

IMPACT OF INSULIN SENSITIZERS ON ANTI-MÜLLERIAN HORMONE IN POLYCYSTIC OVARY SYNDROME

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Abstract

Introduction: Elevated AMH level is considered an indicator of anovulation in PCOS. It is postulated that the use of insulin sensitizers will lead to a reduction in insulin resistance, hyperandrogenism and consequently AMH levels, as a result of ovulation induction in these patients.

Aims: The study aimed to determine the impact of therapy with the insulin sensitizers, Metformin and Myoinositol, on Anti-Mullerian hormone and to determine its association with insulin resistance in women with polycystic ovary syndrome.

Material and methods: A prospective, randomized, clinical study was conducted at the University Clinic for Endocrinology, Diabetes and Metabolic Diseases in Skopje, in the period 2022/2023. The study included 64 women, aged 18 to 40 years, diagnosed with polycystic ovarian syndrome, according to the 2003 Rotterdam criteria. Patients were divided into two groups: group A received Metformin 1500 mg XR, and group B was on Myoinositol 2 gr TID, during 6 months. The parameters BMI, AMH, HOMA-IR (homeostasis model assessment index) and FAI (free androgen index) were monitored.

Results: After completion of treatment, a significant decrease in AMH levels was observed in both groups - group A ($p=0.0012$) and group B ($p=0.0014$). This was accompanied with decline in HOMA IR and FAI values after the end of treatment in both groups.

Conclusion: In line with the results obtained, AMH could be considered a marker of the efficacy of treatment with insulin sensitizers in PCOS.

Keywords: PCOS, insulin senzitisers, Metformin, Myoinositol, Anti-Mullerian hormone

Introduction

Anti-Müllerian hormone (AMH) originates from preantral and small antral follicles, and as such, is an important marker of the remaining follicular reserve (pool) in the reproductive period of a woman. AMH levels are 2-3 times higher in women with PCOS than in healthy women. The reason for the elevated AMH in PCOS is thought to be the result of the increased number of preantral and small antral follicles in these patients, as well as the proven increased intrinsic production of granulosa cells in PCOS patients. Polycystic ovary syndrome is characterized by

hyperandrogenism (clinical and/or biochemical), chronic anovulation or oligomenorrhea and PCOM (polycystic ovarian morphology).

AMH belongs to the transforming growth factor-beta superfamily of proteins. The AMH gene is located on chromosome 19. AMH production begins at 36 weeks of gestation and continues until menopause.

It is known that AMH prevents FSH-mediated estrogen production from preantral and small antral follicles, through inhibition of the aromatase enzyme and reduction of the number of LH receptors. In this way, it prevents follicular growth, maturation and prevents the formation of a dominant follicle, thus preserving the follicular reserve. The level of this hormone begins to decline when the follicle reaches a size of about 10 mm, allowing further maturation of the follicle. AMH levels are 2-3 times higher in women with PCOS than in healthy women. The reason for the elevated AMH in PCOS is thought to be the result of the increased number of preantral and small antral follicles in these patients, as well as the proven increased intrinsic production of granulosa cells in PCOS patients^[1]. Granulosa cells in women with anovulation in PCOS produce 75 times more AMH than granulosa cells in normal women^[2].

The cause of the increased production of AMH is still unknown, but it is thought that hyperandrogenism and insulin resistance, present in PCOS may be involved in the pathophysiological mechanism of the increased secretion of AMH by these cells.

AMH concentrations may be secondary to the action of insulin on androgens, as insulin affects gonadotropin-stimulated steroid production by granulosa and theca cells^[3].

It is thought that elevated AMH levels in patients with PCOS may serve as an additional marker for diagnosing PCOS, with AMH reflecting the severity of the condition.

Along with hyperandrogenism, insulin resistance is considered one of the pathophysiological mechanisms in the occurrence of PCOS, independent of BMI.

