Research Article

Homocysteine Level in the Blood and Follicular Fluid is Higher in Infertile Women with Endometriosis

Kadar Homosistein Darah dan Zalir Folikel pada Perempuan Infertil dengan Endometriosis Lebih Tinggi

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Abstract

Objective: To compare and determine the differences in the level of homocysteine in the blood and follicular fluid in infertile women with and without endometriosis, then analyze the effect of homocysteine levels to oocyte quality.

Methods: This study was cross-sectional study. Fifty-nine subjects following the in-vitro fertilization program are included in the admission criteria were divided into two equal groups, ie groups of endometriosis and without endometriosis consecutively (consecutive sampling). Each subject taken from the blood and follicular fluid then measured the levels of homocystein levels with immuoassay method. The mean of each group was statistically tested with an independent t test.

Result: The mean levels of homocysteine in the blood is higher in the endometriosis group than without endometriosis group and it was statistically significance (8.34 ± 2.68 vs 6.71 ± 1.56, p=0.007; 95% CI: 0.02417-0.14657). Similarly, the levels of homocysteine in follicular fluid , the endometriosis group is higher and statistically significance (6.19 ± 1.67 vs 3.46 ± 1.03; p= 0.000; 95% CI: 0.19310-0.32353). All oocytes are in good quality in both groups, maturation grade 3. There is a correlation between the levels of homocysteine in the blood and follicular fluid in the endometriosis group and assessed with Pearson test, and it found significant (p = 0.002) and the correlation value 0.553 (moderate correlation strength) and direction of a positive correlation.

Conclusion: The mean levels of homocysteine in the blood and follicular fluid in infertile women with endometriosis is higher than without endometriosis and were statistically significantly different. These homocysteine levels does not affect the quality of oocytes. There is a positive correlation between the levels of homocysteine in the blood and follicular fluid in endometriosis group.

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Keywords: endometriosis, homocysteine, infertility, oocyte quality

Abstrak

***Tujuan**: Membandingkan dan menentukan perbedaan kadar homosistein dalam darah dan zalir folikel pada perempuan infertil dengan dan tanpa endometriosis, kemudian menganalisis pengaruh kadar homosistein tersebut dengan mutu oosit.*

***Metode**: Penelitian ini merupakan penelitian potong lintang (cross sectional). Lima puluh sembilan subjek mengikuti program fertilisasi in-vitro yang masuk dalam kriteria penerimaan dibagi menjadi dua kelompok sama besar, yakni kelompok endometriosis dan tanpa endometriosis secara konsekutif (consecutive sampling). Masing-masing subjek diambil percontoh dari darah dan zalir folikel kemudian diukur kadar homosisteinnya dengan metode teraimun CMIA. Rerata masing-masing kelompok diuji statistik dengan uji t independen.*

***Hasil**: Rerata kadar homosistein dalam darah pada kelompok endometriosis lebih tinggi dibandingkan dengan tanpa endometriosis dan secara statisik berbeda bermakna (8,34 ± 2,68 vs 6,71 ± 1,56, p=0,007; 95% CI: 0,02417-0,14657). Demikian pula dengan kadar homosistein dalam zalir folikel, kelompok endometriosis lebih tinggi dan secara statistik berbeda bermakna (6,19 ± 1,67 vs 3,46 ± 1,03; p= 0,000; 95% CI: 0,19310-0,32353). Semua mutu oosit baik pada kedua kelompok, yakni derajat 3. Terdapat korelasi antara kadar homosistein di dalam darah dan zalir folikel pada kelompok endometriosis dan dinilai dengan uji Pearson didapatkan bermakna (p = 0,002) dan nilai korelasi 0,553 (kekuatan korelasi sedang) dan arah korelasi positif.*

