

Research Article

Role of TNF- α and Interleukin 6 Serum against Ovarian Reserve in Endometriosis Cysts

Peran TNF – α dan Interleukin 6 Serum terhadap Cadangan Ovarium pada Penderita Kista Endometriosis

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Abstract

Objective: To investigate the correlation between pro-inflammatory factors (TNF- α and IL-6) with ovarian reserve in patients with endometriosis cysts.

Methods: This is a cross sectional study. The number of samples is 25 people. Sampling technique consecutive sampling. The study began in June 2019 until the number of samples was fulfilled at the Obstetrics and Gynecology Polyclinic of the Dr. General Central Hospital M. Djamil Padang and at the Biomedical Laboratory of the Faculty of Medicine, Andalas University, Padang. To determine the correlation of TNF- α , IL-6, and AMH in patients with endometriosis cysts. Examination of TNF- α , IL-6, and AMH levels was carried out in a quantitative manner, using the ELISA method.

Result: The mean AMH level was 2 ± 0.40 pmol / L and TNF- α level was 11.16 ± 4.79 pg / ml. Correlation of AMH level with TNF- α indicates the value of $r = -0.049$ which has weak strength and negative pattern means that the higher the TNF- α level, the lower the AMH level. The analysis showed that there was no correlation between TNF- α levels and AMH levels in patients with endometriosis cysts ($p > 0.05$). Correlation of AMH levels with IL-6 shows the value of $r = 0.35$ which has moderate strength and positive pattern means that the higher the IL-6 level, the higher the AMH level. The analysis showed that there was no correlation between IL-6 levels and AMH levels in patients with endometriosis cysts ($p > 0.05$).

Conclusions: There is a negative correlation of TNF- α levels with AMH levels in patients with endometriosis cysts, namely the higher TNF- α levels, the lower AMH levels and there is a positive correlation of IL-6 levels with AMH levels in patients with endometriosis cysts. Where the higher levels of IL-6, the higher levels of AMH.

Keywords: AMH, endometriosis, TNF- α , IL-6.

Abstrak

Tujuan: Mengetahui korelasi faktor pro inflamasi (TNF- α dan IL-6) dengan cadangan ovarium pada penderita kista endometriosis.

Metode: Penelitian ini merupakan penelitian analitik kuantitatif dengan desain studi potong lintang. Jumlah sampel sebanyak 25 orang. Teknik Pengambilan sampel consecutive sampling. Penelitian ini dimulai Juni 2019 sampai dengan jumlah sampel terpenuhi di Poliklinik Obstetri dan Ginekologi Rumah Sakit Umum Pusat Dr. M. Djamil Padang dan di Laboratorium Biomedik Fakultas Kedokteran Universitas Andalas Padang. Untuk mengetahui korelasi TNF- α , IL-6, dan AMH pada penderita kista endometriosis. Pemeriksaan kadar TNF- α , IL-6, dan AMH dilakukan dengan cara kuantitatif, menggunakan metode ELISA.

Hasil: Rerata kadar AMH adalah $2 \pm 0,40$ pmol/L dan kadar TNF- α $11,16 \pm 4,79$ pg/ml. Korelasi kadar AMH dengan TNF- α menunjukkan nilai $r = -0,049$ yang memiliki kekuatan lemah dan berpola negatif artinya semakin tinggi kadar TNF- α maka semakin rendah kadar AMH. Hasil analisis menunjukkan bahwa tidak terdapat korelasi antara kadar TNF- α dengan kadar AMH pada penderita kista endometriosis ($p > 0,05$). Korelasi kadar AMH dengan IL-6 menunjukkan nilai $r = 0,35$ yang memiliki kekuatan sedang dan berpola positif artinya semakin tinggi kadar IL-6 maka semakin tinggi kadar AMH. Hasil analisis menunjukkan bahwa tidak terdapat korelasi antara kadar IL-6 dengan kadar AMH pada penderita kista endometriosis ($p > 0,05$).

Kesimpulan: Terdapat korelasi negatif kadar TNF- α dengan kadar AMH pada penderita kista endometriosis, yaitu semakin tinggi kadar TNF- α maka semakin rendah kadar AMH dan terdapat korelasi positif kadar IL-6 dengan kadar AMH pada penderita kista endometriosis. Dimana semakin tinggi kadar IL-6 maka semakin tinggi kadar AMH.

