

## RESEARCH ARTICLE

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## The Difference of Bax Protein Expression between Endometrioma and Ovarian Carcinoma

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**Background:** Endometriosis is a benign disease that has malignant properties such as genetic polymorphism, loss control of cell proliferation, infiltration, and local spread or to distant places. Several endometriosis studies linking endometrioma/ovarian endometriosis with an increased risk of ovarian malignancy give rise to a transformation phenomenon of endometriotic cysts into malignancy. Bax is a pro apoptotic protein whose expression decreases in a malignancy. This decrease is related to the poor prognosis of endometrioma and ovarian carcinoma. This study was aimed to identify the expression and the difference of Bax expression between endometrioma and ovarian carcinoma.

**Materials and Methods:** Fifty of paraffin blocks of endometrioma tissue and ovarian carcinoma (serous, mucinous, clear cell, and endometrioid type) were examined by immunohistochemical using Bondmax Full Automatic with specific monoclonal antibody to identify Bax expression. The difference of Bax expression score between endometrioma tissue and ovarian carcinoma was tested by Mann-Whitney test with significant value was set at  $p < 0.05$ .

**Results:** This study found that mean Bax expression score in endometrioma tissue and ovarian carcinoma was 3.88 and 3.72. No difference of Bax expression between endometrioma tissue and ovarian carcinoma ( $p > 0.05$ ). No difference of Bax expression between the clinical stages and histopathological types of ovarian carcinoma ( $p > 0.05$ ).

**Conclusion:** There are no statistically significant difference in Bax protein expression in ovarian cancer and endometrioma.

**Keywords:** Bax expression, endometrioma, ovarian carcinoma, apoptotic resistance

### Introduction

Endometrioma is a benign gynecological disease characterized by the presence of endometrial tissue in the ovary including the gland and the stromal associated with pelvic pain and infertility.<sup>1</sup> Endometrioma is a pseudocyst that develops from the growth of ectopic endometrial tissue

and progressively invaginates in the ovarian cortex.<sup>1,2</sup> Endometrioma is commonly known as endometriosis in ovaries or chocolate cysts because it contains many brownish blood debris.<sup>3</sup>

Endometriosis often occurs in women of reproductive age (8-10%), but it is possible in premenopausal, menopausal, and postmenopausal ages. Endometriosis also

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found in nullipara and women with infertility. As many as 15-25% of infertile women in Indonesia are caused by endometriosis. Amount of 30% of women with primary infertility have endometriosis and 70-80% of women with idiopathic infertility are due to endometriosis. The incidence of chocolate cysts is 30-40% of the entire population of endometriosis.<sup>2,4</sup>

Several endometriosis studies linking endometrioma or endometriosis with an increased risk of ovarian malignancy give rise to a transformation phenomenon of endometriosis cysts into malignancy (malignant degeneration)<sup>5,6</sup> and result in a poor disease prognosis. Recent study also report that 9.3% of ovarian cancer patients have a history of endometriosis.<sup>7</sup> Endometriosis is a benign disease that has malignant properties such as genetic polymorphism, loss control of cell proliferation, infiltration, and local spread or to distant places.<sup>8-10</sup> The histogenesis theory of endometriotic cysts, including coelomic metaplasia, retrograde menstruation, embryonic cell rest, induction, lymphatic and vascular dissemination, as well as the pattern of chromosomal aberrations in endometriosis.<sup>6,11,12</sup>

One of the molecular characteristics of a malignancy is resistance to apoptosis which is characterized by increased expression of anti-apoptotic protein (Bcl-2), decreased pro apoptosis expression (Bcl-2 associated x protein, Bax), and inactivation of p53 due to gene mutation.<sup>5</sup> Endometriotic pathways have similarities in their development to avoid apoptosis with increased expression of Bcl-2, decrease in Bax<sup>13</sup>, and inactivity of the p53 gene due to mutations.<sup>14-16</sup> The purpose of this study was to compare Bax expression between ovarian endometrioma and ovarian carcinoma.

