

ORIGINAL ARTICLE:**Higher IL-1 β level in the follicular liquid of endometriosis compared with non-endometriosis patients****Cahyono Hadi,^{1*} Djaswadi Dasuki,² Ahmad Hamim Sadewa,³ Indwiani Astuti,⁴ Awal Prasetyo⁵**¹Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Faculty of Medicine Diponegoro University, Indonesia, ²Department of Obstetrics and Gynecology, Faculty of Medicine Gadjah Mada University, ³Department of Biochemistry, Gadjah Mada University, ⁴Department of Pharmacotherapy, Molecular Biology, Faculty of Medicine Gadjah Mada University, ⁵Department of Biomedical Science, Faculty of Medicine Diponegoro University, Indonesia**ABSTRACT****Objective:** To ascertain the IL-1 β level in the follicular liquid of endometriosis sufferers compared with that of non-endometriosis sufferers.**Materials and Methods:** The ELISA method, for detecting the IL-1 β , is taken from follicular fluid of endometriosis as well as non-endometriosis patients that diagnosed according to the ASRM criteria.**Results:** The 54 subjects were dominantly endometriosis <34 year old (88.9%). The IL-1 β levels of endometriosis patients and non-endometriosis patients were 82.86 ± 30.15 and 56.05 ± 23.27 respectively ($p=0.01$). The ROC value of IL-1 β was found at a cut-off point of 26 (with the IL-1 β cut-off point of 65.16). The discriminating value of AUC (area under curve) was 59.3%.**Conclusion:** It was concluded that the IL-1 β level in endometriosis is higher than that in non-endometriosis.**Keywords:** Gene Polymorphism of Interleukin-1 β (IL-1 β) level; endometriosis**ABSTRAK****Tujuan:** Untuk mengetahui kadar IL-1 β dalam cairan folikel penderita endometriosis dibandingkan dengan penderita non endometriosis.**Bahan dan Metode:** Metode ELISA untuk mendeteksi IL-1 β diambil dari cairan folikuler endometriosis serta pasien non endometriosis yang didiagnosis sesuai kriteria ASRM.**Hasil:** 54 subjek didominasi endometriosis berusia <34 tahun (88,9%). Kadar IL-1 β penderita endometriosis dan non endometriosis masing-masing adalah $82,86 \pm 30,15$ dan $56,05 \pm 23,27$ ($p = 0,01$). Nilai ROC IL-1 β ditemukan pada titik potong 26 (dengan titik potong IL-1 β 65,16). Nilai pembeda AUC (area di bawah kurva) adalah 59,3%.**Simpulan:** Disimpulkan bahwa kadar IL-1 β pada endometriosis lebih tinggi dibandingkan pada nonendometriosis.**Kata kunci:** Gene Polymorphism of Interleukin-1 β (IL-1 β) level; endometriosis***Correspondence:** Cahyono Hadi, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Faculty of Medicine Diponegoro University, Indonesia. E-mail: cahyono.hadi007@gmail.com, cahyonohadi@mail.ugm.ac.idpISSN:0854-0381 • eISSN: 2598-1013 • doi: <http://dx.doi.org/10.20473/mog.V28I22020.59-66>

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INTRODUCTION

Endometriosis is a common gynecological disorder marked by the growth of the endometrial tissue (stroma and gland) outside the uterine cavity, and this disease affects at least 10% of women of the reproductive age. The prevalence of endometriosis varies depending on the geographical location and race, and is the highest in Japan.¹ In the United States, as much as 6 – 10 % of endometriosis cases have been documented whereas there are no definite figures regarding this disease in Indonesia.²

In women with endometriosis, the immune system will decline, and this condition causes the peritoneal fluid in the peritoneal cavity to show a change in the levels of prostanoid, cytokine, growth factor, interleukin, and oxidative stress (OS).³ In the immunology system, the cytokine interleukin-1 β (IL-1 β) is known as a product of monocyte activated by macrophage, which has a main role in the regulation of inflammation and immune response. IL-1 β affects the activation of T lymphocytes and differentiation of B lymphocytes as a good receptor agonist. In women with endometriosis, IL-1 β increases in the peritoneal fluid.⁴ In another research, IL-1 β has been reported to be found in human follicular fluid in a certain amount. IL-1 β is assumed to be involved in the ovulation process such as the protonation synthesis of plasminogen activator regulation activity.⁵ These free radicals will cause oxidative stress in the endometrium.