It is known that hyperinsulinemia directly stimulates androgen secretion from the ovaries and adrenal glands, as well as suppresses SHBG synthesis in the liver, which increases the level of biologically active free testosterone.

High levels of androgenic hormones lead to impaired folliculogenesis in the ovaries, contributing to the appearance of PCOS - polycystic ovary morphology, which leads to an increase in Anti-Müllerian hormone than normal. Accordingly, higher levels of AMH reflect anovulation in patients with PCOS.

It is expected that the use of insulin sensitizers such as Metformin and Myoinositol, through the reduction of insulin resistance, and consequently hyperandrogenism, would lead to a reduction in AMH levels and thus to induction of ovulation.

The study aimed to determine the impact of therapy with the insulin sensitizers, Metformin and Myoinositol, on Anti-Müllerian hormone as a prognostic marker for the efficacy of treatment with insulin sensitizers in women with polycystic ovary syndrome.

Material and methods

This prospective, randomized, clinical study was conducted at the University Clinic for Endocrinology, Diabetes and Metabolic Diseases in Skopje, in the period of 2022/2023. In accordance with previously set inclusion and exclusion criteria, the study included women of reproductive age from 18 to 40 years. The selection of subjects was done by a simple random sampling method.

According to the 2003 Rotterdam criteria, all women had a clinical diagnosis of polycystic ovary syndrome (PCOS), with varying duration of the disease, and were without regular treatment

for PCOS for at least three months prior to inclusion in the study. Inclusion in the study was preceded by a signed informed consent.

The diagnosis of PCOS based on the 2003 Rotterdam criteria requires the presence of two of the three criteria: a) oligo and/or anovulation: oligomenorrhea defined as less than 9 cycles per year or three cycles with a duration of more than 36 days over the last year; b) clinical and/or biochemical signs of hyperandrogenism, implying a clinical diagnosis based on the Ferriman-Gallwey (mFG) scale ≥ 8 or the presence of moderate to severe acne, and/or biochemically defined as a total testosterone level > 2.0 nmol/L or a free androgen index (FAI) ≥ 6 ; c) polycystic ovarian morphology determined by ultrasound (8 MHz probe) - presence of 20 or more follicles in one of the ovaries with a diameter of 2-9 mm and/or volume > 10 ml on one of both ovaries.

According to the treatment, the patients were divided into two groups. One group was placed on Metformin therapy of 750 mg XR after a meal, with a gradual introduction of the total dose of 1500 mg XR over two weeks, to minimize side effects from the GIT. The other group received therapy with Myoinositol 2 gr every 12 hours, taken at a certain interval from meals. Patients were monitored before starting the therapy and 6 months after treatment. The parameters monitored were BMI, AMH, HOMA-IR (homeostasis model assessment index) and FAI (free androgen index).

Statistical analysis

SPSS for Windows 26.0 was used for analysis. The distribution of data (BMI, AMH, HOMA-IR and FAI) was tested using the Shapiro-Wilk's W test and their non-normal distribution was determined. Qualitative data were presented as absolute numbers and percentages, and Difference test was used to compare proportions. Between-group comparison of Metformin/ Myo-inositol before the start of treatment and after 6 months of treatment were compared with Mann-Whitney U test. Within-group comparison of Metformin/ Myo-inositol between starting point and at 6 months was done with Wilcoxon-Matched pairs test.

The association of AMH with BMI, HOMA-IR and FAI six months after treatment was performed using Spearman Rank Correlation. A level of $p < 0.05$ was used to determine statistical significance.

Results

The study included 68 women, aged 18 to 36 years, with an average age of 24.2 ± 5.1 years. Patients were divided into 2 groups depending on the type of therapy they received: 34 patients on Metformin therapy, 34 patients on Myoinositol.

