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Effects of Metformin Therapy on Anti-Mullerian Hormone (AMH) Levels in Polycystic Ovary Syndrome Patients with Insulin Resistance at Palembang

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ABSTRACT

Anti-Mullerian Hormone is useful in examining folliculogenesis and ovarian abilities. Previous research found that follicular fluid and serum in PCOS women containing high AMH levels. As therapy in PCOS with insulin resistance, metformin, an insulin sensitizer agent can be given. The efficacy of metformin therapy in inducing ovulation is more due to the local action of metformin on the ovaries rather than the systemic improvements that result both metabolically and hormonally. We conducted this study to evaluate the levels of Anti-Mullerian Hormone (AMH) in women with polycystic ovarian syndrome (PCOS) with insulin resistance before and after metformin therapy. An analytic observational study with cross-sectional design. Seventy PCOS women with insulin resistance, taking metformin for 3 months, were enrolled in this study. Serum levels of AMH was assessed before and after treatment, and the difference between them was analyzed with student t-test. Diagnosis of PCOS is made based on Rotterdam criteria and insulin resistance by HOMA-IR calculation. This study was conducted at the Fertility and Reproductive Endocrinology Polyclinic of Dr. Mohammad Hoesin Palembang hospital and Imaya clinic. The mean age of PCOS patients with insulin resistance ranges from 25-30 years, with a mean age of 28.61±3.883. The mean AMH levels prior metformin therapy was 7.6957±0.6001 ng/ml; mean AMH after therapy was 7.5914±0.57678 ng/ml; and there are significant differences ($p < 0.001$). The mean AMH levels in all patients were 0.10429. Based on body mass index (BMI), a total of 52 respondents (74.29%) met the criteria for obesity and 18 respondents (25.71%) nonobese. There is a relationship between AMH levels and the number of follicles ($p < 0.01$), with an OR of 1.5. AMH levels decreased significantly after metformin therapy in PCOS patients with insulin resistance.

Keywords: Anti Mullerian Hormone, polycystic ovarian syndrome, insulin resistance

1. INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is an endocrine disorder that is often found in reproductive age women. The diagnosis of PCOS is based on the presence of hyperandrogenism and chronic anovulation with exclusion of specific conditions from the adrenal glands, ovaries, or pituitary. The prevalence of PCOS is between 5-10%. [1,2]

The most probable mechanism of pathophysiology of PCOS is impaired dominant follicular selection resulting in anovulation. Impaired selection mechanism results in the accumulation of small antral follicles, which contribute significantly to the production of Anti-Mullerian Hormone (AMH). Anti-Mullerian Hormone decreases follicular sensitivity to FSH, causing impaired follicular selection. It is suspected that the aromatase activity of PCOS patients may decrease because the follicles in PCOS patients do not produce estrogen (E2) in large quantities. AMH also inhibits aromatase activity, so AMH is considered to affect the severity of PCOS. [3]

Anti Mullerian Hormone is useful in examining folliculogenesis and ovarian abilities. Previous research found that follicular fluid and serum in PCOS women containing high AMH levels. Anti-Mullerian Hormone serum levels of PCOS are related to the antral follicles count. An increase of two to three times the number of developing follicles shows an increase of two to three times the level of serum AMH [3]

PCOS is closely related to insulin resistance and hyperinsulinemia, which has been shown in several studies that obesity is also often found in PCOS. [3,4] Barbieri et al. concluded that insulin stimulates the production of androgen binding and invitro ovarian stroma. [5]

The relationship of fasting insulin and free androgens supports the hypothesis that hyperinsulinemia can increase hyperandrogenemia in PCOS. [3] Insulin also suppresses levels of sex hormone binding globulin (SHBG), so that free androgen increases. Increased androgens will affect the ovarian environment to be androgenic, disruption of the androgen aromatization system to estrogen so that it triggers earlier follicular atresia. [6,7] Decreased levels of SHBG can be used as a single predictor in the identification of an increased risk of insulin resistance in nonobese PCOS. [8]

