RESEARCH ARTICLE

High Expression of PR-A and Low Expression of PR-B is Correlated with Inflammation in Endometrioma Cases

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Abstract

BACKGROUND: Progestin therapy has been commonly used in endometriosis. The regulation of progesterone receptors B (PR-B) greatly affects the success rate of therapy in cases of endometriosis. The presence of tumor necrosis factor (TNF)-α in endometriosis triggers PR-B hypermethylation, decreasing PR-B expression and PR-B/A ratio that induce progesterone resistance. It may also occur in endometrioma. Studies regarding the distribution of PR-A and PR-B with TNF-α expression in endometriosis with endometrioma tissue samples has not been elucidated well. Therefore, this study was conducted to measure and compare the distribution of PR-A and PR-B expression, and to assess the effect of PR-B/A ratio on TNF-α in endometrioma and benign cysts.

METHODS: A cross-sectional study was conducted by collecting paraffin blocks of endometriomas and benign cysts as controls, from patients undergoing surgery at Dr. Sardjito Hospital, Yogyakarta. Immunohistochemistry was performed to assess the expressions of PR-A, PR-B, TNF-α and PR-B/A ratio, to compared differences between endometriomas and benign cysts.

RESULTS: Twenty-three endometrioma and 22 benign cyst tissue samples were collected. The mean PR-B expression and PR-B/A ratio were found to be lower in endometriomas than benign cysts, and mean expression of PR-A and TNF-α in endometriomas was higher than in benign cysts. However, there were no significant correlations between the expression of PR-A, PR-B, PR-B/A ratio, and TNF-α with endometriosis severity.

CONCLUSION: In endometrioma cases, the expression of PR-A and TNF-α was higher, while the expression of PR-B and PR-B/A ratio was lower. However, there was no significant relationship between the ratio of PR-B/A and TNF-α.

KEYWORDS: progesterone receptor, tumor necrosis factor-alpha, endometriosis, endometrioma, benign cyst, ovarian cyst

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Introduction

Endometriosis is a chronic, estrogen-dependent inflammation caused by ectopic growth of endometrial tissue. The process of inflammation, bleeding and adhesion of endometriosis nodules results in the emergence of complaints in patients with endometriosis.(1,2) Endometrioma, a benign gynecological disease characterized by the presence of endometrial tissue in the ovary including the gland and the stromal, is the most common type of endometriosis disease, afflicting up to 17-44% of women at reproductive age, yet its pathogenesis is still unclear.(2,3)

In several clinical observations, the role of estrogen in promoting the growth and development of the disease is well characterized, although the etiology of endometriosis remains largely unknown.(4) Progestins have been widely used for the treatment of endometriosis. It is given as an

easier to administer and less expensive alternative therapy. Progestins work by inhibiting the secretion of estrogen. However, not all patients with endometriosis respond well to progestin therapy.(5) Endometriotic lesions do not respond adequately to progesterone although serum progesterone levels in women with endometriosis are similar to those in women without endometriosis, which is because the endometriotic lesions have altered progesterone receptor (PR) expression. The decreased progesterone response in endometriosis is due to aberrant expression of PRs in endometrial cells.(6) Progesterone resistance, a condition of inappropriate response to progesterone in endometriosis lesions which impairs the efficacy of progestin-based therapy is mediated by lower PR levels. Low PR may explain why progestin-containing agents are associated with treatment failure in some patients.(4)

PR-A and PR-B have different mechanisms of action whereas PR-A acts as a suppressor of PR-B.(7) The main characteristics of low endometriosis response to progestin are the lack of transcription, translation, and biological activities of PR-B. This defect could be hereditary, or acquired through epigenetic effects from inflammatory conditions.(8) Therefore, alternatives are considered to restore the epigenetic mechanism that inhibits PR-B synthesis by the addition of anti-inflammatory or immunomodulatory substances as adjuvants of progestin therapy.(9) The methylation of PR-B promoter in endometriosis may cause by increased proinflammatory cytokine release in chronic inflammatory environment.(10)