Table 1. General characteristics of participants

Variable	n (%)
Age/years (mean \pm SD)(min- max)	(24.2 ± 5.1) (18 – 36)
BMI (kg/m ²) (mean \pm SD)(min- max)	(28.34 ± 7.3) (17.5 – 47.3)
MC n (%)	52 (76.47)
irregular	16 (23.53)
regular	

Both groups of patients were homogeneous in terms of age, i.e. the difference in age of patients on Metformin and Myoinositol therapy was statistically insignificant (23.9 ± 5.8 vs. 24.5 ± 4.4 years, $p=0.66$).

Before initiation of therapy, the body mass index for this cohort of patients averaged 28.34 ± 7.3 kg/m² and ranged from 17.5 to 47.3 kg/m².

Patients with irregular menstrual cycles were predominant-52(76.47%) (Table1).

An analysis of the intra-group difference for the examined variables, in relation to BMI (Table 2), was also made. Before treatment, patients with BMI lower and higher than 25 kg/m² had non-significantly different AMH levels ($p=0.49$). Patients with BMI ≥ 25 kg/m² had non-significantly higher levels compared to patients with BMI ≤ 24.9 kg/m² (mean= 17.22 ± 13.7 vs. 12.91 ± 7.5 ng/ml; median= 13.2 vs. 10.91 ng/ml).

Difference of AMH levels between groups was also insignificant after 6 months of treatment based on BMI ($p=0.15$). AMH levels were similar in patients with BMI lower and higher than 25 kg/m² (mean= 7.78 ± 4.4 vs. 6.74 ± 5.5 ng/ml; median= 6.58 vs 6.31 ng/ml).

Before and after treatment, patients with BMI lower and higher than 25 kg/m² did not differ significantly in terms of HOMA IR index values ($p=0.3$ and $p=0.063$, respectively): 3.97 ± 1.3 and 4.32 ± 1.5 were the mean values before therapy, 2.24 ± 0.99 and 2.70 ± 0.98 were the mean values after therapy in the groups with BMI lower and higher than 25 kg/m²; 3.5 and 4 were the median values before therapy, 2.1 and 2.4 were the median values after therapy in the groups with a BMI lower and higher than 25 kg/m².

Body mass index had no significant influence on the FAI index before ($p=0.75$) and after completion of treatment ($p=0.1$). The values of this parameter were insignificantly different in patients with a BMI lower and higher than 25 kg/m² before therapy (mean= 10.94 ± 7.7 , median= 6.7 vs. mean= 13.37 ± 13.8 , median= 10.46) and after therapy (mean= 5.10 ± 3.6 , median= 4.22 vs. mean= 6.44 ± 3.3 , median= 5.5).

Table 2. Intra-group analysis for examined variables based on BMI

Entire group					
variable	Statistical parameters	BMI (kg/m ²) before therapy		BMI (kg/m ²) after therapy	
		≤ 24.9	≥ 25	≤ 24.9	≥ 25
AMH	mean \pm SD	12.91 ± 7.5	17.22 ± 13.7	7.78 ± 4.4	6.74 ± 5.5
start/	median (IQR)	10.91(7.3-18.49)	13.2(7.59-21.1)	6.58(3.69-10.34)	6.31(2.8-6.7)
end	p-level	Z=0.69 $p=0.49$		Z=1.45 $p=0.15$	
HOMA IR	mean \pm SD	3.97 ± 1.3	4.32 ± 1.5	2.24 ± 0.99	2.70 ± 0.98
start/	median (IQR)	3.5(3.2-4.6)	4(3-5.7)	2.1(1.6-2.8)	2.4(2.1-3.4)
end	p-level	Z=1.03 $p=0.3$		Z=1.94 $p=0.063$	
FAI	mean \pm SD	10.94 ± 7.7	13.37 ± 13.8	5.10 ± 3.6	6.44 ± 3.3
start/	median (IQR)	6.7(4.65-15.47)	10.46(5.9-17.54)	4.22(2.82-5.99)	5.5(3.95-8.34)
end	p-level	Z=0.31 $p=0.75$		Z=1.64 $p=0.1$	