## Materials and methods

This study was an observational analytic study with cross-sectional design. The study was conducted in Department of Obstetrics and Gynecology, H. Adam Malik Hospital, Medan, Indonesia, from October to November 2014. The samples used were paraffin blocks of endometrioma tissue and serous type of ovarian carcinoma, mucinous, endometrioid, and clear cells based on histopathology examination of post laparotomy and post laparoscopy patients at H. Adam Malik Hospital, Medan. Our inclusion criteria were no hormonal therapy for endometriomas was given before surgery and no chemotherapy was given before surgery for ovarian cancer cases (chemo naive). We exclude

the sample from damaged paraffin blocks. The sample size used was 25 paraffin blocks of endometrioma tissue and 25 paraffin blocks of ovarian carcinoma which were chosen non-randomly convenient sampling of available paraffin blocks. Information regarding age, parity status, and the disease stages was obtained from patient's medical record.

Expression of Bax was examined by immunohistochemical using Bondmax Full Automatic with specific Bax monoclonal antibody (Cat. No.#6A7, Thermo Fisher Scientific, Rockford, USA). Scoring was based on examination of all cells on the slide using the Allred score guidelines. The score was the sum of the percentage scores of cells that are stained or proportion score (PS) and the intensity of staining or intensity score (IS). The proportion score was graded into six qualitative score groups: 0 = no stained cells; 1 = less than 1% stained cells; 2 = 1-10% stained cells; 3 = 11-33% stained cells; 4 = 34-66% stained cells; and 5 = 67-100% stained cell. The intensity of staining was graded into four qualitative score groups: 0 = no stained cells; 1 = weak staining intensity; 2 = moderate staining intensity; 3 = strong staining intensity. The immunostaining results were quantified as follows: 0-2 = negative expression and  $\geq 3$  = positive expression.<sup>17</sup>

The difference of Bax expression between endometrioma and ovarian carcinoma was tested by Mann-Whitney test with significant value was set at  $p < 0.05$ . The accuracy of reading the Bax expression between two observers was analyzed using the kappa value and was declared valid if more than 75%.

## Results

Most of the endometrioma patients were  $\leq 40$ -years old (68%) and the mean age was 34.12 years, while the ovarian carcinoma patients were  $> 40$ -years old (64%) and the mean age was 44.28 years. Generally, endometrioma groups were nulliparous (76%), while ovarian carcinoma groups were multiparous (44%). Types of ovarian carcinoma based on histopathological examination were serous cancer (48%), endometrioid (24%), mucinous (16%), and clear cells (12%) (Table 1).

A good agreement was found between two pathologists ( $\kappa = 0.84$ ), therefore the analysis of Bax expression score can be used from one pathologist. This study found that mean Bax expression score in endometrioma tissue and ovarian carcinoma was 3.88 and 3.72. These showed that there was an under expression of Bax protein in both cases

**Table 1. Histopathological types of ovarian carcinoma.**

Types	n	%
Serous	12	48
Mucinous	4	16
Endometrioid	6	24
Clear cell	3	12
Total	25	100

based on Allred score. There was no difference between Bax expression in endometrioma tissue and ovarian carcinoma ( $p>0.05$ ). The mean Bax expression score in early ovarian carcinoma was not significantly different from advanced ovarian carcinoma (3.88 vs. 3.65) ( $p>0.05$ ). Due to the Bax expression score of clear cell and mucinous ovarian carcinoma was constant, analysis of the two groups was not carried out. Median value of Bax expression score in serous ovarian carcinoma was 4.00 and in endometrioid was 3.00. However, this difference was not statistically significant ( $p>0.05$ ) (Table 2).

**Discussion**

The transformation phenomenon of endometrioma towards malignancy increases the risk of ovarian carcinoma.<sup>18</sup> Several studies have suggested that ovarian carcinoma arising from endometriosis. Endometriosis have similar

characteristics with cancer cells. One of the genetic similarities of endometrioma with ovarian carcinoma is apoptotic resistance which is characterized by a decrease of pro apoptotic protein expression<sup>5,6</sup> This current study provided a comparison of pro apoptotic protein expression, Bax, in endometrioma tissue and ovarian carcinoma.