***Kesimpulan**: Rerata kadar homosistein dalam darah dan zalir folikel pada perempuan infertil dengan endometriosis lebih tinggi dibandingkan dengan tanpa endometriosis dan secara statistik berbeda bermakna. Kadar homosistein ini tidak berpengaruh terhadap mutu oosit. Terdapat korelasi positif antara kadar homosistein dalam darah dan zalir folikel pada kelompok endometriosis.*

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***Kata kunci**: endometriosis, homosistein, infertilitas, mutu oosit*

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INTRODUCTION

Infertility is the failure of couples to conceive after regular intercourse without any contraceptive method for 12 months. Infertility occurs in about 7-10% of couples. The cause of infertility from female factors of around 50%, the rest is a male factor of 20-26%, and 25-30% is idiopathic.[1] One of the therapies for infertile couples is to follow the In-Vitro Fertilization program (IVF). The success rate of the IVF programs ranges between 30-40% in all programs in IVF centers around the world. There are various factors that influence the success of this program, the genetic factor (congenital), which is

difficult to interventions, and acquired factors that more likely to intervene and prevent. These factors are quite important role in the microenvironment within the ovarian follicle. It is well known, most of every mature follicle there are oocyte, which quality of oocytes can give influence to embryos that will be transfer into the uterus on the IVF programs.

In the last two decades, homocysteine increasingly attracted the attention of researchers because of it has a unique role in the process illness.[2] Homocysteine as a product of the methionine cycle has been known to play an important role in cardiovascular disease, neurological diseases, and embryology, and also in some diseases in the field of obstetrics and gynecology.[3] Levels of homocysteine are found increased in several diseases, especially diseases that involve inflammatory process and autoimmune diseases.[2] The increasing of homocysteine levels may lead to increased levels of free radicals, and it caused imbalance between free radicals and antioxidants. In the field of obstetrics, homocysteine has a role in the pathophysiology of preeclampsia disease. Elevated levels of homocysteine also can lead to increase the risk of recurrent miscarriage in early pregnancy.[3] Various studies about important role of homocysteine metabolism in fertility started much discussed.[4,5] In the homocysteine metabolism, it involve micronutrients, such as vitamin B, folic acid, and pyridoxine act as co-factors. Some of the compounds between the metabolism of homocysteine works directly with synthesis of proteins, DNA synthesis and repair, and balancing the occurrence of oxidative stress (OS). These things are important in the process gametogenesis.[4]

It has long been understood that endometriosis is a disease that can trigger inflammation process, thus allowing the occurrence of elevated levels of homocysteine in the blood. On the other side, one of the main complaints of the patients with endometriosis is infertility. Pathophysiology of infertility in endometriosis has many theoretical bases. One of them is the presence of reactive oxygen species (ROS) that can be trigger oxidative stress that can impair women's fertility. Excessive increase in ROS will increase the incidence of apoptotic cells. These disorders can be impacted to low oocyte quality, which leads to lower embrio quality.[5]

Previous studies have shown an increase in the levels of homocysteine in the follicles liquid in patients with endometriosis who are undergoing in vitro fertilization (IVF), which may affect the quality embrio.[5] However, the levels of homocysteine in the blood and follicular fluid in infertile women with endometriosis compare to infertile women without endometriosis still unknown. Also we want to know the level of homocysteine that can impact the oocyte quality in both groups. When the levels of homocysteine in the blood of women with endometriosis shows meaningful value, the findings are expected to be added to the parameter oocyte quality in infertile women that participate in IVF program.

METHODS

This study use a cross-sectional design and the subjects were recruited from three in vitro fertilization (IVF) centers, the Dr. Cipto Mangunkusumo General Hospital, Yasmin Kencana clinic, Sam Marie Wijaya Invasive Clinic, and Sam Marie Basuki Rahmat (Basra) Mother and Children Hospital. Subjects were taken by a consecutive sampling between 20-40 years old. There were 2 groups in the study, those with endometriosis and the other group without endometriosis, each with 29 subjects. Patients included into the endometriosis group by laparoscopy or laparotomy results, which found the endometriosis lesions, as well as those who has never undergone both procedures but with history, physical examination and ultrasonography (USG) suggestive for endometriosis lesion or disease. When the patients were not matched with the mentioned criteria, the patients were included into non-endometriosis group. The exclusion criteria in this study were patients with polycystic ovary syndrome, a history of heart disease, hypertension, renal disease, autoimmune (such as lupus, rheumatoid arthritis), and body mass index > 25 kg/m^2.

