Research Article

Ovarian Reserve In Infertile Women with and without Endometriosis Measured with Anti Müllerian Hormone

Cadangan Ovarium pada Perempuan Infertil dengan dan tanpa Endometriosis Diukur dengan Anti Müllerian Hormone

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Abstract

Objective: To compare serum Anti Müllerian Hormone (AMH) levels in infertile women with and without endometriosis, and to determine the mean levels of serum AMH in every stage of endometriosis.

Method: We performed a cross-sectional study. Sixty-eight subjects who have undergone laparoscopy and fulfilled both inclusion and exclusion criteria are recruited consecutively. They are divided into two groups, namely group with endometriosis and without endometriosis. Blood samples are taken from each subject before laparoscopy, where serum AMH levels are then measured. The difference in mean levels of each group are tested with Mann-Whitney test.

Result: The mean levels of serum AMH were significantly lower in the endometriosis group than those in the group without endometriosis (2.30 1.8 ng/ml vs 3.75 2.13 ng/ml; p=0.005). Using Kruskal-Wallis test, it was found that there was a statistically significant difference among endometriosis groups based on the severity of endometriosis. There was no significant difference in the mean serum AMH levels between the minimal-mild endometriosis group and without endometriosis group (p=0.34), but the mean levels of serum AMH were significantly lower in the moderate-severe endometriosis compare to the group without endometriosis (p<0.005).

Conclusion: The mean levels of serum AMH in infertile women with endometriosis were significantly lower than those in women without endometriosis. There was no significant difference in the mean serum AMH levels in minimal-mild endometriosis group and those without endometriosis; while in moderate-severe endometriosis group, it was significantly lower than in the group without endometriosis.

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Keywords: endometriosis, infertility, serum AMH

Abstrak

***Tujuan**: Membandingkan rerata kadar AMH serum pada perempuan infertil dengan dan tanpa endometriosis, serta untuk mengetahui rerata kadar AMH serum pada tiap derajat endometriosis.*

***Metode**: Kami melakukan penelitian potong lintang (cross sectional). Enam puluh delapan subjek yang menjalani laparoskopi dan memenuhi kriteria inklusi dan eksklusi direkrut secara konsekutif dan dibagi menjadi dua kelompok yang sama besar, yakni kelompok endometriosis dan tanpa endometriosis. Sampel darah dari masing-masing subjek diambil sebelum dilakukan laparoskopi, kemudian diukur kadar AMH serum. Perbedaan rerata masing-masing kelompok diuji dengan uji Mann-Whitney.*

***Hasil**: Rerata kadar AMH serum pada kelompok endometriosis secara signifikan lebih rendah dibandingkan dengan kelompok tanpa endometriosis (2,30 1,8 ng/ml vs 3,75 2,13 ng/ml; p=0,005). Menggunakan uji Kruskal-Wallis, ditemukan perbedaan yang bermakna secara statistik pada subjek kelompok endometriosis berdasarkan derajat endometriosis (p=0,005). Tidak ditemukan perbedaan kadar AMH serum yang bermakna pada kelompok endometriosis minimal-ringan dengan kelompok tanpa endometriosis (p=0,34), sedangkan pada kelompok endometriosis sedang-berat rerata kadar AMH serum secara signifikan lebih rendah dibanding kelompok tanpa endometriosis (p<0,005).*

***Kesimpulan**: Rerata kadar AMH serum pada perempuan infertil dengan endometriosis lebih rendah dibandingkan dengan yang tanpa endometriosis, dan secara statistik terdapat perbedaan bermakna. Tidak ditemukan perbedaan kadar AMH serum yang bermakna pada kelompok wanita infertil dengan endometriosis derajat minimal-ringan dibandingkan dengan kelompok wanita infertil tanpa endometriosis, tetapi ditemukan perbedaan kadar AMH serum yang bermakna antara kelompok wanita infertil dengan endometriosis derajat sedang-berat dan kelompok wanita infertil tanpa endometriosis.*

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***Kata kunci**: amh serum, endometriosis, infertilitas*

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INTRODUCTION

Endometriosis is implantation of endometrium-like tissue (stroma and glands) outside of the uterine cavity, inducing chronic inflammation. The process can affect all organs, but primarily encountered in pelvic organs, including the ovaries.[1-4] Most commonly, the complaints of endometriosis patients are pain and infertility.[5,6]

The hypothesis that endometriosis causes infertility or decreased fertility remains a controversy. It has been shown that the prognosis of assisted reproductive technique in endometriosis patients is worse than that in patients with tubal occlusion.[7-9] One of the mechanisms causing infertility in endometriosis is the change in folliculogenesis resulting in ovulatory dysfunction and poor oocyte quality.[8] To evaluate the ovarian follicular status, classically, early follicular phase serum FSH, inhibin-B, and E2 levels have to be measured. However, the utility of those measurements and their clinical value is still limited.[10,11] A new way to evaluate the ovarian follicular status is by measuring Anti Müllerian Hormone (AMH).