Kata kunci: TNF- α , IL-6, AMH, endometriosis.

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INTRODUCTION

Endometriosis is one of the common gynecological abnormalities suffered by women of reproductive age in which stromal and endometrial glands can be found outside the normal location. not yet known with certainty.¹ However, some studies suggest that around 6-10% of women of childbearing age experience endometriosis with an average age of patients around 28 years.¹⁻²

Endometriosis is a local pelvic inflammatory process with changes in the function of immune-related cells, so that the serum of women with endometriosis increases the number of active macrophages that secrete products such as growth factors and cytokines.³ Cytokines produced include interleukin 6 (IL-6), vascular endothelial growth factor (VEGF), and tumor necrosis factor α (TNF- α). Interleukin 6 is considered to play a potential role in the growth and / or maintenance of ectopic endometrial tissue. Interleukin 6 is an inflammatory and immune regulator that modulates the secretion of other cytokines, promotes T-cell activation and B-cell differentiation, and inhibits the growth of various cells.⁴ TNF increases in peritoneal and serum search for patients with endometriosis, and it is said that TNF is an essential factor of pathogenesis of endometriosis.⁵ The importance of TNF in the pathogenesis of endometriosis is further supported by the proliferative effect of TNF observed in endometrial cells of women with endometriosis but not in cells of healthy controls.⁶ Also, TNF in high concentrations appears to affect sperm motility in vitro and may have an embryotoxic effect.⁷ These findings suggest that TNF may have an additional role in the development of infertility related to endometriosis.

Anti Müllerian Hormone (AMH) is specifically produced by granulosa cells of preantral follicles and antral follicles. AMH is relatively stable during the menstrual cycle in normoovulatory women. IL-1 β , IL-6, and serum TNF- α levels were significantly higher in women with endometriosis compared with healthy women. in patients with endometriosis increased TNF- α correlates with poor oocyte quality due to damage in the luteal phase due to increased TNF- α . So in patients with endometriosis anovulation will occur.⁸ Chronic inflammation is a pathogenesis of endometriosis.

Several studies have shown that in endometriosis women there is an increase in the volume of peritoneum fluid, as well as an increase in the concentration of prostaglandins, proteases, cytokines including IL-1, IL6, MCP-1, and TNF- α , angiogenic cytokines (IL-8) and VEGF produced by macrophages. Recent studies have found higher chemotactic activity in macrophages in the peritoneum fluid.⁹

Increasing the number of macrophages can damage oocytes and zygocytes thereby disrupting the fertilization process. In addition, endometriotic cells and macrophages are both responsible for high levels of reactive oxygen and nitrogen which adversely affect sperm implantation. Experiments in mice by adding IL6 resulted in decreased embryonic development and decreased sperm motility. The above inflammatory state also has toxic effects on gametes and embryos and interferes with tubal motility.¹⁰

METHODS

This was a cross-sectional study. Subjects were patients suffering from endometriosis cysts who underwent surgery in the FER subsection of Dr. General's Central Hospital M. Djamil Padang who met the inclusion criteria, in June 2019. The inclusion criteria were women of reproductive age (18 - 35 years old) who were diagnosed with endometriosis cysts (grade III-IV) by ultrasound. Exclusion criteria are patients with acute inflammatory diseases such as: acute bronchitis, cough, influenza, skin lesions, acute appendicitis, dermatitis, tonsillitis, infectious meningitis, sinusitis, physical trauma, patients with chronic inflammation such as asthma, gastric ulcer, tuberculosis, rheumatoid arthritis, periodontitis, crohn's disease, sinusitis, active hepatitis, operative laparoscopic procedures on the ovary, hormonal therapy, ovarian removal laparotomy, patients with polycystic ovary syndrome, patients with congenital abnormalities in the ovary and patients who have undergone chemotherapy and radiotherapy . Examination of TNF- α , IL-6, and AMH levels was carried out in a quantitative manner, using the ELISA method. Data were analyzed by T-test to. Data were processed with the help of Statistical Product and Service Solutions (SPSS) for Windows version 22.0