Z(Mann Whitney test)

Table 3. BMI difference in Metformin and Myoinositol groups at baseline and 6 months after treatment

Statistical parameters		Type of therapy		p-level
		Metformin n=34	Myoinositol n=34	
Age/years	mean \pm SD	23.9 ± 5.8	24.5 ± 4.4	$t=0.45$
	min - max	18-36	20-35	$p=0.66$
BMI (kg/m ²) Start	mean \pm SD	29.76 ± 7.2	26.92 ± 7.3	$t=1.6$
	min - max	17.5-40.7	17.6-47.3	$p=0.11$
BMI (kg/m ²) End	mean \pm SD	28.21 ± 6.4	25.40 ± 6.3	$t=1.8$
	min - max	17.8-39.5	17.2-43	$p=0.07$

t (Student t-test)

BMI - The mean BMI before initiation of therapy was 29.76 ± 7.2 kg/m² in the Metformin group, and 26.92 ± 7.3 kg/m² in the Myoinositol group (Table 3).

At the end of treatment, the mean BMI was 28.21 ± 6.4 kg/m² in the Metformin group, and 25.40 ± 6.3 kg/m² in the Myoinositol group.

A significant decrease in BMI was registered in both groups after therapy ($p=0.000001$ and $p=0.000013$ in the Metformin and Myoinositol groups, respectively).

Body mass index did not differ significantly between the two groups before and after completion of the 6-month therapy ($p=0.11$ and $p=0.07$, respectively).

The average decrease in BMI by 1.547 ± 1.45 kg/m² in the Metformin group and by 1.523 ± 1.73 kg/m² in the Myoinositol group was not statistically significant ($p=0.95$).

Regularity of menstrual cycle - Before therapy, patients in the Metformin group had significantly more irregular menstrual cycles than patients in the Myoinositol group (94.12% vs. 58.82%, $p=0.0006$).

After 6-month therapy, 29.41% of patients treated with Metformin and 23.53% of patients treated with Myoinositol had irregular menstrual cycles, with no statistically significant difference between the groups ($p=0.58$) (Table 4).

Table 4. Regularity of menstrual cycles in Metformin and Myoinositol groups at baseline and 6 months after treatment

MC Menstrual cycle		Type of therapy		p-level
		Metformin n (%)	Myonositol n (%)	
start	irregular	32 (94.12)	20 (58.82)	$X^2=11.8$ $p=0.0006$
	regular	2 (5.88)	14 (41.18)	
end	irregular	10 (29.41)	8 (23.53)	$X^2=0.3$ $p=0.58$
	regular	24 (70.59)	26 (76.47)	

X^2 (Chi-square test), ***sig $p<0.0001$

In the Metformin group of 32 patients with irregular menstrual cycles before therapy, 22 (68.75%) had their cycles regulated after treatment (Table 5); in the Myoinositol group, 20 patients had irregular menstrual cycles before therapy, and 12 (60%) of them had regular cycles after therapy (Table 6). The difference between the two groups in the proportion of patients whose menstrual cycles became regular after treatment was statistically insignificant ($p=0.45$), i.e. the type of therapy had no significant difference on the frequency of cycle regularity.

Table 5. Regularity of menstrual cycle in Metformin group

MC Menstrual cycle	n	Metformin	Irregular after therapy n (%)
		Regular after therapy n (%)	
Irregular before therapy	32	22 (68.75)	10 (31.25)
Regular before therapy	2	2 (100)	0

Table 6. Regularity of menstrual cycle in Myoinositol group

MC Menstrual cycle	n	Myoinositol	Irregular after therapy n (%)
		Regular after therapy n (%)	
Irregular before therapy	20	12 (60)	8 (40)
Regular before therapy	14	14 (100)	0

Before treatment, Anti-Müllerian hormone (AMH) had a mean level of 16.86 ± 14.6 and 13.78 ± 7.1 ng/ml in the Metformin and Myoinositol groups, respectively. After completing 6 months of treatment, the mean level of AMH was 7.69 ± 5.4 and 6.84 ± 4.6 ng/ml in the Metformin and Myoinositol groups, respectively. At the two analyzed time points, AMH did not differ significantly between the groups ($p=0.88$ and $p=0.6$, respectively before and after treatment) (Table 7).