Obesity in PCOS is a factor that affects the phenotype of this syndrome and worsens endocrine and metabolic parameters including insulin action. [8] Compared to normal women, obese and nonobese women with PCOS experience insulin resistance and hyperinsulinemia. [9] In a study conducted by Wiweko B and Mulya R at the RSCM stated that 75% of PCOS patients had insulin resistance and from 32 obese patients they found 84% had insulin resistance while only 50% nonobese patients had insulin resistance. [10] Rusnasari (2005) obtained the frequency of insulin resistance using the Homeostasis Model Assessment (HOMA-IR) method which is considered close to the Euglycemic clamp technique, the gold standard for predicting insulin resistance. With this method, insulin resistance of PCOS sufferers was 82.9%. [11]

As therapy in PCOS with insulin resistance, metformin, an insulin sensitizer agent can be given. Palomba showed that administration of metformin had systemic influences by reducing hyperandrogenism in PCOS patients and also worked directly on the ovaries. The efficacy of metformin therapy in inducing ovulation is more due to

the local action of metformin on the ovaries rather than the systemic improvements that result both metabolically and hormonally.[11,12] This study aims to determine the effect of metformin therapy in PCOS patients in Palembang towards AMH levels.

2. METHODS

This study was an observational study with a cross sectional approach, which was conducted at the Reproductive Endocrinology Fertility Polyclinic in dr. Mohammad Hoesin General Hospital Palembang and Imaya Clinic, from February 2015 until the number of samples were met. The number of samples needed was 70 samples. The sample selection method used consecutive sampling. Inclusion criteria included reproductive age women who were diagnosed with PCOS and accompanied by insulin resistance, had no diabetes mellitus, and had no family history of diabetes mellitus. PCOS patients who were undergoing hormonal contraceptive therapy and did not adhere to the therapeutic schedule were excluded from the study. Insulin resistance (IR) was established based on the HOMA-IR method, namely:

$$IR = \frac{\text{fasting insulin (µUI/ml) x fasting glucose (mmol/ml)}}{22.5}$$

The normal value of insulin resistance according to Matthew was <1. Polycystic ovarian syndrome was diagnosed if it fulfilled 2 of the following 3 criterias, namely oligo or anovulation; clinical signs or symptoms of hyperandrogenism, including hirsutism and acne; and polycystic ovary features on transvaginal ultrasound or laparoscopy. Anti Mullerian Hormone was a member of transforming growth factor-b which could be examined from a patient's blood sample using an enzyme immunoassay (ELISA). The level of AMH serum in PCOS patients with insulin resistance were first examined before receiving metformin therapy, then re-examined after 3 months therapy. Normal level of AMH serum ranged from 1.2 to 5.8%.

3. RESULT

From 70 samples, the mean age of the patients was 28.61 ± 3.883. The majority of patients was between 25-30 years namely 42 respondents (60%). Based on body mass index (BMI), 52 respondents (74.29%) met the obesity criteria and 18 respondents were classified as nonobese (25.71%), where the lowest BMI was 19 and the highest BMI was 33. Based on the length of the menstrual cycle, we obtained that 39 respondents experienced 30-60 days of menstrual cycles, while the fewest had menstrual cycles 60-90 days. Based on the antral follicles count, 43 respondents (61.4%) had more than 20 follicles. The distribution of respondent characteristics can be seen in table 1.

Table 1. Distribution of respondent characteristics

Characteristics	Frequency	Percentage (%)
Age		
25-30	42	60
30-35	28	40
Total	70	100
BMI		
Obese	52	74.29
Nonobese	18	25.71
Total	70	100
Menstrual cycle		
30-60 days	39	55.7
60-90 days	11	15.7
90-120 days	20	28.6
Total	70	100
Antral follicle count		
10-20	27	38.6
20-30	43	61.4
Total	70	100

The mean of AMH serum levels before and after metformin therapy were compared and tested with a student t-test. There were significant differences ($p < 0.001$) on the mean of AMH levels before and after metformin therapy. The mean of pretreatment and post treatment AMH level were 7.6957±0.6001 and 7.5914±0.57678, respectively (table 2).