Tumor necrosis factor (TNF)-α and interleukin (IL)-1β directly cause a decrease in levels of both isoforms of the PR, which is possibly done through epigenetic modification.(6) TNF-α, an inflammatory cytokine, is produced by the activation of lymphocytes, macrophages, and natural killer (NK) cells. TNF-α expression is enhanced by IL-1 and it is expressed on ectopic endometrial epithelial cells. It has an increase in concentration in the peritoneal fluid of patients with endometriosis and is related to the severity of the disease. Endometriosis is marked by the down-regulation of PR-B receptors by means of epigenetic inhibition (hypermethylation promoter) of upstream transcription of PRs due to local inflammatory mediators.(11)

The balance between PR-A and PR-B isoforms controls the myometrial response to proinflammatory stimuli.(5) The relative increase in PR-A compared to PR-B in endometriosis contributes to tissue inflammation. However, available evidence suggests that both isoforms of the PR mediate the anti-inflammatory action of progesterone in the endometrium.(5) In a human endometriotic epithelial cell line expressing PR-A or PR-B, dienogest inhibits the transcription of several pro-inflammatory genes independent of the receptor isoform expressed by the cell. Thus, it appears that in endometriosis, sensitivity of the receptors to PR-A does not trigger a local inflammatory response. In conclusion, endometriosis is characterized by downregulation of PRs in general and PR-B in particular through epigenetic (promoter hypermethylation) inhibition of upstream PR transcription by local inflammatory mediators.(4) In endometriotic cells, exposure of TNF-α leads to promoter hypermethylation of the PR-B isoform, which has an impact in decreasing of expression and ratio of PR-B/ PR-A.(8)

Previous research on expression of PR-A and PR-B in endometriosis, found that PR-A is expressed higher in endometriosis.(6) Other studies regarding the decrease in the ratio of PR-B/A in the endometrium by TNF-α and peritoneal fluid from endometriosis patients, decreased ratio of PR-B/A in endometrial cells by tumor necrosis factor-alpha and peritoneal fluid from patients with endometriosis.(12) Studies regarding the distribution of PR-A and PR-B with TNF-α expression in endometriosis with endometrioma tissue samples has not been elucidated well. This study aimed to examine the expressions of PR-A, PR-B, and TNF-α in endometriomas tissue that could be used to assist in clinical practice to determine medical therapy using progestins in patients with endometriosis more accurately after surgery.

Methods

Sample Collection

Forty-five subjects were recruited from patients that were diagnosed with endometriomas and benign cysts through histopathological examination or undergoing surgery between August 2019 and December 2020 at Dr. Sardjito General Hospital, Yogyakarta. Subjects were excluded if they received progesterone treatment for at least six months before surgery, and diagnosed with any type of malignancy. The protocol of this study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (Ref. No: KE/FK/0150/EC/2020, extended Ref. No: KE/FK/0600/EC/2021).

Immunohistochemistry (IHC)

Paraffin blocks that match the histopathological diagnosis of endometriomas and benign cysts (controls) were collected

from subjects and then three sample slides were made for each PR-A, PR-B, and TNF-α. The paraffin blocks were sliced in 6 μm and taken on the glass slide. Then, all of samples deparaffinized, antigen-retrieved, and endogenous-peroxidase-blocked by blocking solution. Then the samples were dried and incubated at 38-40°C for 24 hours.

