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The Effects of Metformin on Hyperandrogenism and Menstrual Functions in Insulin Resistant Adolescents with PCOS

Adolesan Polikistik Over Sendromlu Hastalarda Metformin Tedavisinin Hiperandrojenizm ve Menstrual Fonksiyonlar Üzerine Etkisi

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Abstract

Aim: To investigate the effects of metformin therapy on hyperandrogenism and menstrual functions in adolescent patients with polycystic ovary syndrome (PCOS) and insulin resistance (IR).

Material and Method: This study was conducted with 50 adolescents with PCOS. Hormonal and ultrasonographic assessments were done at the early follicular phase. On the day of 19-21 of the cycle, progesterone levels were measured and patients were questioned for menstrual periods. Body-mass-indexes (BMI), waist-hip ratios, and Ferriman-Gallwey scores were calculated for all patients. IR is diagnosed according to HOMA index and insulin-resistant patients were instructed to use 1500 mg/day metformin for 3 months. After treatment, measurements were repeated by the same researcher.

Results: Mean age and BMI values were similar between groups. Although the values of BMI and waist/hip ratio decreased after treatment, the decrement didn't reach the values of the IR- group. Free testosterone levels were significantly higher in IR+ subjects compared to controls and decreased considerably after metformin. Sex hormone-binding globulin levels were increased with treatment and as a result, free androgen indexes were decreased. 17 OH progesterone levels were significantly higher in the IR+ group and regressed to similar levels with IR negatives with metformin. While mean levels of fasting insulin were 19.21 IU/ml in the IR+ group, it regressed to 13.14 IU/ml after treatment. Also fasting glucose/insulin ratios were increased as expected. Finally, a significant improvement in the treatment group was observed at menstrual irregularity.

Conclusion: Consequently, this study supports the conclusion that metformin reduces hyperandrogenism, regulates menstruation and improves ovulatory functions.

Keywords: Adolescent, polycystic ovary syndrome, insulin resistance, metformin

Öz

Amaç: Çalışmamızda insülin direnci olan adolesan polikistik over sendromlu hastalarda metformin tedavisinin hiperandrojenizm ve menstrual fonksiyonlar üzerine etkisinin değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Çalışmamıza, polikistik over sendromlu 50 adolesan hasta dahil edildi. Erken foliküler fazda hormonal ve ultrasonografik değerlendirmeler yapıldı. Tüm hastaların vücut kitle indeksleri (VKİ), bel-kalça oranları ve Ferriman-Gallwey skorları hesaplandı. Hastalar menstrual fonksiyonlar açısından sorgulanıp ovulasyon tespiti için siklusun 19-21. günlerinde progesteron düzeyleri ölçüldü. HOMA indeksi kullanılarak insülin resistansı (IR) tanısı konulan hastalara 3 ay süreyle 1500 mg/gün metformin kullanması önerildi. Tedavi bitimi tüm ölçümler aynı araştırmacı tarafından tekrarlandı.

Bulgular: Gruplar arasında ortalama yaş ve VKİ açısından anlamlı fark yoktu. İnsülin rezistansı olan grubun bel çevresi kontrol grubuna göre fazla olup, tedavi sonrası kontrollerle benzer seviyelere inemediği gözlendi. Serbest testosteron seviyeleri, IR+ hastalarda IR negatiflere kıyasla anlamlı oranda daha yüksekti ve tedaviden sonra önemli ölçüde azaldı. SHBG düzeylerinin metformin tedavisi ile arttığı, dolayısıyla serbest androjen indeksinin azaldığı izlendi. 17 OH progesteron seviyeleri IR+ grupta anlamlı olarak daha yüksekti ve metformin tedavisi ile IR negatiflerle benzer seviyelere indi. IR+ grubunda ortalama açlık insülin düzeyi 19,21 IU/ml iken tedavi sonrası 13,14 IU/ml'ye geriledi. Beklenildiği gibi açlık glukoz/insülin oranı da metformin tedavisi sonrası artış gösterdi. Son olarak tedavi grubunda menstrual düzensizlikte istatistiksel olarak anlamlı oranda düzelme gözlendi.

Sonuç: Bu çalışma, metforminin hiperandrojenizmi azalttığı, menstruasyonu düzenlediği ve ovulatuar fonksiyonları iyileştirdiği sonucunu desteklemektedir.