On the day of oocyte retrieval, 3-5 ml of venous blood and follicular fluid from the largest follicle was withdrawn from the patient after an informed consent was given. Moreover, the homocysteine levels were evaluated by immunoassay, the CMIA.

RESULTS

The total number of subjects who participated in this study was 58 female infertile patients from the Yasmin Kencana Clinic RSCM (52 subjects), RSIA Sam Marie Basra (3 subjects), and Clinical Invasive

Sam Marie Wijaya (3 subjects). Subjects were recruited by the consecutive method, between March 12, 2012 and October 10, 2012 involved in the IVF program from each clinic. The oldest patient in the study was 40 years old and the youngest patient was 24 years old with the average of 33.79 years old. The age distribution normality was assessed by the Kolmogorov-Smirnov normality test and it was found that the distribution was normal and similar between both groups.

All subjects in current study have never experienced abortion. The types of infertility in almost all subjects were primary infertility (98.3%), whilst only one subject with secondary infertility. The range of infertility period was 1 to 15 years with the median of 6 years and the infertility period distribution curve by the Kolmogorov-Smirnov was not normal.

In this study, the subjects were divided into two groups, which are endometriosis and non-endometriosis group. In the group without endometriosis, the cause of infertility includes sperm factor (31%), idiopathic (10.3%), and tubal factor (8.6%). The characteristics of the two groups were provided in Table 1.

On the oocytes retrieval day, homocysteine levels from the blood serum and follicular fluid were evaluated, and the result were presented in Table 2. More over, the oocytes maturity level was assessed, and it was found that all subjects have the maturity level of 3, which indicate the best oocytes.

Table 2. Mean Plasma Homocysteine Levels and Follicular Fluid in both Groups.

	Homocysteine Levels (μmol/l)		p
	Plasma	Follicular fluid	
Mean	8.34	6.71	<0.001
SD	±2.32	±1.95	

Based on the Shapiro-Wilk normality test, the distribution of plasma homocysteine levels for non-endometriosis group was normal, while in endometriosis group it was not normal. Because the later data distribution was not normal, it was transformed with log 10, so that both groups have normal distribution. Thus, the parametric test requirements are fulfilled and the independent t-test can be conducted. The result shows significant relationship between the types of infertility with plasma homocysteine levels (p = 0.007; 95% CI: 0,02417-0.14657). Comparison of plasma homocysteine levels in both groups, shown in Table 3.

Table 3. Infertility Group Relationships with Plasma Homocysteine Levels (μmol/l).

Hcy level	Infertility group		p
	Endometriosis	Non-endometriosis	
Mean	8.34	6.71	0.007
SD	± 2.68	± 1.56	

Table 1. Comparison of Characteristics of Subjects in the Group with Endometriosis and Infertility without Endometriosis.

Characteristics		Infertility Type		p
		With endometriosis (n = 29)	Without endometriosis (n = 29)	
Age (years)	Mean	34	33	0.44
	SD	± 4	± 5	
Infertility Type	Primary	29	28	0.31
	Secondary	0	1	
Infertility Period (years)	Median	6	6	1.0
	Minimum	1	1	
	Maximum	15	14	
Protocol of ovulation stimulation	Antagonist	26	29	0.23
	Agonist	3	0	
Right follicle count	Mean	5	8	0.06
	SD	± 4	± 4	
Left follicle count	Mean	5	8	0.06
	SD	± 4	± 4	

The study results revealed that the average follicular fluid homocysteine level in the group with endometriosis was higher than the other group. Comparison of these levels was provided in Table 4. Data distribution based on Shapiro-Wilk test for the group with endometriosis was not normal. Therefore, the data was transformed into log 10. After transformed, it was found that data for both groups were normal. Furthermore, independent t-test was performed and found a significant relationship between the two infertility groups with homocysteine follicular liquid level (p = 0.000, 95% CI: 0.19310 - 0.32353)

Table 4. Infertility Group Relationship with Homocysteine Levels Follicular Liquid (µmol/l).