Anti Müllerian Hormone (AMH) is produced by small, early anthral follicles and is more strongly associated with the number of small anthral follicles compared to FSH, E_2, and even inhibin-B levels. In vivo and in vitro studies showed that AMH has an inhibitory effect on primordial follicular recruitment and decreases the sensitivity of follicles for the FSH-dependent selection for dominance. Beside its functional role in the ovary, serum AMH level serves as an excellent candidate marker of ovarian reserve.[11-13]

Hendarto found that infertile women with endometriosis have an increase in TNF- and hyaluronan levels with a decrease in GDF-9 level, causing disturbance in folliculogenesis and low oocyte quality.[14] In another study, Kusuma found that mean level of serum AMH in postpartum women with endometriosis is lower than the control group (non-endometriosis women).[15] The mean level of serum AMH in baby girls born from mother with endometriosis was clinically lower than that in the control group (baby girls with non-endometriosis mother). This study showed that there was a correlation between endometriosis and the low level of AMH, and the decrease of AMH level begins in the postnatal period.

The aims of this study are to compare the levels of serum AMH in infertile women with and without endometriosis, and also to determine the mean serum AMH levels in different stages of endometriosis.

METHODS

Design

A cross-sectional study was conducted at the Infertility Clinic and Yasmin Clinic at Dr. Cipto Mangunkusumo Hospital and Sam Marie Wijaya Invasive Clinic, Jakarta, from January 2011 until June 2013. Patients were consecutively recruited and divided into two groups: group I (study group) consisting of infertile patients with endometriosis as proven by laparoscopy (n=38) and group II (control group) consisting of infertile patients without endometriosis (n=30). All patients underwent routine exploration and laparoscopy for the investigation of infertility and as a part of this study. Endometriosis staging was performed according to the classification of the American Society for Reproductive Medicine 1996.

All patients younger than 35 years old who were infertile and agreed to be included in the study were recruited. The following exclusion criteria were applied: body mass index (BMI) >30 kg/m^2; history of ovarian surgery, including ovarian drilling and/or uterine artery ligation, history of cancer or having therapy that can affect ovarian function, history of severe pelvic infection (frozen pelvis) and patients with previous hormonal therapy for endometriosis.

Venous blood samples were collected (3 ml) from each patient before laparoscopic surgery, to measure the serum AMH levels. AMH levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instruction.

All data were collected in a computerized database. Statistical analysis was performed using SPSS 20.0 for Windows. Data were presented as mean, median (minimal-maximal range), and standard deviation. Independent t-test and ANOVA were used if the sample was normally distributed. Otherwise, Mann-Whitney and Kruskal-Walls tests will be used.

We calculated that a sample size of more than 22 subjects in each group would allow us to show a difference in AMH level between subject and control of 0.76 ng/ml[9] with type I error of 0.05 and type II error of 0.2. A p-value <0.05 was considered as statistically significant.

RESULTS

In the endometriosis group, there were 6 patients (15.8%) with stage 1 endometriosis (minimal endometriosis), 13 patients (34.2%) with stage 2 endometriosis (mild endometriosis), 11 patients (28.9%) with stage 3 endometriosis (moderate endometriosis), and 8 patients (21.1%) with stage 4 endometriosis (severe endometriosis). In the control group there were 21 patients (70%) with tubal occlusion or hydrosalphinx, 6 patients (20%) with subserosal myoma, and 3 patients (10%) with endometrial polyp.

The baseline characteristics of both groups were comparable. The median age in the study group was 33 (range = 23-35) years and 32.5 (range = 25-35) years for the control group (p>0.05). In addition, BMI (22.4 2.06kg/m^2 for the study group and 23.1 2.04kg/m^2 for the controls) were also similar between groups.

As shown in Table 1, the mean serum AMH levels were lower in the endometriosis group than in the control group and it was found to be statistically significant (2.30 1.8 ng/ml vs 3.75 2.13 ng/ml; p = 0.005).

Using Kruskal-Wallis test, it was found that there was a statistically significant difference of serum AMH levels among endometriosis subgroups based on the stages of endometriosis.

We divided the endometriosis group into minimal-mild endometriosis and moderate-severe endometriosis and compared the serum AMH levels with the control group. There was no significant difference in the mean serum AMH level between the group with minimal-mild endometriosis and the control group (3.12 1.97 ng/ml vs 3.75 2.13 ng/ml, p=0.34). Meanwhile, the mean serum AMH level was significantly lower in the moderate-severe endometriosis compared to the control group (1.48 1.30 ng/ml vs 3.75 2.13 ng/ml, p<0.005).