Anahtar kelimeler: Adolesan, polikistik over sendromu, insülin direnci, metformin

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex common gyneco-endocrine pathology of reproductive age.[1,2] It is an entity with many clinical signs of hyperandrogenism such as acne, hirsutism, and menstrual irregularities secondary to anovulation. The diagnosis of the adolescent age group is controversial, temporary functional hyperandrogenism and peripubertal menstrual disorders make the diagnosis difficult.[3] Hyperpulsatile gonadotropin release, hyperactive ovarian and adrenal androgen production are similar events in puberty and adolescents with PCOS. Besides this, the pathophysiological process is often associated with insulin resistance in adolescents with PCOS. Compensatory hyperinsulinemia can be the cause or result of hyperandrogenism.

Since the relationship between hyperandrogenemia secondary to stimulated androgen production from theca cells decreased sex hormone binding globin (SHBG) and IGFBG-1, and exaggerated insulin secretion was discovered; insulin-sensitizing agents have been used for the treatment of both hyperinsulinemia and hyperandrogenemia.[4] Metformin has been studied extensively in many phenotypes of PCOS in these agents. Although the adolescent group is a sensitive population, studies must be designed with increased scrutiny; metformin has been used in adolescents with type 2 diabetes and found in a safe area on the risk-benefit ratio.[5] But it is still not well documented in insulin-resistant adolescents and also its effects not have been presented on hyperandrogenism signs and symptoms in adolescents with PCOS. This study, it is aimed to evaluate the effects of metformin treatment on clinical and biochemical hyperandrogenism and menstrual functions in insulin-resistant adolescent patients with PCOS.

MATERIAL AND METHOD

This study was conducted with 25 patients between the age of 12 and 18 who were diagnosed with PCOS accompanying insulin resistance in the Gynecology and Obstetrics Department of a tertiary center between January 2012 and April 2013. 25 PCOS patients of the same age without insulin resistance were included as control group. PCOS was diagnosed as the presence of two of the following three findings: hyperandrogenism, ovulatory dysfunction, and polycystic ovaries, after exclusion of the other endocrine disorders like congenital adrenal hyperplasia and hypothyroidism. At the first visit, all patients' heights, weights, waist and hip circumferences were measured and physical examination for hirsutism was done by only one observer. Body mass indexes (BMI), waist-hip ratios, and Ferriman-Gallwey scores were calculated for all patients.

Transabdominal ultrasounds were applied between the 2nd and 5th days of the menstrual cycle. Increased ovarian volume (>10 cm^3) and presence of 2-9 mm follicles more than 12 in number on each ovary of patients were considered as polycystic ovaries. Blood samples were collected early in the morning after 8-10 hours of fasting on the 2-4th day of the cycle. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, thyroid-stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), prolactin (PRL), free testosterone (FT), total testosterone (TT), dehydroepiandrosterone sulfate (DHEA-S), 17 hydroxyprogesterone (17 OH progesterone), insulin, glucose, HDL cholesterol, LDL cholesterol, VLDL cholesterol, total cholesterol, and triglyceride measurements were recorded. For fasting blood glucose and fasting insulin, HOMA and QUICKI indexes were calculated. Scores higher than 2.1 for HOMA and lower than 0.357 for QUICKI were considered as insulin resistance.[6,7] Patients were questioned for menstrual periods and patterns. On the day of 19 – 21 of the cycle progesterone levels were measured for evaluation of ovulation and scores higher than 3 ng/ml were considered as ovulation.[8,9]

Insulin resistant 25 patients were instructed to use 2x850 mg/day metformin for 3 months (Glucophage®, Merck, Germany). After treatment ended all measurements were repeated by the same researcher. Menstrual periods and patterns after treatment were recalled from patients. The study was approved by the Local Ethics Committee of the Fatih University with the approval number of 2013/100.1. All patients and their legal representatives provided written informed consent to participate.

Statistical Analysis

The data of both groups were statistically analyzed with SPSS 17.0 (Statistical Package for Social Sciences) program. While evaluating the study data, besides descriptive statistical methods, the student t-test was used for comparing the parameters with normal distribution between groups in comparison of quantitative data. Pearson's correlation test was used to examine the relationships between parameters. If $P < 0.05$, at the 95% confidence interval, the result was considered statistically significant.