The role of IL-1 β in stimulating angiogenesis in the endometriotic lesion is assumed to be caused by induction in the angiogenic factor (vascular endothelial growth factor and interleukin-6) of the endometriosis tissues, but not in the normal endometrium.⁴ In the ovary, several researchers stated a hypothesis that IL-1 β is the paracrine factor involved in ovulation.⁶ IL-1 β is secreted in the human ovary.⁷ An in vitro research shows a correlation between the intrafollicular stage of IL-1 β and the quality of oocytes.⁶ Several other studies demonstrated directly and indirectly that IL-1 β may obstruct the maturation and ovulation processes.

Various researches on IL-1 β in follicular fluid have been reported with different results, but research on IL-1 β in follicular fluid and blood serum in humans with endometriosis are extremely limited. This research is expected to ascertain the interleukin-1 β (IL-1 β) level in follicular fluid of endometriosis sufferers in comparison with that of non-endometriosis.

MATERIALS AND METHODS

This study was done at the Permata Hati Clinic of Dr. Sardjito Hospital, Yogyakarta, Indonesia. The indepen-

dent variable was measured during the diagnosis of endometriosis based on clinical examination and laparoscopy, and normal patients who were undergoing sterilization. The ELISA method was used for research on the IL-1 β level. Data collection was done from endometriosis sufferers and normal patients. During laparoscopy, follicular fluid was taken from endometriosis sufferers.

Sample analyses

Samples for research were patients with endometriosis who were diagnosed according to the ASRM criteria and non-endometriosis patients who were undergoing tubectomy. The sampling was done from the 12th up till the 15th day using Storz aspirating needle®, whereas the sampling of non-endometriosis patients was done using syringe. All the endometriosis patients and the controls who showed IL-1 β value were involved as respondents. The research variables comprised of a dependent variable: IL-1 β ; an independent variable: endometriosis; and external variables: ethnic group, age, dysmenorrhea, BMI, and a family history of endometriosis. The laboratory used was the Biochemis-try Laboratory in the Medical Faculty of Gadjah Mada University to study the IL-1 β level using the PCR-RFLP method. Various methods were used in the examination process to ascertain the IL-1 β level using ELISA and PCR-RFLP.

Statistical analysis

Statistical analysis was performed using the Stata intercooled version 11 software program with the following steps: 1) univariate analysis; 2) bivariate analysis; 3) multivariate analysis. The statistical test used were chi-square and multiple linear regression and multinomial logistic regression with a confidence interval (CI) of 95% with $\alpha=0.05$.

RESULTS

This research was conducted at the Permata Hati Fertility Clinic of Dr. Sardjito General Hospital by involving endometriosis patients who joined the laparoscopy program. The research population was 64 patients, all of whom were taken as samples or research subjects. Out of these 64 subjects, 54 were able to join the laboratory examination, and 10 did not continue participating in the research for various reasons, one of which was insufficient samples of ovarian fluid for examination.

Univariate analysis

This research involved 54 subjects consisting of 53 women of the Javanese ethnic group and one woman of

the Padang ethnic group. Figure 1 shows the demographic characteristics and epidemiological distribution of the independent variable. The patients aged 34 years and below were dominantly those with endometriosis, i.e. 88.9%, whereas those >35 years of age were dominantly patients with non-endometriosis, i.e. 59.3%. The majority of endometriosis subjects had parity of 0-1 and all of them were endometriosis patients who joined the ingin anak (want children) program. For non-endometriosis patients, majority had a parity of 2 to 6, who were patients taking the MOW contraception. From the examined patients, 27 with endometriosis had no children.