For the detection of PR-A, the slides were incubated for 24 hours using the mouse monoclonal antibody progesterone receptor NCL-L-PGR-AB (Leica Biosystems, Newcastle, United Kingdom). For PR-B detection, the progesterone receptor monoclonal antibody Alpha PR6 MA1-411 antibody (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA) was used. Meanwhile, for the detection of TNF-α, the TNF-α Rabbit pAb A11534 polyclonal antibody (ABclonal Technology, Woburn, MA, USA) was used. After incubation for 24 hours period, the slides were washed with PBS 3 times for 5 minutes, then drops of Trekki ink were added for 10 minutes followed by washing using pH 7.4 PBS 3 times for 5 minutes. HRP conjugate drops were then added and allowed to stand for 10 minutes before being washed again using pH 7.4 PBS 3 times for 5 minutes, then followed by the addition of diaminobenzidine (DAB) drops for 15 minutes before rinsed. After that, counterstaining was done by hematoxylin Mayer staining, then washed with running water, dehydrated with increasing concentrations of alcohol, and mounted with mounting medium and cover glass. Using a light microscope Olympus type CX33 (Olympus, Tokyo, Japan), protein expression can be seen which is characterized by the appearance of brown color on the plasma membrane or cell membrane.

Histo-score (H-score) Calculation

The expressions of PR-A, PR-B, and TNF-α proteins were considered to be positive if there was a brownish color in the cell membrane and cytoplasm. Evaluation with microscopic assessment was conducted at 400x magnification in five different fields of view using an ImageJ application (National Institutes of Health, Bethesda, MD, USA). An H-score for immunohistochemical staining was determined for each slide. H-score calculation was done independently by two different observers by concealing the identity of the observed sample. Then, the intra-class correlation testing was done to confirm between the observers (the estimated of cells). The score is about 0 to 3, with mean percentage of negative, weakly positive, moderate, and strongly positive, respectively. The percentages of this formula were multiplied with the scores and summed. The relation and degree of correlation between proteins were described by the Spearman correlation score.

Statistical Analysis

Univariate analysis was performed using independent T-tests for PR-A characteristics and Mann-Whitney tests for PR-B characteristics, PR-B/A ratio, and TNF-α. Bivariate analysis was used to see the expressions of PR-A protein with TNF-α, PR-B with TNF-α, and expression ratio between PR B/A with TNF-α which is shown in the 2x2 table and analyzed using Pearson and Spearman correlation analyses ($p<0.05$). Data were analyzed with SPSS ver. 24 (IBM Corporation, Armonk, NY, USA).

Results

Characteristics of Research Subjects

From the obtained samples, total of 23 endometrioma tissues and 22 benign cyst tissues were obtained. The Kolmogorov-Smirnov normality test was done on the variables of age, body mass index (BMI), parity, and endometriosis grade and it was found that the data distribution was not normal. Based on the mean comparison test results, there was no statistically significant difference ($p>0.05$) in all variables between the two groups.

From 45 study subjects, it was found that the mean age of the subjects was 36 ±5.40 years old in the endometrioma group and 38.41±11.62 years old in the benign cyst group, with a mean BMI of 22.71±3.72 kg/m^2 in the endometrioma group and 24.92±4.95 kg/m^2 in the benign cyst group. Thus, most of the subjects with endometriosis and benign cysts who underwent surgery at Dr. Sardjito General Hospital had normal BMI and underwent surgery in their late 30s.

All subjects with endometriosis who underwent surgery at Dr. Sardjito General Hospital were assessed for severity grades during surgery according to the classification of the American Society for Reproductive Medicine (ASRM) into grades 1, 2, 3, and 4. Among 23 endometriosis cases diagnosed with endometrioma histopathologically, most of the research subjects were classified in severe endometriosis with a total of 91.3%. While 73.9% were grade 4, 17.4% were grade 3 and 8.7% were grade 2 (Table 1).

PR-A and PR-B Expressions

The IHC examinations of PR-A and PR-B expressions represent the activity of PR-A and PR-B. PR-A and PR-B staining scores were obtained in the form of a percentage of the number of stained nuclei (immunostained) compared to the total number of nuclei in the infected tissue observed under a microscope. The H-score of PR-A and PR-B expressions obtained was 1.00. The intra-class correlation

Table 1. Characteristics of research subjects.