Table 7. AMH levels at baseline and 6 months after treatment in Metformin and Myoinositol groups

AMH	Statistical parameters	Type of therapy		p-level
		Metformin	Myoinositol	
AMH (ng/ml) start	mean \pm SD	16.86 ± 14.6	13.78 ± 7.1	Z=0.15
	median (IQR)	11.48(5.6 – 24.14)	10.91 (9.27 – 20.1)	p=0.88
AMH (ng/ml) end	mean \pm SD	7.69 ± 5.4	6.84 ± 4.6	Z=0.5
	median (IQR)	6.4 (4.1 – 9.6)	5.02 (3.56 – 8.82)	p=0.6

Z (Mann Whitney test), *sig $p<0.05$

Metformin and Myoinositol therapy significantly reduced AMH levels ($p=0.000007$ and $p=0.000024$ in the Metformin and Myoinositol groups, respectively). The mean decrease in AMH was greater in the Metformin group, but statistically insignificant (9.17 vs. 6.94 ng/ml, $p=0.4$) (Table 8).

Table 8. Average reduction differences in AMH level at baseline and 6 months after treatment in Metformin and Myoinositol groups

AMH	Statistical parameters	Type of therapy	
		Metformin	Myoinositol
AMH (mg/ml) start	mean \pm SD	16.86 ± 14.6	13.78 ± 7.1
AMH (mg/ml) end	mean \pm SD	7.69 ± 5.4	6.84 ± 4.6
difference		9.17	6.94
p-level		Z=4.23 *** $p=0.000024$	Z=4.5 *** $p=0.000007$

Z (Wilcoxon Matched Pairs Test), ****sig $p<0.0001$

HOMA IR – Before therapy, the HOMA IR index had significantly higher values in the Metformin group ($p=0.017$). The mean and median values of this parameter were 4.52 ± 1.4 and 4.1 in the Metformin group, 3.81 ± 1.3 and 3.5 in the Myoinositol group. After treatment, the two groups of patients did not differ significantly in terms of HOMA IR values ($p=0.35$). HOMA IR had a mean and median value of 2.60 ± 1.2 and 2.4 in the Metformin group; 2.34 ± 0.8 and 2.2 in the Myoinositol group (Table 9).

Table 9. HOMA and FAI values at baseline and 6 months after treatment in Metformin and Myoinositol groups

Variables	Statistical parameters	Type of therapy		p-level
		Metformin	Myoinositol	
HOMA IR start	mean \pm SD	4.52 ± 1.4	3.81 ± 1.3	Z=2.38
	median (IQR)	4.1(3.4 – 5.7)	3.5 (3.0 – 4.0)	* $p=0.017$
HOMA IR end	mean \pm SD	2.60 ± 1.2	2.34 ± 0.8	Z=0.9
	median (IQR)	2.4 (1.7 – 3.9)	2.2 (1.7 – 2.8)	$p=0.35$
FAI start	mean \pm SD	14.06 ± 14.2	10.54 ± 7.7	Z=1.1
	Median (IQR)	10.46 (5.99 – 15.47)	7.45 (4.5 – 14.6)	$p=0.27$
FAI end	mean \pm SD	6.34 ± 3.8	5.21 ± 3.2	Z=0.85
	Median (IQR)	4.66 (3.26 – 9.44)	5.1 (2.88 – 5.99)	$p=0.39$

Z (Mann Whitney test), *sig $p<0.05$

FAI-Patients treated with Metformin and Myoinositol did not differ significantly in terms of FAI index values before the start of therapy ($p=0.27$) and after therapy ($p=0.39$). Before therapy, the mean and median FAI values were 14.06 ± 14.2 and 10.46 in the Metformin group, and 10.54 ± 7.7 and 7.45 in the Myoinositol group. After therapy, the mean and median FAI values were 6.34 ± 3.8 and 4.66 in the Metformin group, and 5.21 ± 3.2 and 5.1 in the Myoinositol group (Table 9).