Characteristics and clinical distribution of the independent variable

Figure 2 shows the Body-Mass Index (BMI) was divided into two, i.e. BMI \geq 23 and < 23. As many as 24

patients (88.9%) with endometriosis and 17 patients (63%) of non-endometriosis had a BMI of \geq 23, while three patients (11.1%) with endometriosis and 10 patients (37%) with non-endometriosis had a BMI of < 23. As many as 25 subjects (92.6%) with endometriosis and 24 normal subjects (88.9%) had a normal menstrual cycle, while two subjects (7.4%) with endometriosis and three normal subjects (11.1%) had an abnormal menstrual cycle. Most of the endometriosis patients (85.2%) had dysmenorrhea. The first menstruation (menarche) occurred to 55.6% of the endometriosis and non-endometriosis patients at the age of 11-13. Menarche at the age of 14-16 occurred to 44.4% of both endometriosis and non-endometriosis patients. These were more endometriosis patients than non-endometriosis patients who complained of pain during sexual intercourse. 11.1% of endometriosis patients had a family history of endometriosis, but none of the non-endometriosis patients had a family history.

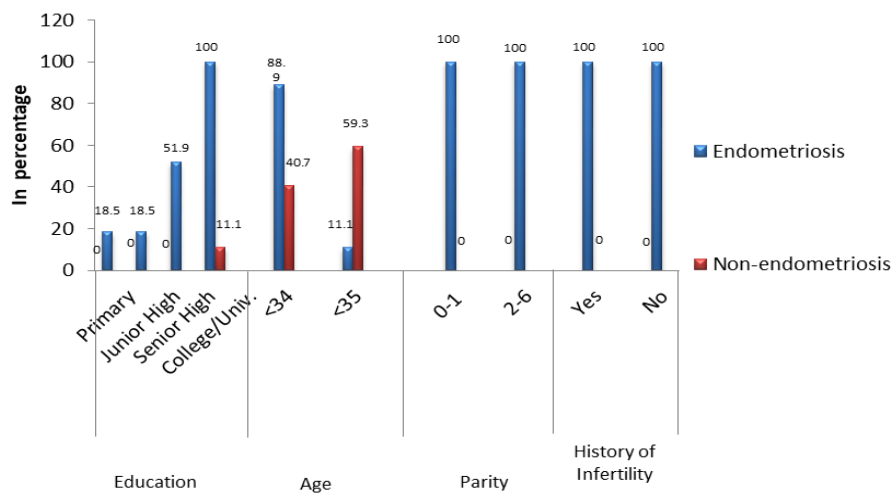


Figure 1. Demographic characteristics and epidemiological distribution of the respondents

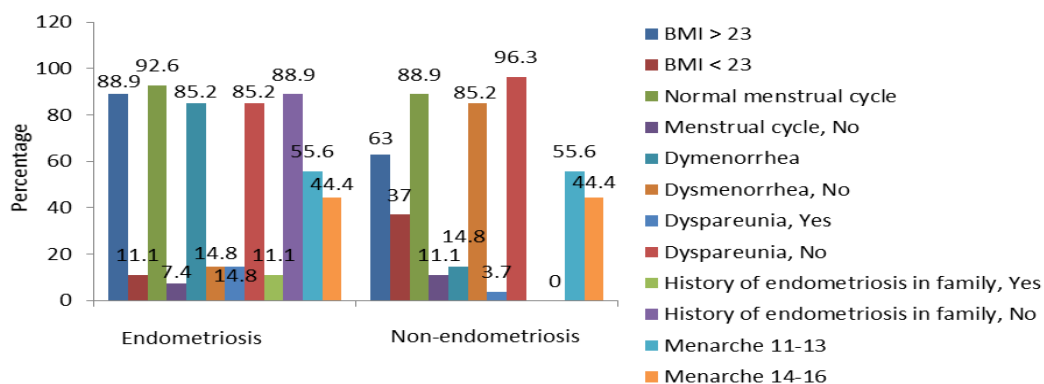


Figure 2. Clinical characteristics of respondents

Clinical characteristics of endometriosis patients

Figure 3 shows the clinical characteristics and distribution of endometriosis patients. Laparoscopy examination for endometriosis of grades 2, 3, and 4 showed that the disease dominantly occurred at grade 4, i.e. 48.15%. Endometriosis occurred more in patients who had chocolate cyst (+), i.e. 59.26%.

