RESEARCH ARTICLE

# Evaluation of the Pathogenesis of Tumor Development from Endometriosis by Estrogen Receptor, P53 and Bcl-2 Immunohistochemical Staining

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## Abstract

**Objective:** Endometriosis, one of the most common estrogen dependent gynecological disorders, can present as both benign and malignant disease. The prevalence of tumoral transformation is 0.7-1.6% and the most common tumors are clear cell and endometrioid carcinomas. Unfortunately, the pathogenesis of transformation is unknown. For this purpose, we examined molecular alterations in ovarian endometriosis and endometriosis-associated tumors. **Methods:** Using the data bank of Alzahra hospital pathology department and paraffin blocks from appropriate cases were identified. Sections were cut and stained for 3 markers: estrogen receptor, P53 and bcl2. Correlations between findings were investigated**. Results:** Nineteen cases of endometriosis-associated tumor and 19 cases of endometriosis were identified. Staining for bcl2 was documented in 14 of 19 (73.7%) of endometriosis-associated tumor cases and also 7 of 19 (36.8%) endometriosis cases (P=0.02). Only 3 of the 19 (15.8%) endometriosis-associated tumors exhibited positive staining for estrogen receptors, compared with 14 of 19 (73.7%) endometriosis cases (P<0.001). Positive staining for P53 was noted in 5 of 19 (31.6%) endometriosis-associated ovarian tumor samples but was absent in endometriosis samples (0%), (P =0.008). **Conclusions:** Endometriosis-associated tumors appear to be associated with overexpression of bcl2 and P53 and reduced expression of Estrogen receptor. These finding may help to diagnose tumoral transformation with a background of endometriosis.

**Keywords:** Ovarian tumor- endometriosis- estrogen receptor- P53- Bcl2

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## Introduction

Endometriosis is one of the most common estrogen dependent gynecological disorders, which is reported in 5-15% of reproductive age women (Giudice and Kao, 2004; Kobayashi et al., 2007; Bulun, 2009; Salmassi et al., 2011). It is describe as presence of endometrial stroma and glands in any site out of uterine cavity (Munksgaard and Blaakaer, 2012). Endometriosis behaviors as benign and malignant disease (Thomas and Campbell,2000). The endometriosis tumoral transformation was first reported in 1925 by Sampson (Sampson, 1925) and the prevalence of this transformation is 0.7-1.6% (Kobayashi et al., 2007). The most common tumors are clear cell and endometrioid carcinoma, other types are endometrial stromal sarcoma, malignant mixed müllerian tumor, borderline and benign tumors of serous and endometrioid type (Rosai, 2011). Unfortunately, the pathogenesis of this transformation is unknown (Tereda, 2012). Recent investigations have suggested the role of many genes mutation like estrogen receptor, P53 and bcl-2 in this phenomenon (Nezhat et al., 2007; Kriplani and Patel, 2013; Kreizman-Shefer et al., 2014). So the aim of this study is to evaluation of tumoral transformation pathogenesis in endometriosis by estrogen receptor, p53 and bcl-2 imunohistochemical staining.

## Materials and Methods

Retrospectively, from January 2004 till July 2014, 19 patients with endometriosis-associated ovarian tumor which detected and diagnosed at pathology department of Alzahra hospital (Tabriz, Iran), selected. Also, 19 patients with final histopathologic diagnosis of endometriosis (without tumoral transformation) evaluated for comparison and hematoxylin and eosin stained sections of all patients were reviewed (Figure1). The inclusive criteria consist of the presence of both benign endometriosis and tumoral tissues in the same ovary; presence of histopathologic criteria of endometriosis; excluding metastasis and invasion of other primary tumors,

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and simultaneous ovarian cancer in endometriosis group. Unstained slides with 4-µm thickness were prepared from paraffin blocks and baked at 60°C for 30 minutes in a Lab oven then stained by the envision two-steps method (DAKO Company), using primary antibodies of Estrogen receptor (ER), P53 and bcl-2. The known positive cases were used as positive control. ER was stained brown in nuclei of endometrial tissue. The expression levels of ER were scored according to the number of positive cells (< 50% = one point, 50–80% = two points, > 80% = three points) and intensity (no stain = 0 point, light yellow = one point, brownish yellow = two points, pure brown = three points). Multiplying the scores of these two indices, 0-1 point reported to negative expression (-); 2–9 points reported to positive expression (+) (Shen et al., 2008). The P53 antibody was identified when golden brown intranuclear imunohistochemical staining occurred (Xiao et al., 2012). Bcl-2 stains the plasma membrane and membranes of intracellular organelles. The expression levels were assessed according to intensity and percentage of positive cells. Intensity was scored with 1+ indicating weak staining, 2+ indicating moderate staining, and 3+ indicating strong or dark brown staining. We reported a sample as positive when ≥ 10% of the cells demonstrated staining of at least 1+ to 2+ (weak to moderate) intensity (Nezhat et al., 2011).