Hcy level	Infertility group		p
	Endometriosis	Non-endometriosis	
Mean	6.19	3.46	0.000
SD	± 1.67	± 1.03	

In current study, all subjects in both groups had a level of oocyte maturity of third degrees. Because the value of this level was constant, the data was not available to be tested statistically.

Kolmogorov-Smirnov test for homocysteine levels distribution in plasma and follicle liquid was not normal. The data were then transformed with log 10, resulting in normal data distribution. The Pearson test was then performed to determine the correlation of homocysteine levels in plasma and follicular fluid and gave a significant result (p = 0.000) with correlation value of 0.496 (moderate) and a positive correlation direction.

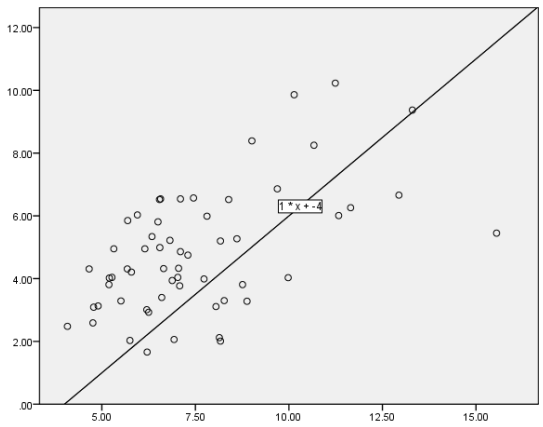

Figure 1. Correlation between Plasma Homocysteine Levels and Follicle.

Based on Shapiro-Wilk test, the plasma and follicular fluid homocysteine levels in endometriosis group were not well distributed. Therefore, data was transformed into log 10. After transformation, normal data distribution was formed. The Pearson test revealed a significant correlation (p = 0.002) between homocysteine levels in plasma and in the follicular fluid with the correlation value of 0.553 (moderate) and a positive direction.

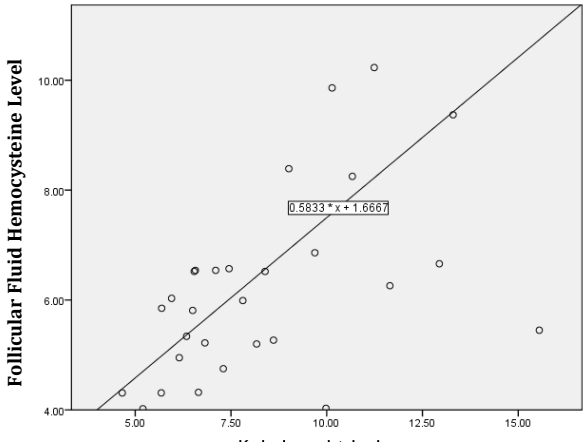

Figure 2. Correlation of Homocysteine Levels in Plasma and in Follicular Fluid in the Endometriosis Group.

Based on Shapiro-Wilk test, the plasma and follicular fluid homocysteine levels in non-endometriosis group were not well distributed. Therefore, data was transformed into log 10. After transformation, normal data distribution was formed. The Pearson test revealed a not significant correlation (p = 0.129) between homocysteine levels in plasma and in the follicular fluid with the correlation value of 0.288 (weak).