Table 2. Comparison of AMH levels before and after receiving metformin therapy

Variables	Mean	SD	Min	Max	Delta mean	P
Pre-treatment AMH	7.6957	0.6001	6.3	9.2	0.10429	<0.001
Post-treatment AMH	7.5914	0.57678	6.2	9.1		

A significant decrease in the average AMH level after metformin treatment was found in 52 samples of obese group namely 0.05 ($p = 0.022$), which showed that metformin was able to reduce AMH levels in obese PCOS patients significantly and effectively in the majority of cases (table 3). A decrease in the average AMH levels after metformin treatment was found in 18 samples of nonobese group by 0.02778. The statistical calculation found that the reduction in AMH levels after metformin therapy was not significant ($p = 0.384$) (table 4).

Table 3. Comparison of AMH levels before and after metformin therapy in obese PCOS patients

AMH Level (pre vs post treatment)	AMH serum levels of obese PCOS patients			Δ Mean of AMH serum level	p
	N	Mean	Std. Deviation		
Pre treatment AMH	52	7.9231	0.51359	0.05 ± 0.01527	**0.022**
Post treatment AMH	52	7.8731	0.43572		

Table 4. Comparison of AMH levels before and after metformin therapy in nonobese PCOS patients

AMH Level (pre vs post treatment)	AMH serum levels of nonobese PCOS patients			Δ Mean of AMH serum level	p
	N	Mean	Std. Deviation		
Pre treatment AMH	18	7.0389	0.23549	0.02778 ± 0.00131	0.384
Post treatment AMH	18	7.0111	0.26544		

To find out whether there is a decrease in AMH levels before and after metformin treatment in obese and nonobese PCOS patients, a student t-test was calculated. From table 5, it was known that the mean AMH level before metformin therapy in obese PCOS patients is 7.9231 ± 0.51359 and the mean AMH level before therapy in nonobese patients was 7.0389 ± 0.23549. There was a difference in the mean AMH level before metformin therapy in the obese group and nonobese group is 0.88419.

From table 6, the mean AMH level after metformin therapy in obese PCOS patients was 7.8731 ± 0.43572 and the mean AMH level after therapy in nonobese PCOS patients is 7.0111 ± 0.26544. There was difference in the mean AMH levels after metformin therapy in the obese group and nonobese namely 0.86197.

Table 5. Comparison of AMH levels before metformin therapy

BMI Category (obese vs non obese)	Pretreatment AMH serum level			Δ Mean of AMH serum level	p
	N	Mean	Std. Deviation		
Obese	52	7.9231	0.51359	0.88419	0.002
Nonobese	18	7.0389	0.23549		

Table 6. Comparison of AMH levels after metformin therapy

BMI Category (obese vs non obese)	Post treatment AMH serum level			Δ Mean of AMH serum level	P
	N	Mean	Std. Deviation		
Obese	52	7.8731	0.43572	0.86197	<0.001
Nonobese	18	7.0111	0.26544		

Table 7. Differences of AMH levels in obese and non-obese PCOS patients

BMI Category (obese vs non obese)	Pretreatment -Post treatment AMH serum level			Δ Mean of AMH serum level	P
	N	Mean	Std. Deviation		
Obese	52	0.05	0.01527	0.02222	0.058
Non-Obese	18	0.0278	0.00131		

From table 7, it is known that the delta mean of AMH levels in obese patients was 0.05 ± 0.01527 and the delta mean of AMH levels in nonobese patients was 0.0278 ± 0.00131. There was no significant difference (p> 0.05) between these two groups and the mean difference between the two groups was 0.02222.

Based on the chi square analysis (x^2), there was no relationship between AMH level category and BMI category (p> 0.05) which could be seen in table 8. Spearman's correlation showed a weak negative correlation (r = -0,244), it could be seen in Image 1.