Characteristics	Endometrioma (n=23)	Benign Cyst (n=22)	*p* -value[a]
Age (years), Mean±SD	36.00±5.40	38.41±11.62	0.374
BMI, Mean±SD	22.71±3.72	24.92±4.95	0.080
Parity, Mean±SD	0.65±0.88	1.09±1.23	0.252
Endometriosis Grade, n (%)			
1	0 (0.00)	-	
2	2 (8.7)	-	
3	4 (17.4)	-	
4	17 (73.9)	-	
Dermoid	-	5 (22.7)	
Functional	-	7 (31.8)	
Serous	-	10 (45.5)	
Endometriosis, n (%)			
Severe-moderate	21 (91.3)	-	
Mild	2 (8.7)	-	

[a]Tested with Mann Whitney test.

value of 0.8 indicated that there was a strong match between the two observers. A brownish change of color of the cell nucleus with varying intensity indicates the expressions of PR-A and PR-B, as follows: negative (0), weak intensity (1), moderate intensity (2), or strong intensity (3). PR-A and PR-B receptors are confirmed positive if more than 15% of cells show that PR-A and PR-B expressions in the cell membrane or cytoplasm were found. Figures 1 and 2 were the results of semi-quantitative measurements using the H-scoreof PR-A and PR-B expression in slide samples observed from the stromal and glandular layers.

Based on the normality test results, PR-A was found to be normally distributed so independent t-tests were performed to calculate the mean ratio and SD. Meanwhile, for PR-B, PR-B/A, and TNF-α ratio, Mann Whitney tests were conducted because the data were not normally distributed. Based on the results of the mean comparison test, there was no statistically significant difference (p>0.05) in all variables between the two groups.

Mean of PR-A expressions in endometriomas group (252.00±55.45) was higher than in benign cysts group (245.01±60.85), but there was no significant difference between the two groups (p=0.689). Meanwhile, the PR-B expression in endometriomas group (144.98±27.09) was lower than in benign cysts (158.84±64.95), yet there was also no significant difference between the two groups (p=0.482). And for the PR-B/A ratio, there was a slight difference in the mean of the two groups, with a mean of 0.60±0.17 in endometriomas group and 0.68±0.28 in benign cysts group, but it was not statistically significant (p=0.555) (Table 2).

TNF-α Expression

TNF-α expression involves proinflammatory cytokines found in the cytoplasm. Assessment of IHC staining is defined as a positive expression if a brownish appearance was found on the cytoplasmic and intracytoplasmic membranes. The IHC examination of TNF-α represents the inflammatory activity of endometriosis. After the IHC examination, TNF-α stain scoring was done. Brownish discoloration of the cytoplasm with varying intensity indicates the expression of TNF-α.

The results of the IHC examination of TNF-α showed that the color intensity appears as staining on the cytoplasm and cell membranes (Figure 3). Although there were not any statistically significant differences between the mean values of TNF-α expression found, it was found that the mean of TNF-α expression was higher in endometriomas group with a mean value of 6.65±4.43 compared to the benign cysts group with a mean value of 4.23±4.19 (Table 2).

Mean Differences of PR-A, PR-B, PR-B/A Ratio, and TNF-α in Grade 4 and ≤3 Endometriosis

In this study, the endometriosis grade was classified as grade 4 and grade ≤3. To depict the diagnostic accuracy and to determine the optimal cut-off value the receiver operating curve (ROC) curve was used to classify the grade of endometriosis on the H-score value from PR-A, PR-B, PR-B/A ratio, and TNF-α. H-score assessment shows a value of 0-300. From the assessment using the ROC curve, the cut-off value of PR-A expression H-score was 233.9 with a sensitivity of 37.5% and a specificity of 85.7 %, PR-B was 154 with a sensitivity of 43.8% and a specificity of 100%,