Clinical Assessment and Hormone Assay

Height and weight were measured to calculate the BMI with weight(kg) / $[height(m)]^2$ formula. Hirsutism was evaluated according to the Ferriman-Gallwey score system. According to this system, each patient was evaluated in 9 anatomical regions by the same researcher and a score between 0 (no terminal hair growth) and 4 (maximum hair growth) was given for each region. A score below 8 was considered normal, while a score above 8 was considered pathological.[10] Waist and hip circumference was measured by tape measure, to the nearest 0.5 cm for waist circumference, the narrowest diameter between crista iliaca and the costa were used as reference. The hip circumference was measured at the level of maximal anteroposterior excursion at the level of thighs.[11]

Transabdominal ultrasounds were applied with a full bladder using a 3.5 MHz convex probe (General Electric Logiq 7, USA) by the same researcher on the day of 2-5 of the menstrual cycle.[12,13] Plasma levels of FSH, LH, estradiol, progesterone, TSH,

free T4, free T3, prolactin, total testosterone, free testosterone, DHEA-S, 17 OH progesterone, insulin were measured using radioimmunoassay (Roche Diagnostics, Cobas Integra, France). Fasting glucose, HDL, LDL, VLDL, cholesterol, and triglyceride measurements were performed using calorimetric studies (Roche Diagnostics, Cobas Integra, France). QUICKI indexes calculated as 1/[log(Insulin)+log(Glucose)] and the HOMA-IR was determined from [Insulin X Glucose (mmol/L) X 0.055/22.5] formula.[14,15] The free androgen index (FAI) was the equation of (testosterone/SHBG) x 100).[16]

RESULTS

Mean age and BMI values were similar between IR+ and IR- groups. Both BMI and mean waist circumference values were higher in the insulin-resistant group compared to the control group, while differences at mean waist circumference were significant (77.84 vs 71.68, p<0.05). Although the values of BMI and waist/hip ratio decreased after the treatment, the decrement didn't reach the values of the control group (67.20 vs 64.92, p>0.05 and 77.84 vs 76.36, p>0.05)(**Table 1**).

FSH, LH, E2, DHEA-S, and total testosterone levels were similar between IR+ and IR- groups and didn't affected significantly after the treatment. 17 OH progesterone levels were significantly higher in the IR+ group compared to the control group and regressed to similar levels with IR negatives after metformin. Free testosterone levels were significantly higher in IR+ subjects compared to control subjects (2.67 vs 1.98, P<0,05) and decreased considerably after the treatment (p>0.05). Although didn't reach statistical significance; SHBG levels increased with metformin treatment and as a result, the free androgenic indexes decreased (38.82 vs 42.45 and 0.92 vs 0.80, p>0.05)(**Table 2**). HDL, LDL, total cholesterol, and triglyceride values were similar between the insulin-resistant and non-resistant groups and didn't significantly differ in IR patients after the treatment (**Table 3**).

Table 1. Comparison of demographic characteristics and anthropometric measurements between groups

	IR (+) Group		IR (-) Group	P		
	Before Treatment (a)	After Treatment (b)	Control (c)	a-c	b-c	a-b
Age	16.28±1.30	16.28±1.30	16.00±1.71	0.764	0.764	1.00
Weight(kg)	67.20±8.11	64.92±7.00	63.16±7.71	0.061	0.264	0.322
Height(m)	1.64±0.04	1.64±0.04	1.63±0.06	0.407	0.407	1.00
BMI(kg/m^2)	24.86±2.70	24.02±2.35	23.77±2.55	0.128	0.509	0.299
Waist(cm)	77.84±8.89	76.36±6.63	71.68±5.60	0.011	0.013	0.793
Hip(cm)	105.0±9.27	103.28±7.41	100.68±4.77	0.132	0.231	0.634
Waist/Hip	0.75±0.09	0.74±0.05	0.71±0.04	0.057	0.070	0.793

Body-mass-indexes; BMI.