Relation of the endometriosis variable to IL-1β level

Table 1 shows the examination results of the IL-1β level in endometriosis and non-endometriosis patients, which was obtained by aspiration of the follicular fluid from the ovary using the ELISA method. The IL-1β levels of endometriosis patients and non-endometriosis patients were 82.86 ± 30.15 and 56.05 ± 23.27 respectively with p=0.01. The IL-1β level of endometriosis patients was statistically higher than that in non-endometriosis patients.

The mean differences of endometriosis patients according to age, parity, dysmenorrhea, family history of endometriosis, types of endometriosis, and grades of endometriosis with regard to IL-1β are shown in Table 2.

As for the endometriosis variable, the mean IL-1β levels of endometriosis and non-endometriosis patients were 82.86 up to ± 30.15, and 56.05 up to ± 23.27. The mean difference in the IL-1β levels of endometriosis and non-endometriosis patients was 26.81 with p value=0.01 and CI 95%=12.09-41.51. The conclusion from these results was that the differences in the IL-1β levels between endometriosis and non-endometriosis patients were significant. As for parity, dysmenorrhea, types of endometriosis of chocolate cyst (+), and endometriosis grade 4, the differences in the IL-1β levels between endometriosis and non-endometriosis patients were also significant.

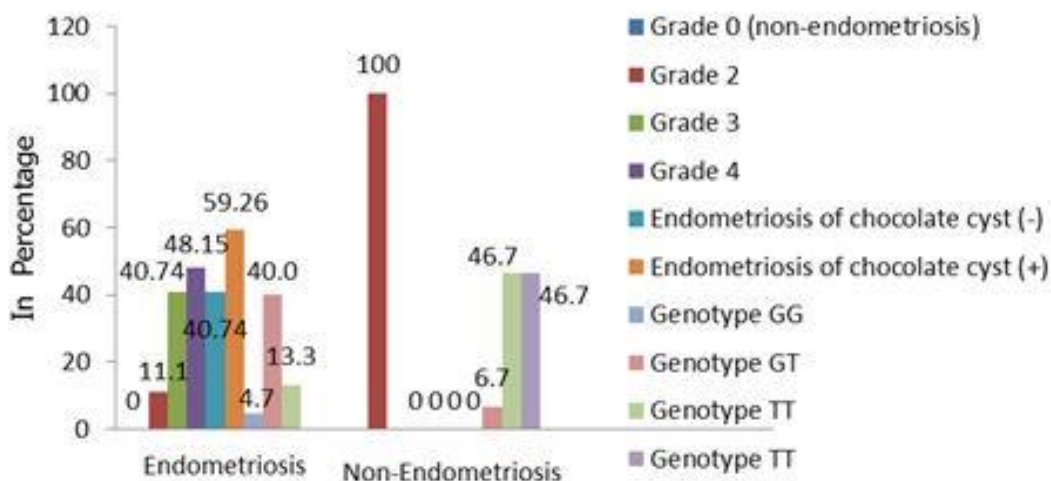


Figure 3. Clinical characteristics and distribution of endometriosis

Table 1. T-test of IL-1β level between endometriosis and non-endometriosis patients

Variable	Endometriosis	Non-Endometriosis	p	Δ	CI 95%
	Mean ± SD	Mean ± SD			
IL-1β	82.86 ± 30.15	56.05 ± 23.27	0.01*	26.81	12.09-41.51

Significant p < 0.05

Table 2. The mean difference of endometriosis patients based on age, parity, dysmenorrhea, family history of endometriosis, types of endometriosis and grades of endometriosis