RESULTS

Table 1. Subject Characteristic

Characteristics	f (%)	Mean \pm SD
Age		29.40 \pm 3.62
BMI		23.16 \pm 2.51
Marriage history		
Married	23 (92)	
Single	2 (8)	
Infertile		
Yes	22 (88)	
No	3 (12)	
Cyst size > 3cm		
Yes	25 (100)	
No	0 (0)	
Kind of cyst		
Monolokulare	20 (80)	
Multilokulare	5 (20)	

Interpretation: The mean age of the respondents was 29.40 \pm 3.62 years and BMI 23.16 \pm 2.51. A total of 23 respondents (92%) were married, experienced infertility, namely 22 respondents (88%), all respondents (100%) had cyst sizes of more than 3 cm, and 20 respondents (80%) had monolokulare cyst types.

Table 2. Correlation of TNF- α Level dan AMH Level

Variable	Mean \pm SD	r	P-value
AMH level	2 pmol/L \pm 0.40	-0.049	0.82
TNF- α level	11.16 pg/ml \pm 4.79		

Interpretation. The mean AMH level was 2 \pm 0.40 pmol / L and the TNF- α level was 11.16 \pm 4.79 pg / ml. Correlation of AMH level with TNF- α indicates the value of r = -0.049 which has weak strength and negative pattern means that the higher the TNF- α level, the lower the AMH level. The analysis showed that there was no correlation between TNF- α levels and AMH levels in patients with endometriosis cysts (p > 0.05).

Table 3. Correlation of IL-6 Levels and AMH Levels in Patients with Endometriosis Cysts

Variable	Mean \pm SD	r	P-value
AMH level	2 pmol/L \pm 0.40	0.35	0.09
IL-6 level	17.15 pg/ml \pm 3.48		

Interpretation. The mean AMH level was 2 \pm 0.40 pmol / L and the IL-6 level was 17.15 \pm 3.48 pg / ml. Correlation of AMH levels with IL-6 shows the value of r = 0.35 which has moderate strength and positive pattern means that the higher the IL-6 level, the higher the AMH level. The analysis showed that there was no correlation between IL-6 levels and AMH levels in patients with endometriosis cysts (p > 0.05).

RESULTS

Endometriosis is a local pelvic inflammatory process with changes in the function of immune-related cells, so that the serum of women with endometriosis increases the number of active macrophages that secrete products such as growth factors and cytokines.⁶ Cytokines produced include interleukin 6 (IL-6), Vascular Endothelial Growth Factor (VEGF), and tumor necrosis factor α (TNF- α).¹¹

Interleukin 6 is considered to play a potential role in the growth and / or maintenance of ectopic endometrial tissue. Interleukin 6 is an inflammatory and immune regulator that modulates the secretion of other cytokines, promotes T-cell activation and B-cell differentiation, and inhibits the growth of various cells.⁴ TNF increases in peritoneal and serum search for patients with endometriosis, and it is said that TNF is an essential factor of endometriosis pathogenesis.¹²

Until now, the exact mechanism of decreased ovarian reserve in patients with endometriosis cysts is still being debated. One important factor that occurs in endometriosis cysts is the presence of a chronic inflammatory process that involves an immunocellular mechanism and an increased apoptotic mechanism. It is suspected that the chronic inflammatory process that occurs in endometriosis cysts will cause damage to the follicles contained in the ovaries. Damage to the ovarian follicle, especially to the antral follicle, will cause a decrease in the number of follicles or ovarian reserves.¹³ Women with endometriosis have a higher incidence of oocyte apoptosis, more cell cycle changes, and a higher incidence of oxidative stress than women with infertility caused by other pathologies such as the fallopian, male, and idiopathic factors. Patients with advanced stage endometriosis show a higher incidence of apoptosis in granulosa cells.¹¹

Endometriosis contributes to low pregnancy rates, both in the normal ovulation cycle, as well as intrauterine insemination, in vitro fertilization, and embryo transfer. The fertility cycle rate (CFR) in endometriosis women is 2-10% and the prevalence of endometriosis is higher in infertile women than in fertile women. TNF- α values in follicular fluid correlate with poor oocyte quality¹⁴. Tumor necrosis factor α is secreted by activated macrophages, and has a strong inflammatory, cytotoxic and angiogenic

effect. High TNF concentrations also have an effect on sperm motility *in vitro*, and may have an embryotoxic effect, causing an infertility effect on endometriosis.¹⁴ Other studies also propose several mechanisms for the emergence of fertility disorders in endometriosis including tubal-ovarian dysfunction (anatomic disorders in the ovaries and tubes), ovulation disorders, hyperprolactinemia, luteinizes un-ruptured follicles, abnormal follicular development, decreased follicular development, decreased estrogen production and increased estrogen production granulosa cell apoptosis), impaired immunity (anti - endometrial antibodies), abnormal peritonium environment (increased peritonium fluid and high cytokine concentrations and macrophage activation), dysregulation of endometrial function.¹⁵