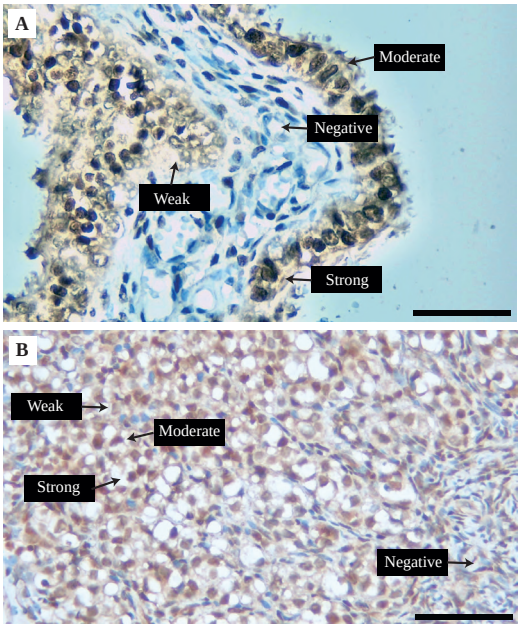

Figure 1. Immunostaining of the PR-A expression in endometriomas group (A) and benign cysts group (B). Brown: positive immunostaining; blue, negative immunostaining. Black bar: 100 μm.

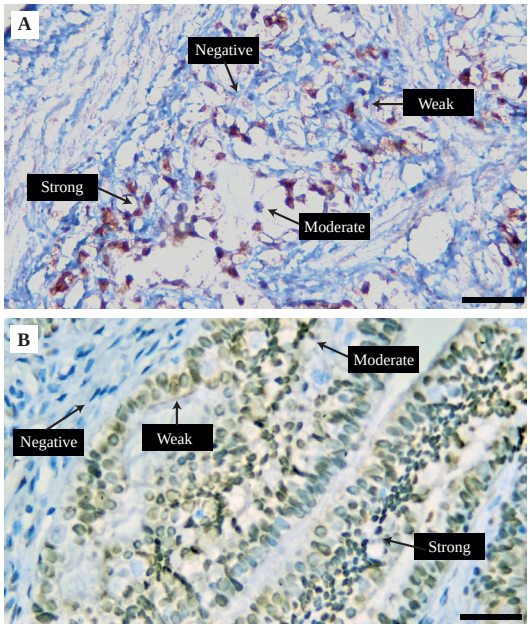

Figure 2. Immunostaining of the PR-B expression in endometriomas group (A) and benign cysts group (B). Brown: positive immunostaining; blue, negative immunostaining. Black bar: 100 μm.

the PR-B/A ratio was 0.65 with a sensitivity of 50.0% and a specificity of 100%, while for TNF-α, it was 7.5 with a sensitivity of 75% and a specificity of 85.7%.

Independent t-tests were performed to calculate the mean values of PR-A, PR-B, PR-B/A ratio, and TNF-α expressions in grade 4 and ≤3 endometriosis (Table 3). There was a significant difference in the mean TNF-α expression level found in grade 4 and ≤3 endometriosis (p=0.012), where the mean value of TNF-α was found higher in grade ≤3 endometriosis compared to grade 4. Meanwhile, the mean expressions of PR-A, PR-B, and the ratio of PR-B/A did not show any significant differences. PR-A expression in grade 4 endometriosis had a mean value 251.85±61.28, which was lower than in grade ≤3 with a mean of 252.34±43.40. However, there was no significant difference between the two groups (p=0.985). PR-B expression in grade 4 endometriosis had a mean value of 149.67±29.94, which was higher than grade ≤3 with a mean of 134.27±16.04, yet no significant difference between the two groups (p=0.218). The PR-B/A expression ratio in endometriosis grade 4 (0.63±0.197) was higher than in grade ≤3 (0.54±0.65), but there was no significant difference between the two groups (p=0.245).