Variables	IL-1 β Level		P	Δ	CI 95%
	N	Mean \pm SD			
Endometriosis					
Endometriosis	27	82.86 \pm 30.15	0.01*	26.81	12.09-41.51
Non endometriosis (Ref)	27	56.05 \pm 23.27	-	-	-
Mother's age					
\leq 34 years of age	35	74.63 \pm 32.53	0.08	14.71	-2.06-31.49
\geq 35 years of age	19	59.91 \pm 22.08	-	-	-
Parity					
0-1	27	82.86 \pm 30.15	0.01*	26.81	23.09-41.51
2-6	27	56.05 \pm 23.27	-	-	-
Dysmenorrhea					
Yes	27	79.25 \pm 28.96	0.01*	19.60	4.03-35.17
No	27	59.65 \pm 28.03	-	-	-
Family History of Endometriosis					
Yes	3	101.53 \pm 31.19	0.05	33.97	-0.77-68.71
No	51	67.56 \pm 29.05	-	-	-
Endometriosis (Types)					
Chocolate (-)	11	70.91 \pm 28.33	0.11	14.86	-3.96-33.70
Chocolate (+)	16	91.07 \pm 29.40	0.01*	35.02	18.40-51.63
Not Endometriosis (Ref)	27	56.05 \pm 23.27	-	-	-
Endometriosis (Grade)					
0 (Ref)	27	56.05 \pm 23.27	-	-	-
2	3	78.18 \pm 29.54	0.18	22.13	-10.73-55.00
3	11	74.81 \pm 27.23	0.06	18.76	-0.55-38.08
4	13	90.74 \pm 32.78	0.01*	34.69	16.45-52.92

Ref = non-endometriosis, * Significant $p < 0.05$

Multivariate analysis

Multiple linear regression test results for types of endometriosis, grades of endometriosis, dysmenorrhea, and family history of endometriosis with regard to IL-1 β can be seen in Table 3.

Model 1 analysis shows that in the endometriosis variable, the coefficient value obtained was 24.47 (CI 95%=9.39-39.55; $p=0.01$). In the history of endometriosis variable, the coefficient value obtained was 21.01 (CI 95%= -11.89-53.92; $p=0.20$). Endometriosis patients who are known to have a history of endometriosis in the family are likely to be at risk of a 23% increase ($R^2=23\%$) in the IL-1 β level. Model 2 analysis shows that patients with endometriosis of chocolate cyst (-) had a coefficient value of -4.46 (CI 95%=29.93-21.00; $p=0.72$), whereas endometriosis of chocolate cyst (+) had a coefficient value of 26.08 (CI 95%=5.07-47.10; $p=0.01$). In the variable family history of endometriosis, the coefficient value was 25.25 (CI 95% = -5.38-55.90; $p=0.10$). Considering a family history of endometriosis, endometriosis patients were at risk of a 14% increase ($R^2=14\%$) in the IL-1 β level. Model 3 analysis of endometriosis grade 2, the coefficient value obtained was -11.18 (CI 95%= -46.22-23.84; $p=0.52$). In endometriosis grade 3, the coefficient value obtained was 6.17 (CI 95%= -13.99-26.35;

$p=0.54$). In endometriosis grade 4, the coefficient value obtained was 18.77 (CI 95%=0.01-37.53; $p=0.05$). In dysmenorrhea, family history of endometriosis, and interactions of all the above variables, the p values obtained were 0.14, 0.14, and 0.01 respectively. As for patients with endometriosis grade 2, after taking into account dysmenorrhea, family history of endometriosis, and interactions of all the above variables, there was an increase of 38% in the IL-1 β level.

Multinomial logistic regression analysis presented in Table 4 showed the relation of IL-1 β taking into account the BMI between stadium 2 and non-endometriosis between stadium 3 and 4. It also shows the results of a multinomial logistic regression analysis on the relation of IL-1 β level taking into account the BMI variable between stadium/grade 2 of endometriosis and non-endometriosis as well as a combination of stadium 3, 4 with non-endometriosis. The endometriosis of stadium 2 had an insignificant IL-1 β value and BMI. For the combined value of endometriosis of grade 3 and 4, the IL-1 β level had an OR value=1.04 (CI 95%=1.01-1.08 with $p=0.01$), whereas BMI had an OR value=13.63 (CI 95%=1.35-137.13 with $p=0.03$). The above variables showed that statistically, the IL-1 β value and BMI were significant with $p < 0.05$.