Statistics and analysis Data were analyzed by SPSS 16 software using t– test and chi-square test. A p value of < 0.05 was assessed as significant.

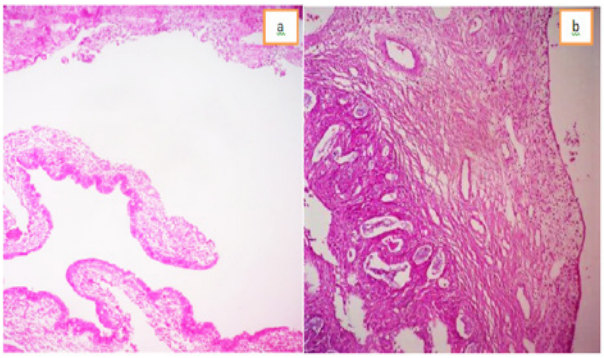


Figure 1. Hematoxylin and Eosin Sections of Ovarian Endometriosis (a) and Ovarian Endometrioid Adenocarcinoma(b)

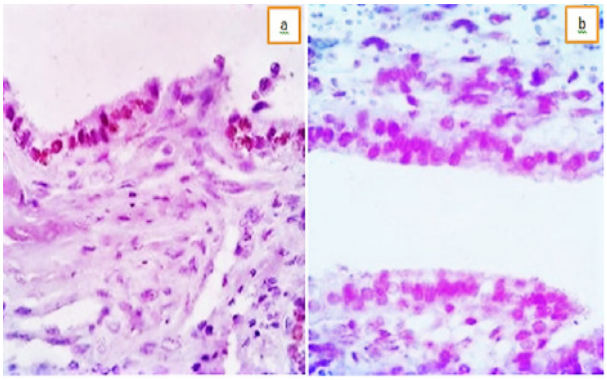


Figure 2. Expression Patterns of P53; P53 Protein Expressed (in Brown) in Cell Nuclei in Endometrioid Adenocarcinoma(a), but Not in Ovarian Endometriosis (b)

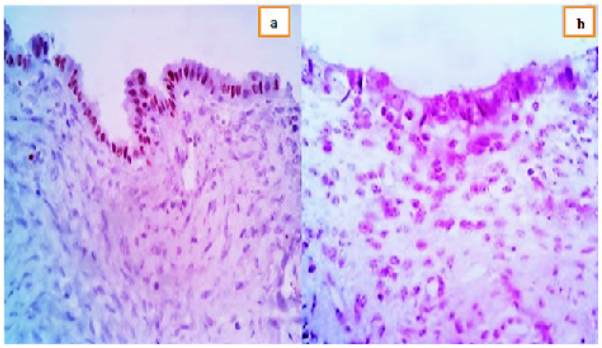


Figure 3 . Expression Patterns of ER; ER Protein Expressed (in Brown) in Cell Nuclei in Ovarian Endometriosis (a), but Not in Endometrioid Adenocarcinoma (b)

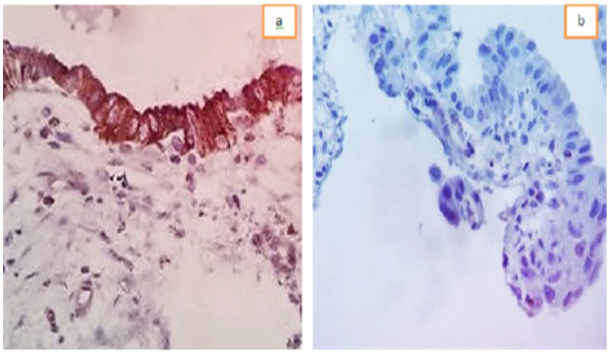


Figure 4. Expression Patterns of BCL2; BCL2 Protein Expressed (in Brown) in Cell Cytoplasm in Endometrioid Adenocarcinoma(a), but Not in Ovarian Endometriosis (b)