A reduction in HOMA IR and FAI was significant after treatment in both groups: $p=0.000001$ and $p=0.000003$ in the Metformin and Myoinositol groups for HOMA IR, respectively; $p=0.000029$ and $p=0.0007$, in the Metformin and Myoinositol groups for FAI, respectively.

The difference in the mean reduction in HOMA IR of 1.92 in the Metformin group and 1.47 in the Myoinositol group was not statistically significant ($p=0.14$).

The average reduction in FAI was also statistically insignificant: by 7.72 in the Metformin group and by 5.32 in the Myoinositol group ($p=0.36$) (Table 10).

Table 10. Average reduction differences of HOMA IR and FAI at baseline and 6 months after treatment in Metformin and Myoinositol group

Statistical parameters		Type of therapy	
		Metformin	Myoinositol
HOMA IR			
start	mean \pm SD	4.52 ± 1.4	3.81 ± 1.3
end	mean \pm SD	2.60 ± 1.2	2.34 ± 0.8
differences		1.92	1.47
p-level		Z=4.9 *** $p=0.000001$	Z=4.64 *** $p=0.000003$
FAI			
start	mean \pm SD	14.06 ± 14.2	10.54 ± 7.7
end	mean \pm SD	6.34 ± 3.8	5.21 ± 3.2
differences		7.72	5.32
p-level		Z=4.18 *** $p=0.000029$	Z=3.97 *** $p=0.00007$

Z (Wilcoxon Matched Pairs Test), ***sig $p<0.0001$

Discussion

SRMA suggests limited or no evidence that high serum AMH levels in patients with PCOS are causally linked to the development of IR (insignificant correlation between AMH and HOMA-IR)^[6].

Meta-analysis provided quantitative evidence demonstrating that Metformin significantly decreased AMH levels, particularly in young patients (age less than 28) and in those with baseline AMH levels higher than 4.7 ng/ml, treated for no longer than 6 months with Metformin, and with a dose not exceeding 2000 mg/day^[7].

Some studies indicate that Metformin regulates insulin and androgen levels, leading to reduced ovarian hyperandrogenism and, ultimately, lower AMH levels. Insulin resistance acts as a moderating factor, as PCOS patients with higher insulin resistance experience a more significant reduction in AMH following Metformin therapy compared to those without insulin resistance^[10].

There is less information about the influence of Inositol on AMH in PCOS. Recent meta-analysis conducted by Greff *et al.* showed non-inferiority of Inositol to Metformin regarding its effect on free and total testosterone, androstenedione, and SHBG^[8]. Facchinetti *et al.* in their meta-analysis found no difference between Metformin and Myoinositol on fasting insulin, HOMA index, testosterone, SHBG levels and body mass index^[9].

Although BMI is thought to negatively affect AMH levels, their mutual relationship is still controversial. Some studies indicate a clear negative correlation between AMH and BMI, whereas

other studies have not confirmed this association^[4,5]. Reduced production of AMH in antral follicles under the influence of hyperinsulinemia and insulin resistance on granulosa cells has been implicated, as well as the possible effect of elevated leptin levels in obese women, which may directly suppress AMH production.

Given that BMI affects the degree of insulin resistance, as well as the level of AMH, our study included patients with normal and elevated BMI in both groups divided according to treatment.