Table 3. The multiple linear regression test for types of endometriosis, grades of endometriosis, dysmenorrhea, and family history of endometriosis

Variables	Model 1	Model 2	Model3
	<i>P</i> Coef CI 95 %	<i>P</i> Coef CI 95 %	<i>P</i> Coef CI 95 %
Endometriosis			
Endometriosis	0.01* 24.47 (9.39-39.55)	- - -	- - -
Non-Endometriosis (Ref)			
Endometriosis (Types)			
Chocolate (-)	- - -	0.72 -4.46 (29.93-21.00)	- - -
Chocolate (+)	- - -	0.01* 26.08 (5.07-47.10)	- - -
Not Endometriosis (Ref)			
Endometriosis (Grade)			
2	- - -	- - -	0.52 -11.18 (-46.22-23.84)
3	- - -	- - -	0.54 6.17 (-13.99-26.35)
4	- - -	- - -	0.05 18.77 (0.01-37.53)
Non-Endometriosis (Ref)			
Dysmenorrhea			
Yes	- - -	- - -	0.14 15.29 (-47.63-27.98)
No			
History of Endometriosis			
Yes	0.20 21.01 (-11.89-53.92)	0.10 25.25 (-5.38-55.90)	0.14 15.29 (-47.63-27.98)
No			
Interaction			
Endometriosis X history of endometriosis			
Chocolate (-) X history of endometriosis, Yes	- - -	- - -	0.01* 94.44 25.49-163.40
R ²	0.23	0.14	0.38
N	54	54	54

Ref = non-endometriosis, *Significant $p < 0.05$ Table 4. Multinomial logistic regression analysis : Relation of IL-1 β taking into account the BMI between grade 2 and non-endometriosis grade 3 and 4.

Stadium/Grade	Variables	OR	CI 95%	<i>P</i>
2	IL-1 β	1.03	0.98-1.09	0.17
	BMI			
	≥ 23 <23 (Ref)	2.61 -	0.09-71.95 -	0.56 -
3 4	IL-1 β	1.04	1.01-1.08	0.01*
	BMI			
	≥ 23 <23 (Ref)	13.63 -	1.35-137.13 -	0.03* -
R ²				0.36
Deviance				60.05

Ref = non-endometriosis, * Significant $p < 0.05$

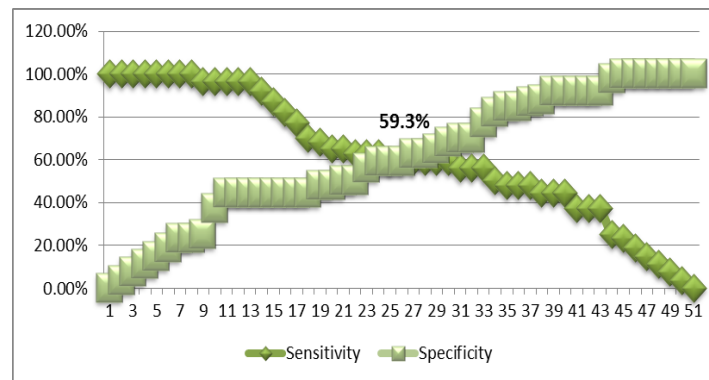


Figure 4. Graphics of the ROC value of IL-1 β

Results of Receiver Operating Characteristics (ROC) analysis

Figure 4 shows that the ROC value of IL-1 β was found at cut-off point 26 (with the IL-1 β cut-off point at 65.16). The discriminating value of AUC (area under curve) was 59.3% and therefore it could be deduced that by using the IL-1 β level with a cut-off point of 65.16, it could detect >59.3% of individuals with endometriosis (AUC value = 59.3% was assumed to have a very weak strength as an filtering test instrument).

The results of this research was not far different from the research results by Hadisaputra and Prayudhana who concluded that the cut-off point of IL-6 level in individuals with endometriosis was 60.3%.⁸ However, this was different from the research results by Boomsma et al., 2009 who found that the cut-off point of IL-1 β was 23.1-31.5.

DISCUSSION

The increased cytokine in the peritoneum of endometriosis sufferers was IL-1 β and this cytokine had an important role in the pathophysiology of endometriosis such as pain and infertility. The mean IL-1 β level in endometriosis was higher than that in non-endometriosis. This increased IL-1 β level will affect folliculogenesis,⁹ which is a developmental process of oocytes in the ovary. If the IL-1 β level in the follicular fluid is high, it is presumed to cause destructions to the oocytes that it cannot be fertilized by sperms and will inhibit pregnancy from occurring (infertility).