## Results

Five patients with endometrioid carcinoma, 3 patients with border-line musinous tumor, 2 cases of each mucinous carcinoma, serous carcinoma and serous cystadenoma, 1 case of mucinous cystadenoma, serous cystadenoma, clear cell carcinoma, seromucinous cystadenoma and borderline serous tumor were identified. The age of the solitary endometriosis group ranged from 19 to 48 years, with mid of 33 years and in EAOT group, ranged from 32 to 77 years with mid of 45 years. P53 overexpression (Figure 2) was found in 31.6% (6/19) of EAOT but was not found in endometriosis (p=0.008). The expression of ER (Figure 3) was detected in only 15.8% (3/19) of EAOT specimens and in 73.7% (14/19) of endometriosis (p<0.001). Bcl2 overexpression (Figure 4) was detected in 73.7% (14/19) of EAOT and in 36.8% (7/19) of endometriosis (p=0.02)

## Discussion

Patients with Endometriosis-associated ovarian tumor (EAOT) are younger, were diagnosed in earlier-stage disease, and have longer survival without disease than do patients with primary epithelial ovarian carcinoma.so the behavior EAOT may be more favorable than the behavior of other ovarian carcinoma (Del et al., 2003; Bulun et al., 2009). Accordingly its malignant potential, endometriosis needs particular vigilance during diagnosis. Routine imaging studies have not been able to diagnose either endometriosis or tumoral transformation of endometriotic

disease (Nezhat et al., 2002; Xiao et al.,2012). If patients were diagnosed in earlier stage of endometriosis, may cause to improvements in the treatment and in the survival of patients with ovarian carcinoma (Del et al., 2003; Giudice et al., 2004). IHC staining can be helpful in screening and detecting of endometriosis. Endometriosis is a sex hormone-dependent disease (Shen et al., 2008; Kreizman-Shefer et al., 2014). The expression of ER protein is detected in normal endometrium (Apostolou et al., 2014). When tumoral transformation occurred, the receptors of epithelium lose the ability of recognizing sex hormones and change from sex hormone dependent to sex hormone-independent receptors (Carcangiu et al., 1997; Shabani et al., 2007). Subsequent researches should try to evaluate differences in receptor subunit expression between endometriosis and EAOT. One of the limitations of our study is the small sample count. In our research, the positive rates of ER were lower in tumoral transformation group than in endometriosis group, so decreasing of ER expression is possibly involved in tumoral transformation of ovarian Ems (Shen et al., 2008; Ho, 2003). Apoptosis has been suspected in the pathogenesis of neoplastic diseases. Many gene products such as bcl-2 family and p53 can be associated with its process. Bcl-2 protects cells from becoming apoptosis (Nezhat et al., 2002). Approximately 23% of benign endometriotic cysts, 67% of endometrioid carcinomas, stain positively for bcl-2 (Mhawech et al., 2002; Nezhat et al., 2008). Our results are approximately similar to those reported by Skirnisdottir (2001), which showed decreasing of Bcl-2 expression in benign endometriotic cysts compared with tumoral cases. P53 is involved in cell proliferation phenomena that are important in tumoral transformation of cells. Immunohistochemical staining have shown overexpression of p53 in many tumors, including ovarian carcinoma (Apostolou et al., 2014). In current study staining for p53 was negative in endometriotic cysts, but positive in 31.6% (6/19) of tumoral cases, and in 2 /4 of borderline tumors. These findings are nearly similar to those of Nezhat (2002) who reported positive P53 staining in malignant epithelial tumors, but negative staining in benign endometriosis. In another study by Chan (2000) positive staining for P53, was seen in malignant ovarian epithelial tumors and negative staining was detected in benign and borderline tumors and normal surface epithelium. Detection of the markers that may be involved in the transition from endometriosis to EAOT may use as a screening and diagnostic tool for identification of patients with endometriosis who are at risk of developing tumoral disease. Also, this data may be helpful in treatment algorithms (Den et al., 2003)

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*Conflicts of interest*

None of the authors have any commercial or financial involvement in connection with this study that represents or appears to represent any conflict of interest.

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