Correlation between Expressions of PR-A, PR-B, PR-B/A Ratio with TNF-α in Benign Cysts

There were no significant correlation of the benign cysts group between PR-A and TNF-α (p=0.232) after the the Pearson correlation test. Meanwhile the Spearman correlation test results between PR-B and TNF-α as well as between the ratio of PR- B/A with TNF-α also showed no significant correlations (p=0.242, p=0.676, respectively).

Table 2. Mean values of PR-A, PR-B, PR-B/A ratio, and TNF-α expression.

Parameters	Mean±SD		*p* -value
	Endometrioma (n=23)	Benign Cyst (n=22)	
PR-A	252.00±55.45	245.01±60.85	0.689[a]
PR-B	144.98±27.09	158.84±64.95	0.482[b]
TNF-α	6.65±4.43	4.23±4.19	0.055[b]
PR-B/A Ratio	0.60±0.17	0.68±0.28	0.555[b]

[a]Tested with Independent t-test; [b]Tested with Mann-Whitney test.

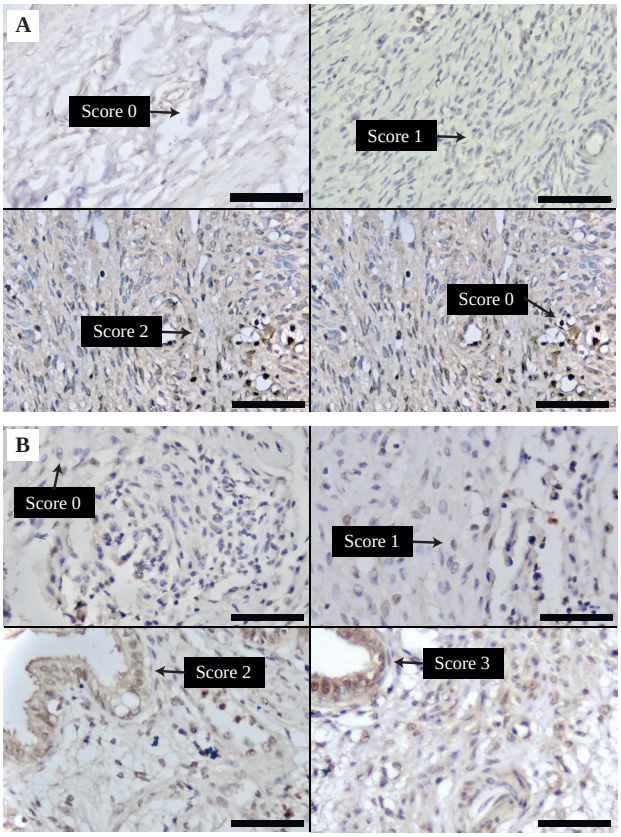

Figure 3. The staining intensity and TNF-α expression of endometriomas group (A) and benign cysts group (B). Brown: positive immunostaining; blue, negative immunostaining. Black bar: 100 μm.

Thus, this study found no significant correlations between the expressions of PR-A, PR-B and the ratio of PR-B/A with TNF-α in the benign cysts (Table 4).

Correlation Test between Expressions of PR-A, PR-B, PR-B/A Ratio with TNF-α and Endometriosis Grade

In the endometrioma group, no significant correlation between PR-A and TNF-α was found (p=0.950). Meanwhile, there was also no significant correlation between PR-B and TNF-α as well as between PR-B/A ratio and TNF-α (p=0.834, p=0.950, respectively) (Table 5). Additionally, no significant correlations were found in the correlation tests performed between PR-A, PR-B, PR-B/A ratio and the grade of endometriosis.

Discussion

The age characteristics of subjects in this study is similar with the characteristic of retrospective population-based study involving 6.146 women aged 15-55 where the incidence rate of endometriosis per year is 7.2 per 10.000 women with the highest incidence in the age range of 25-39 (13), with the mean age when patients are given the definitive diagnosis through surgery in this study was 38.41 years old. Although theoretically the pathogenesis of endometriosis occurs since adolescence. The long delay in diagnosis between the early pathogenesis and the time of diagnosis can be influenced by various factors. For example, these include the high cost of diagnosis and the delayed appearance of accompanying symptoms that may lead to misdiagnosis.(14) This delay in diagnosis is also related to the severity of endometriosis at the time of diagnosis. As can be seen in Table 1, most of the patients who underwent the surgery showed severe endometriosis with grades 3-4, with 73.9% were patients with grade 4, grade 3 was 17.4%, and grade 2 was 8.7%.