Several studies have shown that the transformation of endometriosis towards ovarian malignancy often occurs in type I ovarian carcinoma (endometrioid, clear cell, serous, and well differentiated mucinous). Histopathology of ovarian carcinoma derived from endometriosis cysts was clear cell type, endometrioid, serous, and mucinous.<sup>19-24</sup> Accordingly, the samples taken in this study were endometrioid, clear cell, serous, mucinous adenocarcinoma.

This current study showed that there was no statistically significant difference between Bax expression score in endometrioma and ovarian carcinoma. Bax protein was under expression in both tissues. This result indicated that although endometrioma has benign features, the expression score of Bax proteins looks like a malignancy. This was in line with several recent studies. Fauvet, *et al.*, found that Bax expression in endometrioma was significantly difference with benign ovarian tumors, however there was no difference with ovarian carcinoma.<sup>25</sup> Meresman, *et al.*, and Nezhat, *et al.*, also found that there was a decrease in Bax expression in endometrioma tissue and endometriotic cysts.<sup>13,26</sup> A recent study comparing Bax expression in endometrioma with ovarian carcinoma also

**Table 2. The difference of BAX expression score in endometrioma and ovarian carcinoma, clinical stages, and histopathological type.**

Groups	Expression BAX score					p- value
	Mean	Median	SD	95% CI		
				Low	Up	
Carcinoma Category						
Endometrioma	3.88	4	0.44	3.7	4.06	0.224
Ovarian carcinoma	3.72	4	0.45	3.53	3.91	
Clinical stages						
Early ovarian carcinoma (stage I)	3.88	4	0.35	3.58	4.17	0.246
Advanced ovarian carcinoma (stage II-IVA)	3.65	4	0.49	3.39	3.9	
Histopathological type						
Endometrioid	3.33	3	0.51	2.79	3.88	0.097
Serous	3.75	4	0.45	3.46	4.04	

showed no significant difference<sup>27</sup>, while another study found that Bax expression in endometrioma was higher than in well differentiated serous ovarian carcinoma.<sup>28</sup> The discrepancy of the results may be due to the samples used in this study not only serous type ovarian carcinoma tissue, but also endometrioid, clear cell, and mucinous types which are forms of the endometrioma transformation into ovarian carcinoma. Bax expression is associated with stages of endometrioma and ovarian carcinoma.<sup>18</sup> The higher stage of both diseases, the lower expression of Bax.

An insignificant difference between Bax expression in endometrioma and ovarian carcinoma in this study shows that endometrioma has molecular similarities with ovarian carcinoma in its resistant properties to apoptotic signals. Its clinical meaning is endometrioma has similar malignancy characteristics as ovarian carcinoma. Therefore, endometriomas tend to be transformed to malignant specially to type I ovarian carcinoma. A recent study found as much as 0.6-1.7% of endometriomas transform into a malignancy and the incidence of endometrioma and ovarian carcinoma together was 4-29%.<sup>29</sup> Several studies showed that women with endometrioma have 1.4-1.9 times risk greater to become ovarian carcinoma than healthy women.<sup>8,20,30</sup>

The current study also showed that there was no difference in Bax expression between the clinical stage of ovarian carcinoma. The result was only a statistical representation, not a relationship between the significance level of Bax expression based in clinical stage in ovarian carcinoma, therefore, other techniques and research methods are needed. The result also was not in line with the recent study which found that Bax expression in stage I and II of ovarian carcinoma was higher than in stage III and IV.<sup>31</sup> However, this study results were in line with previous studies comparing Bax expression in histopathological types of ovarian carcinoma. There was no statistical difference between Bax expression score in histopathological types of ovarian carcinoma.<sup>18,31,32</sup>

## Conclusion

There are no statistically significant difference in Bax protein expression between ovarian cancer and endometrioma. The results also expected to be the basis for further study in finding a shared pathway between endometrioma and ovarian cancer especially in ovarian cancer that comes from malignant transformation of endometrioma tissue.

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