DISCUSSION

In this study, we found that patients with endometriosis have lower serum AMH level than those in the control group, and AMH level decreases in relation to the severity of endometriosis. Our finding was concordant with results from a study by Streulli et all.[16] They found that the difference in serum AMH levels between women in endometriosis and controls did not reach significance (3.6 3.1 ng/ml vs 4.1 3.4 ng/ml, p=0.06). The AMH level was only found to be significantly lower in patients aged >40 years old or patients with history of surgery for endometriosis cyst (either laparotomy or laparoscopy).

Table 1. Serum AMH Levels in Infertility Groups (ng/ml)

Serum AMH levels	Infertility Groups		p*
	With Endometriosis (n = 38)	Without Endometriosis (n = 30)	
Mean SD	2.30 1.8	3.75 2.13	0.005
Median (range)	2.35 (0.16-7.20)	3.0 (0.71-8.30)	

* *Mann-Whitney test*

Table 2. Serum AMH Levels in Endometriosis Group, based on Stages of Endometriosis (ng/ml).

Stage of Endometriosis		n	Mean SD	Median (range)	p*
AMH	Minimal	6	4.13 2.05	3.3 (230-9.25)	0.005
	Mild	13	2.65 1.82	2.6 (0.41-7.0)	
	Moderate	11	1.97 1.30	2.3 (0.2-4.18)	
	Severe	8	0.82 1.04	0.2 (0.16-2.8)	

* *Kruskal-Wallis test.*
Post-hoc test with ANOVA-LSD: minimal vs moderate endometriosis p=0.007; minimal vs severe endometriosis p<0.001; moderate vs severe endometriosis p=0.048

In women, serum AMH significantly declines with increasing age. After reaching 37 years old, the number of oocytes is reduced with decreased fertility potential, even when menstrual cycles are still regular. This condition will continue until menopause, when the remaining follicles were approximated to be only 1000 follicles.[17-19]

In this study we discovered that the mean serum AMH levels is decreasing in relation with the severity of endometriosis. After we did post-hoc analysis and divided them into 3 groups (the mild endometriosis is considered to be the level between minimal and moderate endometriosis), we found that there are significantly different AMH levels between minimal and moderate endometriosis, minimal and severe endometriosis, and also between moderate and severe endometriosis. This result is similar to the study conducted by Youssef and Anwar.[20] They concluded that AMH levels could be used as a sensitive marker to predict stage of endometriosis in infertile women.

We divided the endometriosis group into minimal-mild endometriosis and moderate-severe endometriosis and compared the serum AMH levels with the group without endometriosis. There was no difference in the mean serum AMH levels between groups with minimal-mild endometriosis and without endometriosis, but the mean serum AMH level was lower in the group with moderate-severe endometriosis compared to the group without endometriosis and it was statistically significant. This finding shows that not all of infertile women with endometriosis become poor responder. Moreover, it should be considered that in infertile women with minimal-mild endometriosis it is not necessary to provide any intervention since the serum AMH level is not significantly different from the levels in infertile women without endometriosis.

This result is contradictory with the study conducted by Lemos et al[9], where they found that the serum AMH levels in infertile women with minimal-mild endometriosis were significantly lower than serum AMH levels in infertile women with tubal factor. They also found that the number of basal anthral follicles in both groups were comparable. The rationale for this result was the fact that patients with minimal/mild endometriosis had a more heterogeneous follicular cohort, with a mean follicular diameter larger than that of patients with tubal obstruction.

Moreover Shebl et al[16] found that the serum AMH levels in minimal-mild endometriosis did not show any difference from serum AMH levels in infertile women without endometriosis (3.28 1.93 ng/ml vs 3.44 2.06 ng/ml, p=0.61). In moderate-severe endometriosis, however, the serum AMH levels was significantly lower (2.38 1.83 ng/ml vs 3.58 2.46 ng/ml, p<0.0001). Further, Campos et al[21] found that follicular-fluid AMH concentration in infertile women with minimal-mild endometriosis was similar to the concentration in infertile women without endometriosis (1.8 0.3 ng/ml vs 1.5 0.1 ng/ml, mean difference=0.33 ng/ml, 95% CI -0.21 to 0.88). AMH in the follicular fluid plays an important role in oocyte development and fertilization. High levels of follicular fluid AMH had a positive correlation with successful oocyte fertilization; this result showed that follicular fluid AMH plays a role in oocyte development and fertilization stimulated by exogenous FSH.

When serum AMH is compared to follicular AMH, some authors have demonstrated a positive correlation between them, indicating that peripheral AMH concentrations are modulated not only by the number of follicles, but also by the individual ability of the follicle itself to produce AMH.[22] This mechanism is not applicable to endometriosis patients, considering that these patients represent a heterogeneous follicular cohort as has already been shown.[12]

In conclusion, the mean serum AMH levels in infertile women with endometriosis were significantly lower than that in the group without endometriosis. There was no difference between the mean levels of serum AMH in minimal-mild endometriosis group and the levels in women without endometriosis, while in the moderate-severe endometriosis group, serum AMH level was significantly lower than the group without endometriosis.

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