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Comparative Study of BAX/BcL2 Expression Ratio in Menstrual Blood between Endometriosis Women and Women without Endometriosis

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ABSTRACT

Introduction: Endometriosis is characterized by the presence of endometrial tissue outside the uterus and is commonly associated with chronic pelvic pain and infertility. One of the endometrial changes that appear in the eutopic and ectopic endometrium of women with endometriosis is the regulation of apoptosis. The proportion value of the BAX/BcL-2 ratio is a crucial factor in determining the ability of cells to stimulate apoptosis. Higher apoptotic activity is linked to an increased BAX/BcL-2 ratio. The aim of this study was to compare the BAX/BcL-2 ratios in menstrual blood of endometriosis and non-endometriosis patients.

Material and Methods: This research is a quantitative observational study with a cross-sectional method at dr. Saiful Anwar Malang. The case group was postoperative women with ovarian cysts with anatomical pathology examination results indicating endometriosis. While the control group was patients without endometriosis. Data analysis was carried out by using the Independent T Test with SPSS for Windows 19.0 software.

Results: Each group consisted of 17 patients. The normality test for each variable shows p> 0.05. BAX average in patients with endometriosis 4556.29 ± 2338.33 and 6488.06 ± 2649.08 in patients without endometriosis (p = 0.031). The average BcL-2 in endometriosis patients was 6483.82±2489.36 and 4877.18±2023.82 in patients without endometriosis (p=0.047). The average BAX/BcL-2 ratio in patients with endometriosis was 0.698±0.19 and 1.367±0.26 in patients without endometriosis (p=0.000).

Conclusion: The BAX/BcL-2 ratio in women with endometriosis cysts has a lower average rate than women without endometriosis cysts.

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INTRODUCTION

Endometriosis is a disease commonly found in adolescents and women of reproductive age, characterized by the presence of endometrial tissue outside the uterus and is generally associated with chronic pelvic pain and infertility [1]. It is estimated that more than 70 million women worldwide have endometriosis. In the United States it is estimated that 5-10% of women of reproductive age have endometriosis. Other data shows that endometriosis affects women of reproductive age around 6-10% and 30-45% affects women with infertility [2]. In the general female population, 76–90% of women experience menstrual blood backflow, but only about 10% of these women have endometriosis.

Apoptosis is one of the components of the eutopic endometrium's ability to survive outside the uterine cavity after menstrual blood flow returns, thus enabling the eutopic endometrium's ability to attach to the outer area of the uterine cavity, the eutopic endometrium's ability to invade and form blood flow with angiogenesis

and develop into endometriosis [3]. The mechanism of reducing apoptosis in eutopic endometrium in women with endometriosis is related to BcL-2, which has antiapoptotic properties and BcL-2 bound to X protein (BAX) which has proapoptotic properties [4]. Tumor cells can acquire resistance to apoptosis by expression of antiapoptotic proteins such as BcL-2 or by downregulation or mutation of proapoptotic proteins such as BAX. Because BcL-2 and BAX expression is regulated by the p53 tumor suppressor gene, several forms of human B-cell lymphoma have BcL-2 overexpression [5].

The balance between BAX and BcL-2 is believed to play an important role in the process of apoptosis. Higher apoptotic activity associated with an increased BAX/BcL-2 ratio. In women with endometriosis, a decrease in the apoptotic factor can be seen, thereby reducing the ability to apoptotic. Cells that influence apoptosis in the endometrium can be found in endometriosis tissue, peritoneal fluid, and menstrual blood [6]. This study aims to compare the ratio of endometrial BAX/BcL-2 to the menstrual blood of women with endometriosis and without endometriosis. In addition, this study can add scientific information about differences in the ratio of endometrial BAX/BcL-2 to menstrual blood of women with and without endometriosis.

MATERIAL AND METHODS

This research is a quantitative observational study with a cross-sectional method was performed between September – October 2023. The study population was women who visited the obstetric polyclinic at General Hospital dr. Saiful Anwar Malang met the inclusion and exclusion criteria. The inclusion citeria of this study are woman who still have a menstruation, post-operative women with anatomical pathology examination results showing endometriosis, postoperative women with ovarian cysts whose anatomic pathology examination shows no malignancy, and also woman who have not used hormonal therapy in the last 3 months.

The research group was divided into two, the first group were women after ovarian cyst surgery with the results of anatomic pathology examination showing endometriosis. while controls were women without endometriosis. The independent variable in this study is the Endometriosis. The dependent variable in this study is BAX/BcL-2 ratio. Data analysis with SPSS software for Windows 19.0.

RESULTS

This study was conducted on 34 patients and produced data as shown in Table 1. Based on the results of the data normality test using Shapiro Wilk, the data for all variables in each group (endometriosis and non-endometriosis) have an equal distribution ($p>0.05$). Following that, the t test was used to compare the two groups.

Based on Table 2, the average BAX level in Endometriosis group is significantly lower than the control group ($p<0.05$). The average of BcL-2 levels in Endometriosis group were also higher than the control group ($p>0.05$). The average BAX/BcL-2 ratio levels in Endometriosis group were significantly lower than controls ($p>0.05$).