Our results did not confirm an impact of BMI on AMH levels in patients with PCOS. In an intra-group analysis of the investigated variables, before the start of therapy, although AMH was lower in patients with a higher BMI, no statistically significant difference was found in AMH levels in patients with a BMI lower and higher than 25 kg/m² (mean=7.78 ± 4.4 vs. 6.74 ± 5.5 ng/ml; median=6.58 vs. 6.31 ng/ml). The difference of AMH levels between the two groups was also insignificant after 6 months of treatment based on BMI (p=0.15).

Regarding BMI, the two groups of subjects did not differ significantly before initiation of therapy, that is, they were homogeneous in terms of body mass index. After completing the 6-month treatment, the average BMI was 28.21 ± 6.4 kg/m² in the Metformin group, and 25.40 ± 6.3 kg/m² in the Myoinositol group.

In both groups, a significant decrease in BMI was registered after treatment (p=0.000001 and p=0.000013 in the Metformin and Myoinositol groups, respectively). It did not differ significantly between the two groups after the completion of 6-month therapy (p=0.07), implying that both Metformin and Myoinositol had a comparable, statistically significant effect on weight loss in patients with PCOS.

Although the HOMA IR index values were higher in the group with a BMI ≥ 25 kg/m², no statistically significant difference was found in the groups with a BMI lower and higher than 25 kg/m² (p=0.3 and p=0.063, respectively): 3.97 ± 1.3 and 4.32 ± 1.5 were the average values before therapy, 2.24 ± 0.99 and 2.70 ± 0.98 were the average values after therapy.

Body mass index had no significant effect on FAI index before (p=0.75) and after treatment (p=0.1).

Regarding menstrual cycle regularity, before treatment, although patients in the Metformin group significantly more often had irregular menstrual cycles than patients in the Myoinositol group (94.12% vs. 58.82%, p=0.0006), after 6 months of therapy, 29.41% of patients treated with Metformin and 23.53% of patients treated with Myoinositol had irregular menstrual cycles, with no statistically significant difference between the two groups (p=0.58). The difference in the percentage of patients whose menstrual cycle became regular after treatment was statistically insignificant (p=0.45). According to our results, a comparable effect of both treatments on the frequency of cycle regulation was observed.

Regarding Anti-Müllerian hormone, although after the end of treatment with Metformin and Myoinositol, a significant reduction in AMH levels was observed (p=0.000007 and p=0.000024, respectively in the Metformin and Myoinositol groups). The average decrease in AMH was greater in the Metformin group, but statistically insignificant (9.17 vs. 6.94 ng/ml, p=0.4). In our analysis, a comparable effect of Metformin and Myoinositol in terms of AMH reduction was observed.

Before treatment, the HOMA IR index had significantly higher values in the Metformin group (p=0.017). After treatment, the two groups of patients did not differ significantly in terms of HOMA IR values (p=0.35). The decrease in HOMA IR was significant after therapy in both group (p=0.000001 and p=0.000003 in the Metformin and Myoinositol groups, respectively). In our

study, although Metformin group had greater decrease in HOMA IR, it was statistically insignificant. Approximately equal efficacy in reducing HOMA IR values (1.92 in the Metformin group and 1.47 in the Myoinositol group, with $p=0.14$) was observed for both therapeutic modalities. These results indicate that both Metformin and Myoinositol are equally effective in reducing the degree of insulin resistance expressed through HOMA IR, as a surrogate marker.

Patients treated with Metformin and Myoinositol did not differ significantly in terms of FAI index values before the start of therapy ($p=0.27$) and after therapy ($p=0.39$).

The reduction in FAI was significant after therapy in both groups ($p=0.000029$. and $p=0.0007$ in the Metformin and Myoinositol groups, respectively), with the same efficacy of both treatments in reducing free androgen index values ($p=0.36$).