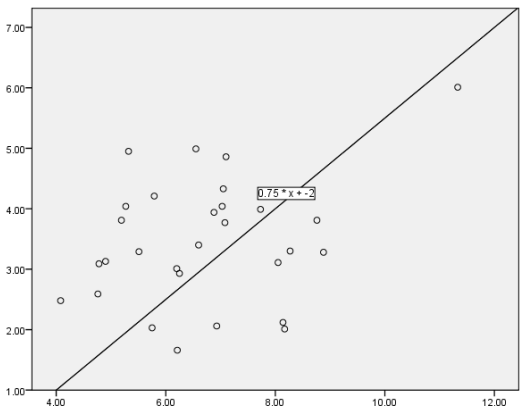

Figure 3. Correlation of Homocysteine Levels in Plasma and in Follicular Fluid in the non-Endometriosis Group.

DISCUSSION

The study was conducted to 58 subjects matched with the inclusion criteria. Subjects were infertile patients included in the In-Vitro Fertilization (IVF) program in three places, namely Yasmin Kencana Clinic RSCM, Sam Marie Wijaya Invasive Clinic, and Sam Marie Basra Mother and Children Hospital. Study subjects were recruited consecutively and then classified into two groups, one group with the diagnosis of endometriosis and the other group without endometriosis. In the current study, subjects were included into the endometriosis group when she has a history of surgery resulted in endometriosis lesion findings. Those with no history of surgery but has an evidence of endometriosis lesion from history, physical examination, and ultrasound, were also placed in endometriosis group. Subjects without any complaints related to endometriosis and had no endometriosis lesion finding in physical and ultrasound examination, were then put in the non-endometriosis group. The age, type of infertility, and prolonged infertility, shown an almost similar situation in both groups.

Mean comparison between homocysteine level in plasma and follicular fluid

This study shown that the plasma and follicular fluid homocysteine level in endometriosis infertility group was statistically higher and significantly different than in non-endometriosis infertility group. It was comparable with the study lead by Ebisch et al[5] that measures thiol compound in follicular fluid in men and women with infertility. From four thiol compound examined, homocysteine had the most significant difference between groups. Follicular fluid homocysteine level in infertile women with endometriosis was higher than those in infertile women caused by idiopathic or tubal factor. Unfortunately, the homocysteine levels were only measured from follicular fluid, not from blood plasma. Similarly, the levels of homocysteine in the spermatozoa were also higher in the infertile group due to male factors. That research also studied the relationship between homocysteine levels in follicular and ejaculate fluids with embryo quality on third day of culture. The results shown a negative relationship between the two; a high homocysteine levels can reduce the embryo quality.

A homocysteine level can be increased hereditary or acquired. In patients with hyperhomocysteinemia, there is a gene mutation causes a deficiency of methyltetrahydrofolic reductase (MTHF R) and cystationin beta-synthase (CBS) enzymes.[6] Acquired high level of homocysteine usually caused by autoimmune or immune cells disorders, such as systemic lupus erythematosus, Crohn's disease, and other diseases causing chronic inflammation, such as rheumatoid arthritis (RA), and ulcerative colitis. High homocysteine level can also be caused by the lack of the nutrient that metabolize homocysteine compounds, such as folic acid, vitamin B_{12}, vitamin B_2, vitamin B_6, and zinc.[6,7]

As reported previously, endometriosis is a disease that causes chronic inflammation and immune cells disruption. The inflammation occurs in the pelvic cavity by interfering immune cells function and increasing the number of active macrophages in the peritoneum, thus increasing the inflammatory mediators, such as growth factors and cytokines.[8] Elevated homocysteine levels may also be caused by micro-environment inflammation that results in impaired follicular development in the ovaries.