Table 1. Demographic Characteristic

Demographic Characteristics	*n*	%
Age		
≤20 years old	3	8,82
21 – 30 years old	13	38,24
31 – 40 years old	12	35,29
>40 years old	6	17,65
Endometriosis Group		
Bilateral Chocolate cyst	1	5,88
Cystoma ovarii unilateral	1	5,88
Endometriosis	1	5,88
Unilateral Brown Cyst	4	23,53
Bilateral Brown Cyst	1	5,88
Uterine Fibroid + Endometrial cyst	1	5,88
Ovarian Cyst	4	23,53
Ovarian Cyst unilateral	3	17,65
Non-Endometriosis Group		
Cystoma ovarii	9	52,94
Cystoma ovarii bilateral	3	17,65
Cystoma ovarii unilateral	3	17,65
No malignant cells were found	1	5,88

DISCUSSION

In the results of the study, it was found that the BAX levels in the menstrual blood of women with endometriosis had a lower average than the BAX levels in the menstrual blood of women without endometriosis with a significant difference. The mechanism of BAX as a proapoptotic is by binding to the Mitochondrial Apoptosis-induced Channel (MAC) to form oligomeric pores on the outer membrane of mitochondria so that membrane permeability increases and initiates cell apoptosis [7]. Previous research also stated that the expression of BAX in the incidence of endometriosis decreased significantly with p=0.003 [8]. The average BAX level in endometrioma tissue is 3.88, because of its anti-apoptotic process which resembles malignancy, BAX levels in endometriomas do not have a significant difference with BAX levels in ovarian carcinoma [9]. In experimental mice induced by endometriosis lesions and tested for proteins that affect apoptosis by western blot, BAX expression was found to be significantly lower than the control group [10].

In the results of the study, it was found that the BcL-2 levels in the menstrual blood of women with endometriosis had a higher average than the BcL-2 levels in the menstrual blood of women without endometriosis with a significant difference. BcL-2 inhibits the release of cytochrome C which has an apoptotic function from mitochondria into the cytoplasm [11]. BcL-2 regulates cell transmembrane transport thereby affecting the distribution of calcium in which calcium itself can activate endonuclease to initiate apoptosis. BcL-2 can also be used as an antioxidant to change the oxidation-reduction state of cells so that the cell components are inhibited by apoptosis [12]. Estrogen is able to increase the expression of BcL-2 as an anti-apoptotic which causes endometriosis tissue to experience proliferation, migration and invasion [13]. BcL-2 expression is significantly increased in the secretory phase of the uterus in patients with endometriosis. In female endometriosis tissue, the BcL-2 protein has a significant increase and is positively associated with CD147 expression which further reduces the degree of apoptosis of a cell [13–15].

In the results of the study, it was found that the BAX/BcL-2 ratio in the menstrual blood of women with endometriosis had a lower average than the BAX/BcL-2 levels in the menstrual blood of women without endometriosis with a significant difference. The BAX/BcL-2 ratio indicates the susceptibility of a cell to undergo apoptosis [16]. The lower the value of the BAX/BcL-2 ratio indicates that the cell is more resistant to apoptosis. BAX/BcL-2 ratio can affect the progression and aggressiveness of a tumour. A low BAX/BcL-2 ratio has a poor prognosis and indicates an infiltrative growth [17].

When the ratio of pro-apoptotic/anti-apoptotic factors (BAX/BcL-2) increases, it will cause disruption of the mitochondrial membrane which in turn will trigger the release of cytochrome C from intra-mitochondria into the cytosol. Then cytochrome C forms a complex with protease-activating factor-1 (Apaf-1) and pro-caspase-9 in the presence of dATP or ATP. This complex then activates caspase-9, an initiator caspase which in turn can activate an effector caspase, namely caspase-3 which then causes the cell to undergo apoptosis [18]. In human endometrial epithelium, the BcL-2 protein is active during the proliferative phase and gradually disappears during the secretory phase. Meanwhile, BAX protein will be found to increase when the endometrium is in the secretory phase so that menstruation occurs due to an increased BAX/BcL-2 ratio. There are low values for the BAX/BcL-2 ratio in the secretory phase of patients with endometriosis. This incident can be used as a warning sign of poor progress in endometriosis. This indicates that endometriosis tissue is associated with resistance to apoptosisah [7,19].

Table 2. T-test of BAX, BcL-2, and BAX/BcL-2 Ratios in Endometriosis and Non-Endometriosis Patient Groups

	Endometriosis Subjects			Non-endometriosis Sbjects			*p value*
	mean	**±**	**sd. dev.**	**mean**	**±**	**sd. dev.**	
BAX level	4556.29	±	2338.33	6488.06	±	2649.08	0.031
BcL-2 level	6483.82	±	2489.36	4877.18	±	2023.82	0.047
BAX/BcL-2 ratio	0.698	±	0.19	1.367	±	0.26	0.000

CONCLUSION

The BAX/BcL-2 ratio in women with endometriosis cysts has a lower average rate than women without endometriosis cysts.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest regarding the publication of this article.

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