Conclusion

Our study showed that treatment with insulin sensitizers in both groups of patients led to a significant comparable decrease in elevated AMH values observed in patients diagnosed with PCOS. At the same time, the regularity of the menstrual cycle, which was observed in the majority of subjects from both groups, is considered an indirect indicator of ovulation induction in these patients. In this regard, equal, comparable efficacy was observed for both therapeutic modalities. This was accompanied with decline in HOMA IR and FAI values after the end of treatment in both groups, which indicates that both pathogenetic mechanisms could play a role in the occurrence of chronic anovulation in PCOS, and thus in the elevated AMH values in these patients. In line with the results obtained, AMH could serve as a prognostic marker of the efficacy of treatment with insulin sensitizers in PCOS.

Conflict of interest statement. None declared.

References

1. Pellatt L, Hanna L, Brincat M, Galea R, Brain H, *et al.* Granulosa cell production of anti-Müllerian hormone is increased in polycystic ovaries. *J Clin Endocrinol Metab* 2007; 92(1): 240-245. doi: 10.1210/jc.2006-1582.
2. Catteau-Jonard S, Jamin SP, Leclerc A, Gonzalès J, Dewailly D, di Clemente N. Anti-Müllerian hormone, its receptor, FSH receptor, and androgen receptor genes are overexpressed by granulosa cells from stimulated follicles in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2008; 93(11): 4456-4461. doi: 10.1210/jc.2008-1231.
3. Willis D, Mason H, Gilling-Smith C, Franks S. Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulosa cells of normal and polycystic ovaries. *J Clin Endocrinol Metab* 1996; 81(1): 302-309. doi: 10.1210/jcem.81.1.8550768.
4. Gorzko A, Melnyk M, Nawrocka-Rutkowska J, Starczewski A, Marciniak A, Szydlowska I. Evaluation of Anti-Müllerian Hormone (AMH) Serum Levels in Patients with Polycystic Ovary Syndrome (PCOS) Depending on Body Mass Index (BMI). *J. Clin. Med.* 2025;14(8): 2677. doi: 10.3390/jcm14082677.
5. Kolcsar M, Szabó L, Mihály R, Vass ER, Gáll Z. Anti-Müllerian Hormone Level Determinants among Non-Polycystic-Ovary-Syndrome Women Undergoing In Vitro Fertilization: A Retrospective Cross-Sectional Study. *Medicina (Kaunas)* 2024; 60(9): 1387. doi: 10.3390/medicina60091387.

6. Zhou Z, Chen H, Chu L, Zou Q, Chen Q, Yang J, et al. The effects of metformin on anti-Müllerian hormone levels in patients with polycystic ovary syndrome: a systematic review and meta-analysis. *J Ovarian Res* 2023; 16(1): 123. doi: 10.1186/s13048-023-01195-1.
7. Greff D, Juhász AE, Váncsa S, Váradi A, Sipos Z, Szinte J, et al. Inositol is an effective and safe treatment in polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. *Reprod Biol Endocrinol* 2023; 21(1): 10. doi: 10.1186/s12958-023-01055-z.
8. Facchinetti F, Orrù B, Grandi G, Unfer V. Short-term effects of metformin and myo-inositol in women with polycystic ovarian syndrome (PCOS): a meta-analysis of randomized clinical trials. *Gynecol Endocrinol* 2019; 35(3): 198-206. doi: 10.1080/09513590.2018.1540578.
9. Bhide P, Pundir J, Gudi A, Shah A, Homburg R, Acharya G. The effect of myo-inositol/di-chiro-inositol on markers of ovarian reserve in women with PCOS undergoing IVF/ICSI: A systematic review and meta-analysis. *Acta Obstet Gynecol Scand* 2019; 98(10): 1235-1244. doi: 10.1111/aogs.13625.
10. Mehdinezhad Roshan M, Sohouli MH, Izze da Silva Magalhães E, Hekmatdoost A. Effect of metformin on anti-mullerian hormone levels in women with polycystic ovarian syndrome: a systematic review and meta-regression analysis of randomized controlled trials with. *BMC Endocr Disord* 2024; 24(1): 43. doi: 10.1186/s12902-024-01570-z.