Table 8. The relationship between BMI category and changes in AMH levels

BMI Category	AMH Category				Total		P	r
	1.5 – 4		>4					
	N	%	N	%	N	%		
18.5-25	4	5.7	17	24.3	21	30	0.423	-0.244
25.1-27	3	4.3	17	24.3	20	28.6		
>27	2	2.9	27	38.6	29	41.4		
Total	9	12.9	61	87.1	70	100		

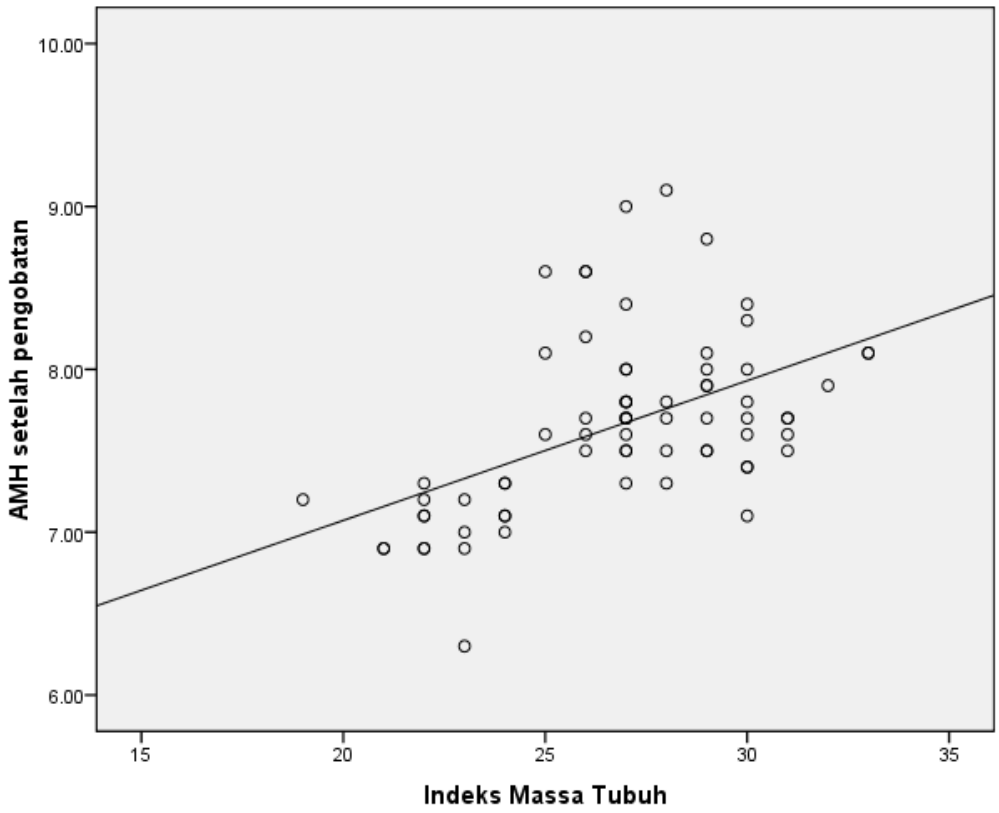

Figure 1. Relationship between BMI and AMH levels

The relationship between the categories of menstrual cycle length and AMH levels was shown in Table 9. Based on the chi square (x^2) analysis, it was found that no relationship was found between the categories of AMH levels and menstrual periods (p> 0.05). Spearman's correlation showed a weak negative correlation (r = -0.112).

Table 9. Relationship between menstrual cycle length and changes in AMH levels

Menstrual cycle length	AMH Category				Total		p	r
	1.5 – 4		>4					
	N	%	N	%	N	%		
30 – 60	4	5.7	35	50	39	55.7	0.526	-0.112
60 – 90	1	1.4	10	14.3	11	15.7		
90 – 120	4	5.7	16	22.9	20	28.6		
Total	9	12.9	61	87.1	70	100		

The relationship between categories of antral follicles count and AMH levels was calculated based on chi square (x^2) analysis. It was found that there was a relationship between AMH levels and follicles count (p <0.01), with OR 1.5. Spearman's correlation showed a medium positive correlation (r = 0.485), where the higher the AMH level of the patient, the number of follicles is 1.5 times more than normal. This correlation is statistically significant (p <0.05), could be seen in table 10.