The result of this research is corroborated by another study that an increase in the IL-1 β concentration level in the follicular fluid is followed by a decrease in the vascular endothelial growth factor (VEGF) in endometriosis patients.¹⁰ The immunology changes in

the peritoneal fluid and serum of endometriosis patients may also have a role in the pathological changes associated with infertility.¹¹

The bivariate analysis in this research shows that the IL-1 β level between endometriosis and non-endometriosis with the variables of parity, types of endometriosis, dysmenorrhea, family history of endometriosis, types of endometriosis of chocolate cyst (+) and grade 4 endometriosis show a significant difference, both clinically and statistically.

In connection with the IL-1 β level, the endometriosis variable in the types of endometriosis had a higher IL-1 β level than that of non-endometriosis; mothers aged \leq 34 years had a higher IL-1 β level compared with mothers aged $>$ 35; parity of 0-1 had a higher IL-1 β level than parity of 2-6; the presence of dysmenorrhea had a higher IL-1 β level than the absence of dysmenorrhea; and the presence of family history of endometriosis had a higher IL-1 β level than the absence of history of endometriosis in the family.

In the multiple linear regression test, subjects with a family history of endometriosis and subjects without a family history had no significant difference in the mean IL-1 β level, and was likewise for the types of endometriosis between chocolate cyst (+) and chocolate cyst (-). The endometriosis of chocolate cyst (+) had a higher IL-1 β level than endometriosis with chocolate cyst (-) with $p=0.01$. If endometriosis interacted with a family history of endometriosis, the incidence of IL-1 β would increase by 23%. In the division by grades, the IL-1 β expression that showed a significant increase statistically occurred only in grade 4.

In the multinomial regression test to study the effect of IL-1 β on the grades of endometriosis, the effect of IL-1 β on the grade 2 and in the combination of grade 3 and

4 had no significant increase. However, in the dysmenorrhea and age variables, the effect of IL-1 β on the combination of grades 3 and 4 had a significant difference. Endometriosis of chocolate cyst (+) had a significant difference in the IL-1 β level, whereas chocolate cyst (-) had no significant difference in the IL-1 β level, with $p=0.002$. For the dysmenorrhea and age variables <34 years, both showed a significant difference. For endometriosis of chocolate cyst (+), the variable that showed a significant difference in the IL-1 β level was the dysmenorrhea variable, whereas for endometriosis of chocolate cyst (-), both dysmenorrhea and age <34 years showed a significant difference in IL-1 β level.

In this research, the mean IL-1 β of 86 pg/mL was higher than the research carried out by Butcher et al. (1999) in Berlin who found the mean IL-1 β of 27 pg/mL and was lower than the research done by Tao et al. (1997) in Japan with the mean value of measurement of 121-146 pg/mL.

In Indonesia, the research results by Hadisaputra and Prayudhana (2013) who researched the IL-6 level concluded that the IL-6 cut-off point in individuals with endometriosis was 60.3%. The results of this research was not far different from the research results by the current author, but Hadisaputra and Prayudhana (2013) suggested that IL-6 examination is recommended as a laboratory examination for individuals suspected of having endometriosis.

A research by Kyama et al (2008) shows that IL-1 β in endometriosis patients will increase compared with patients without endometriosis.¹² These accords with the results of this research with regard to the IL-1 β level in endometriosis subjects. As mentioned above, the increased IL-1 β level has an effect on the destruction of oocytes. This also corresponds to a research,¹³ which states that the oocyte donation program shows a decreased degree of pregnancy when the oocyte is taken from a donor who suffers from endometriosis. The degree of success is lower when fertilization is done with the IVF method. This is presumably caused by the low quality of oocytes and increased apoptosis.

CONCLUSION

The IL-1 β level in endometriosis is higher than that in non-endometriosis. This research also arrived at an additional finding that the IL-1 β level is higher in endometriosis patients of chocolate cyst (+) than of chocolate cyst (-).

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