Most of the patients with endometriosis and benign cysts who underwent surgery at Dr. Sardjito General Hospital in the period of August 2019 to December 2020 were patients with a normal BMI. More than 50% of patients with endometriosis are also classified as normal BMI, with a mean BMI of 24.1±6.4 kg/m^2.(15) The relation between BMI and the pathogenesis of endometriosis is still controversial, with evidence showing that the majority of patients with endometriosis have a normal BMI or were even underweight. In a meta-analysis involving studies

Table 3. Mean value differences of PR-A, PR-B, PR-B/A ratio, and TNF-α in grade 4 and ≤ 3 endometriosis.

Parameters	Grade	n	Mean±SD	p-value[a]
PR-A	4	16	251.86±61.28	0.985
	2-3	7	252.34±43.40	
PR-B	4	16	149.69±29.95	0.218
	2-3	7	134.27±16.05	
TNF-α	4	16	5.25±3.55	0.012*
	2-3	7	10.00±4.47	
PR-B/A Ratio	4	16	0.63±0.20	0.245
	2-3	7	0.54±0.06	

[a]Tested with Independent t-test, *significant if p<0.005.

Table 4. The correlation between expression of PR-A, PR-B, PR-B/A ratio with TNF-α in benign cysts.

Parameters	TNF-α	
	r	*p* -value
PR-A	0.266	0.232[a]
PR-B	0.260	0.242[b]
PR-B/A Ratio	0.094	0.676[b]

[a]Tested with Pearson correlation test; [b]Tested with Spearman correlation test.

of various ethnicities on several continents, it was shown that an increase in BMI of 5 kg/m^2 reduced the risk of developing endometriosis by 33% (OR: 0.67; 95% CI: 0.53-0.84). Thus, the meta-analysis found an inverse relationship between BMI and the incidence of endometriosis.(16)

Maximal PR-A and PR-B in expressions in the late proliferative phase and stromal PR-A expression increased temporarily in the mid-secretory phase, followed by its gradual decrease and reached its lowest point at the end of the secretory phase.(5) Studies that evaluate superficial peritoneal endometriosis lesions or ovarian endometriomas showed that PR expression loses the menstrual cycle pattern (17), with low PR-B level (17,18), and even sometimes was not detected (7). Also, so far, PR-A receptors are the dominant PR isoforms that are expressed in endometriosis lesions.(7,18) It is obvious that PR-B levels are reduced much more in the lesions than in the eutopic endometrium of women with endometriosis.(7,10,18) However, if compared with normal endometrium, immunoreactivity of PR-B is suppressed in deep infiltrating endometriosis and even more so in endometriomas.(18) Those theories match the results from this study which obtained PR-A expression with a mean value of 252.00±55.45 which was higher in endometriomas than in benign cysts with 245.01±60.85, PR-B expression with a mean value of 144.98±27.09 which was lower in endometriomas than in benign cysts with a mean of 158.84±64.95. While for the PR-B/A ratio there was a slight difference with a mean of 0.60±0.17 in endometriomas which were slightly lower than in benign cysts 0.68±0.28, although not statistically significant. In this study, the menstrual phase data were incomplete in both groups, so it was not possible to assess the PR-A and PR-B expressions according to the menstrual phase, especially in the benign cyst group where the PR-A and PR-B expressions still matched with the pattern of the menstrual cycle. And also, the IHC assessment of PR-A and PR-B did not differentiate samples in the glandular or stromal areas. It is possible that this also causes the expressions of PR-A and PR-B, especially in benign cysts, to not be assessed optimally which leads to no significant difference from the assessment of the expressions of both PRs. Endometriosis is linked by deviations of cellular and humoral resistance. (19) The peritoneal liquefied of women with endometriosis contains of cytokines and growth factors, which take a part in endometriotic progress and preservation.(5)