Table 10. Relationship between the antral follicle count categories and AMH level

Follicle count category	**AMH Category**				**Total**		**p**	**r**
	1.5 - 4		**>4**					
	N	**%**	**N**	**%**	**N**	**%**		
10 – 20	0	0	43	61.4	43	61.4	<0.001 (OR = 1.5)	0.485 (p = <0.001)
20 – 30	9	12.9	18	25.7	27	38,6		
Count	9	12.9	61	87.1	70	100		

4. DISCUSSION

The mean of pretreatment AMH level was 7.6957 ± 0.6001 ng/ml and the mean of post treatment AMH level was 7.5914 ± 0.57678 ng/ml. There was a significant difference (p <0.001) and the mean AMH level in all patients was 0.10429. The mean AMH level before metformin therapy in obese PCOS patients was 7.9231 ± 0.51359 ng/ml while the mean AMH level before therapy in PCOS nonobese patients was 7.0389 ± 0.23549 ng/ml. It was concluded that the difference in the mean AMH level before metformin therapy in the obese and nonobese group was 0.88419. When we calculated the mean AMH levels after metformin therapy in the obese PCOS group, we obtained 7.8731 ± 0.43572 ng/ml and the mean AMH level after therapy in nonobese PCOS patients was 7.0111 ± 0.26544 ng/ml. The differences in the mean AMH levels after metformin therapy in the obese and nonobese groups were 0.86197.

There was significant decrease namely 0.05 (p = 0.022) of the mean AMH level after metformin therapy in 52 obese patients, which showed that metformin was able to reduce AMH levels in obese PCOS patients effectively in the majority of cases. Statistical calculations on nonobese PCOS patients showed that the mean decrease of AMH level after metformin therapy in 18 samples of nonobese group was 0.02778 which showed that metformin was able to reduce the AMH levels in nonobese PCOS patients but was not statistically significant.

Velazquez, et al. studied 26 obese women treated with metformin 1500 mg/day for 8 weeks and concluded that metformin reduced serum insulin concentration and free serum testosterone significantly by 52%. Three of the 26 sufferers even got pregnant. [13]

Nestler and Jacubowicz (1996) in a placebo-controlled trial that used metformin 1500 mg/day for 4-8 weeks in 24 obese PCOS women obtained similar results in a

decrease in circulating insulin levels in women treated with metformin. The study also found a reduction in LH stimulated by GnRH and reduced ovarian androgen production and also free testosterone serum concentration by 44%. Whereas eight other studies using metformin showed improved menstruation or reduction of androgens in women with PCOS. [13]

Metformin played a direct role in PCOS by ovarian sensitization. In a study conducted by Falbo, et al. it was said that AMH levels were significantly higher in PCOS patients compared to healthy controls. A possible hypothesis in these patients was that AMH is produced as a result of an increase in the number of ovarian follicles. [13]

Falbo, et al. said that in PCOS patients who were given metformin therapy, there were significant changes in AMH levels. Several studies had shown that metformin can improve insulin resistance and ovarian morphology. [13] A retrospective study by Piltonen, et al. showed that AMH levels, antral follicle counts, and ovarian volume decreased after metformin administration.

5. CONCLUSION

AMH levels after metformin therapy significantly decreased compared to AMH levels before therapy in overall PCOS patients. AMH levels after metformin therapy also decreased significantly in obese PCOS patients, but not significantly in nonobese PCOS patients. There is a significant relationship between the antral follicles count and AMH level. The higher AMH levels were, the number of antral follicles would be 1.5 times more than normal.

Further research was recommended to find out the optimal time of metformin therapy which could reduce AMH levels to reach normal levels and examined the effects of metformin therapy that could improve menstrual cycles to become regular and in ovulation.

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