after primary treatment. The reported median age at the diagnosis is about 49, which is compatible with our current study (Hung et al., 2014).

It has two types that is adult type and the juvenile type. Juvenile type was histologically distinguished from adult type by the lack of Call-Exner bodies which small punched out spaces lined by granulosa cells giving a follicle-like appearance. Adult type accounts 95% of all GCTO and juvenile type approximately 5% (Vyas et al., 2013). In our study, juvenile type GCTO was detected in only one woman (3%).

The most common presenting symptoms are abdominal pain and enlargement among the women with GCTO. Dysfunctional uterine bleeding and menstrual irregularities are also frequently seen in these women, especially who had hormonally active tumor (Pectasides et al., 2008). Many other studies showed that there was a close relationship between GCTO and excess of estrogen. Tumoral estrogen secretion can cause uterine enlargement and endometrial thickening. 40% of the adult forms are associated with endometrial hyperplasia and 10% is associated with endometrial adenocarcinoma, so some authors suggested that a preoperative endometrial sampling is required (Ohel et al., 1983; Chua et al., 2001). In our study we found that abnormal uterine bleeding is the most common presenting symptom, and also serum estradiol levels were higher in GCTO group, but the difference was not statistically significant.

Imaging techniques including tomography and sonography cannot reliably distinguish these tumors from the other ovarian malignancies. The appearance varies widely. A large multiloculated cystic mass with solid components is the most common sign during sonographic evaluation. The adult form shows more variability due to cystic component and it can appear as a solid mass. Hemorrhage, necrosis and fibrinoid degeneration can result in a heterogeneous solid appearance (Outwater et al., 1998). Calcifications and papillary projections are not seen in the sonographic evaluation of these patients (Gittleman et al., 2003). MRI is more distinctive. T1 sequence can demonstrate intracystic high signal suggesting intratumoral hemorrhage, T2 sequence demonstrates better sponge like appearance indicating solid and cystic areas. There are limited data evaluating the appearance of GCTO on imaging techniques in the literature. Stine et al. (2013) evaluated one hundred and fifteen patients with GCTO retrospectively, and they concluded that preoperative appearance of GCTO and CA125 failed to predict GCTO In contrast to their study, when we used RMI score which consists of the combination of ultrasonographic characteristics, serum CA125 levels and menopause status, we have obtained the method with the best predictive value.

Preoperative serum parameters, including estradiol, inhibin A, inhibin B, anti-mullerian hormone, transforming growth factor family secreted by granulosa cells reported to be predictive markers for prognosis and diagnosis (Hung et al., 2014). It has been suggested that molecular testing for FOXL2 mutation might be a useful tool in the diagnosis of adult-type granulosa cell tumors (Kommoss et al., 2013). It is classically known that preoperative serum level of CA125 is an important diagnostic and prognostic factor for the ovarian malignancies. We also found that elevated levels of serum CA125 is a valuable predictive test in diagnosis of GCTO. These findings may be related to the tendency to hemorrhage of these tumors, and peritoneal irritation may be a cause of this condition. Hematogeneous metastasis is more common in GCTO, lymphogeneous metastasis is much rarer. Thus, routine lympadenectomy is controversial (Brown et al., 2009; Thrall et al., 2011). Metastasis is rare at the first admission, but peritoneal implants and liver metastasis may be seen such an epithelial ovarian neoplasm.

In the literature studies have focused on prognostic factors in GCTO. Li et al. (2009) reported that stage, nuclear atypia and mitotic count are the important factors on survival rates. They found that only risk factor associated with recurrence is rupture of the tumor.

In recent studies, NLR had been a popular marker in different diagnostic and prognostic procedures. To the best of our knowledge this is the first study which was evaluating NLR in the diagnosis of GCTO preoperatively. Previously, Williams et al. (2013) examined the parameters of complete blood count and NLR among ovarian cancer patients in relation to tumor characteristics, risk factors, serum levels of CA125, and survival rates. They found that NLR before treatment predicts more aggressive disease and correlates with risk factors and results in poor survival rates for the patients with ovarian cancer. They also found that NLR positively correlated with CA125, and they made it relate to cytokines and neutrophil growth

factor released by the cancer cells. In our study we also found that NLR was statistically significantly higher in women with GCTO than the control group. But there was no correlation between NLR and CA125 levels, and the predictive role of NLR is weak in patients with GCTO. The limitation of our study is its retrospective design and the small number of the patients. But it is the first study evaluating NLR in women with GCTO.

In a recent metaanalysis different types and approaches of surgery, including conservative surgery, as well as adjuvant chemotherapy or radiotherapy, and they could not obtain an evidence based consequence (Gurumurthy et al., 2014).

In conclusion, the lack of specific symptoms, slowly growth and the absence of specific tumor markers make it diffcult to diagnose GCTO before surgery and lead to large tumors at diagnosis. We found that GCTO is larger in size than the other benign masses. Therefore, we think that a careful preoperative workshop including diameter of the mass, serum estradiol (E_2), Ca125 levels, RMI and NLR with clinical signs may predict GCTO and may prevent incomplete approaches.

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