Effect of homocysteine levels with oocyte quality

In this study, we also assessed the quality of oocyte derived from the same follicle, which the homocysteine level was measured from the follicular fluid. Veeck method grade 1, 2, and 3 were used and shown a similar quality from both groups, 3rd degree (good quality), regardless the levels of homocysteine in the plasma and in follicular fluid. Homocysteine level in the follicular fluid was reported previously as a parameter of oocytes quality, embryo quality, and the success of pregnancy. High homocysteine level can cause oxidative stress and damage the oocyte, giving a poor quality of oocytes, which furthermore leading to decrease embryo quality and ultimately lower the pregnancy success. This was already proved by studies from Barker, Boxmeer, and Ocal.[9-11] Barker assessed the influence of homocysteine levels in follicular fluid in 52 infertile patients with polycystic ovary syndrome (PCOS) underwent IVF program. The homocysteine levels in follicular fluid, oocyte, and embryo quality were then measured. The study, which includes 94 follicles, higher levels of homocysteine reduced the quality of oocytes and embryos. A

similar study conducted by Ocal also showed a negative correlation between homocysteine levels with the pregnancy rates in IVF program.[11] Mean follicular fluid homocysteine levels in patients with PCOS in Ocal's study was higher than current study. Thus, there is an estimation that homocysteine has a treshold to give a negative effect on the oocytes quality. However, there is still no recent study which can determine the treshold level.

In Boxmeer study,[10] the levels of homocysteine in follicular fluid of infertile women who were given supplements and without any supplementation was assessed and its influence in embryo quality was determined. The results showed that mean levels of homocysteine in the group given supplements were lower compared to the group without supplementation. An interesting finding of this research was that there is a negative correlation between homocysteine levels with embryo quality in the group without the supplement, whereas in group which supplement were given there was a positive correlation, that means, high level of homocysteine in the group does not affect the quality of oocytes. From these results, it was thought that there must be an optimal homocysteine level needed in the follicular micro-environment. Homocysteine causes an increase level of reactive oxygen species (ROS) and leads to oxidative stress, but the value of the physiological or normal ROS is still required for oocyte maturation, ovulation and fertilization.

In our study, all subjects in both groups were given folic acid supplements during their IVF program. This is the basic reason of why homocysteine level in follicular fluid does not affect the oocyte quality.

Correlation between homocysteinein the plasma levels and in follicular fluid

Increased cytokines in the peritoneal cavity caused by the endometriosis lesions has also added the systemic inflammation mediators, such as C-reactive protein (CRP), tumor necrosis factor-α (TNFα), monocyte chemo-attractant protein-1 (MCP-1), interleukin-6 (IL-6), and IL-8 in the peripheral blood flow in patients with endometriosis. This fact referred that endometriosis is a local disease with subclinical systemic manifestations.[8] Increased homocysteine levels in blood plasma is also assumed to have a similar mechanism as mentioned, that the inflammation mediators has triggered the local and systemic inflammation. Furthermore, the level of homocysteine in plasma has a positive significant correlation with those in follicular fluid. These findings strengthen the idea that inflammation occurred in the peritoneum due to endometriosis lesions affects the increase of systemic inflammation mediators.

Limitation of the study

The limitation of this study is that most of all subjects were included based on the past surgical history resulting an endometriotic lesions by laparotomy or laparoscopy. There are two subjects who were included in the infertility with endometriosis group only based on the clinical examination and ultrasonography, because on inspection we found suspected endometriosis lesions. The ovarian stimulation mostly used the antagonist protocol, and only three patients in the endometriosis group with infertility used the agonist protocol. This study did not distinguish the protocols used, because no literature stated that stimulation protocols can affect the levels of homocysteine in the blood plasma and in follicular fluid. In our study, we only measured levels of homocysteine in follicular fluid from a single dominant follicle of each subject. This method is thought to affect the mean levels of homocysteine in follicular fluid and oocyte quality assessment. Oocytes other than the dominants, havepoor quality, therefore they were not measured in this study.

CONCLUSION

From this research we found the homocysteine level in infertile with endometriosis women are higher than without endometriosis. Homocysteine level did not contribute the oocytes quality. There were positive correlation between homocysteine level in blood and follicular fluid in endometriosis group.

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