IL-1 β , IL-6, and serum TNF- α levels were significantly higher in women with endometriosis compared with healthy women, where TNF- α levels of 300 pg / ml could be used as initial screening values for endometriosis in adolescents (7a) and Gulden Halis added in their study that in endometriosis patients increased TNF- α correlated with poor oocyte quality due to damage in the luteal phase due to increased TNF- α . so in patients with endometriosis anovulation will occur.¹⁶ TNF- α values in follicular fluid correlate with poor oocyte quality¹⁷. Tumor necrosis factor α is secreted by activated macrophages, and has a strong inflammatory, cytotoxic and angiogenic effect. High TNF concentrations also have an effect on sperm motility *in vitro*, and may have an embryotoxic effect, thereby causing an infertility effect on endometriosis.¹⁸

According to research that IL-6 and TNF- are good markers of endometriosis, in this study it is also mentioned that IL-6 and TNF can be used to distinguish between patients with or not suffering from endometriosis with a high level of sensitivity and high specificity. Hwu et al reported an association between endometrioma and serum AMH levels. in a retrospective study compared to 141 women with endometrioma, there was an increase in AMH in women with endometrioma. In addition, women with bilateral endometrioma had lower AMH levels compared to unilateral endometrioma.¹⁹

In a journal written, endometriosis patients, both eutopic and ectopic, are involved in increasing estrogen production. In addition, an increase and pro-inflammatory cytokines including IL-1 β , TNF- α , IFN- γ and IL-17,

cyclooxygenase-2 (COX-2) and COX-2 will catalyze the synthesis of prostaglandin E2 (PGE2). Prostaglandin and cytokine production has been widely cited as a cause of infertility in women with endometriosis.²⁰ There were six biomarkers selected (plasma concentrations of IL-6, IL-8, TNF- α , high sensitivity C-reactive protein, and cancer antigens CA125 and CA19-9) during the secretory phase or during menstruation that can diagnose minimal-mild and moderate-severe endometriosis with high sensitivity and specificity.²¹

In a study conducted by Folconer et al, this study compared TNF- α levels with AMH concentrations, in patients with endometriosis there was an increase in TNF- α accompanied by a decrease in AMH concentrations which is a marker that there was a decrease in the number of eggs in endometriosis patients.²² Research stated the relationship of TNF- α with oocyte quality, in patients with endometriosis an increase in TNF- α was correlated with poor oocyte quality due to damage in the luteal phase due to increased TNF- α , so that in endometriosis patients anovulation would occur.²³

The results of this study showed that more than half of the respondents (92%) were married, 88% were infertile, all respondents had cyst sizes of more than 3 cm, and most (80%) types of monolokulare cysts. This study shows that there is no correlation between TNF- α levels and AMH levels. From the analysis it is known that TNF- α and AMH levels are negatively correlated where an increase in TNF- α is followed by a decrease in AMH levels. This is in line with research which states that there is no significant difference in TNF- α levels between patients with endometriosis and no endometriosis. Likewise with research where there are no differences TNF- α levels were significant between the group with endometriosis and the control group. From their results it was found that there were no cytokines which showed a significant correlation with the endometriosis phase.²⁴ The results of this study are in line which states that circulating IL-6 and TNF- α are influenced by exogenous hormones for ovarian stimulation. From this study it was concluded that there was no relationship between cytokine circulation with mild endometriosis.

CONCLUSIONS

There is a negative correlation of TNF- α levels with AMH levels in patients with endometriosis

cysts, namely the higher TNF- α levels, the lower AMH levels but there is a positive correlation of IL-6 levels with AMH levels in patients with endometriosis cysts. Where the higher levels of IL-6, the higher levels of AMH.

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