TNF-α is a pro-inflammatory cytokine. The TNF-α level was found to be increased in peritoneal fluid and serum in women with endometriosis but several cell types are now known to produce it, including endometriotic lesion cells. *In vitro*, show that cytokine-stimulated cellular favor the construction and development of endometriosis, for example about the adhesion, proteases induction, and inflammatory mediators (20). Patients with endometriosis exhibit significantly higher levels of TNF-α in the peritoneal fluid compared to controls (p<0.0001). This theory is in accordance with the results of this study where the mean TNF-α expression in endometriomas was found to be higher with a mean of 6.65±4.43 compared to benign cysts with a mean of 4.23±4.19, even though there were no significant differences. Previous studies revealed that higher TNF-α was obtained from peritoneal fluid samples, while in this study TNF-α was taken from endometrioma samples. Thus, there is a chance that this is also causing only weak correlations between TNF-α with PR-A, PR-B, and PR-B/A and also with the severity of endometriosis that were found in this study. A non-significant result is possible due to the samples are not distinguished by the endometrial phase,

Table 5. The correlation between the expression of PR-A, PR-B, PR-B/A and TNF-α with grade of endometriosis.

Parameters	TNF-α		Endometriosis Grade	
	r	*p* -value	r	*p* -value
PR-A	0.014	0.950[a]	-0.047	0.832[a]
PR-B	0.046	0.834[b]	0.152	0.489[b]
PR-B/A	-0.014	0.950[b]	0.283	0.191[b]
TNF-α	-	-	-0.389	0.067[b]

[a]Tested with Pearson correlation test; [b]Tested with Spearman correlation test.

according to the PR theory (PR-A and PR-B) are found in large quantities in the early proliferative phase, then will overflow in the late proliferative phase and decrease in the early secretory phase and minimal expression in the end secretory phase and did not differentiate PR-A and PR-B in the glands and stroma, where it is known that PR-A and PR-B are more abundant in the gland area.(4) Sampling by differentiating the endometrial phase and calculating IHC by differentiating the stromal and glandular areas will probably give more significant results.

Progesterone resistance is defined as a subnormal cellular response to the natural effect of progesterone and to therapeutic progestin. It is characterized by low PR-B transcription such as the one found in endometriosis tissue, where a lack of PR-B transcription, translation and biological activity were found. This defect may be hereditary, or acquired through epigenetic effects from inflammatory conditions.(6,21) Thus, it is concluded from this theory that a low PR-B/A ratio shows the presence of progesterone resistance.(6) The condition of progesterone resistance will predict the possibility of therapeutic failure of using progesterone in endometriosis.(9)

This study will be able to assist in clinical practice to determine medical therapy using progestins in patients with endometriosis more accurately, by examining PR-A and PR-B expressions of endometrial tissue after surgery. This can be done by using histochemistry staining methods which are relatively easier than other methods and can be done in almost any type of hospital with anatomical pathologists and using ImageJ application which is easily accessible and used to assist H-score assessment.

Conclusion

Based on the H-score value in endometrial tissue, the PR-A expression is higher than PR-B expression and PR-B/A ratio is lower than in benign cysts. Whereas TNF-α expression was higher than in benign cysts. Furthermore, it is known that there were no significant correlations found between the expressions of PR-A and PR-B nor the ratio of PR B/A with TNF-α expression in patients with endometrioma.

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Authors Contribution

EY and SW were involved in the research conception and design. EY processed the experimental data, analysis, and drafted the manuscript. AD provided input and manuscripts reviews. All authors discussed the results and commented on the manuscript.

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