Table 2. Comparison of hormonal parameters between groups

	IR (+) Group		IR (-) Group	P		
	Before treatment (a)	After treatment (b)	Control (c)	a-c	b-c	a-b
FSH (mIU/mL)	4.62±2.07	4.25±1.33	5.41±2.01	0.130	0.023	0.698
LH (mIU/mL)	7,00±4.06	6.36±3.62	6.11±3.00	0.516	0.771	0.594
LH/FSH	1.82±1.21	1.56±0.75	1.20±0.54	0.071	0.086	0.594
E2	50.82±53.89	43.39±31.70	50.81±43.40	0.554	0.712	0.669
DHEA-S (µg/dL)	228.56±107.38	233.72±112.54	210.56±100.79	0.567	0.388	0.884
17 OH Progesteron (ng/mL)	2.00±0.94	1.43±0.72	1.40±.726	0.024	0.869	0.026
Total Testosteron (ng/mL)	0.33±0.14	0.31±0.14	0.30±0.21	0.248	0.372	0.698
Free Testosteron (ng/mL)	2.67±1.19	2.24±0.89	1.98±0.69	0.012	0.225	0.181
SHBG (nmol/L)	38.82±11.53	42.45±12.50	41.20±17.27	0.854	0.547	0.327
FAI (U/mL)	0.92±0.50	0.80±0.39	0.86±0.81	0.133	0.308	0.560

FSH; Follicle-stimulating hormone LH; luteinizing hormone, E2; estradiol, DHEA-S; dehydroepiandrosterone sulfate, 17 OH progesterone; 17 hydroxyprogesterone, SHBG; sex hormone-binding globin, FAI; free-androgen index.

Table 3. Comparison of lipid values between groups

	IR (+) Group		IR (-) Group	P		
	Before treatment(a)	After treatment(b)	Control (c)	a-c	b-c	a-c
Total Cholesterol (mg/dL)	183.68±25.61	174.60±32.19	169.16±28.18	0.087	0.621	0.207
HDL (mg/dL)	48.28±8.52	49.0±7.52	51.04±8.54	0.190	0.268	0.676
LDL (mg/dL)	102.23±21.03	98.92±22.98	94.44±19.24	0.240	0.479	0.669
VLDL	20.64±8.85	18.66±6.02	16.71±5.71	0.052	0.190	0.342
Trigliseride (mg/dL)	103.18±44.27	93.32±30.12	83.56±28.54	0.052	0.190	0.342

HDL; High-density lipoprotein, LDL; Low-density lipoprotein, VLDL; Very low-density lipoprotein.

While fasting insulin levels were 19.21 IU/ml in the group with insulin resistance, they regressed to 13.14 IU/ml (p<0,05) after treatment. Also, the fasting glucose/ insulin ratio was increased from 5.36 to 8.35 after metformin (p<0,05) (**Table 4**). When treatment efficiency regarding menstrual function, ovulation, and hirsutism scores were compared between groups, significant improvement in the treatment group was observed at menstrual irregularity (22 vs 12, p<0.05). The ovulatory cycle rate increased slightly but it was not statistically significant (52% vs 32%, p>0.05). Hirsutism scores measured by the modified Ferriman-Gallwey scoring system were similar between the two groups when compared before and after the treatment (**Table 5**).

DISCUSSION

PCOS is still a controversial status in terms of etiopathogenesis, diagnosis, and treatment. It is a disease that should be evaluated in a wide range with its fairly broad spectrum. The primary clinical symptoms may occur in the late phase or immediately after puberty. It is difficult to diagnose of PCOS in the adolescent age group, but reaching a definitive diagnosis at an early age is very important for the attentive lifelong medical follow-up due to the long-term risks of the disease.

Increased obesity and impaired body fat distribution, which are characteristic of adult women with PCOS, could not be shown in adolescent PCOS patients in studies. While Rosenfield et al. found the obesity prevalence similar in adolescents with adult PCOS,[17] Van Hoff et al. showed that BMI is lower in adolescents.[18] In this study, both insulin resistant and IR- groups BMI were under 25 kg/m^2. When the BMI values were compared, there was no statistically significant difference between the IR- group and the study group (p>0.05). Although a specific diet program was not applied to the patients included in the study, an average weight loss of 2.1 kg was observed in the group using metformin, but no significant difference was observed when compared with the pre-treatment and control groups.

Studies have shown that 30% to 50% of women with PCOS have an increased waist/hip ratio, and this android type obesity is predisposed to glucose intolerance, lipid abnormalities, and hyperandrogenism.[19] In the study, it was observed that the waist circumference measurements of the group with insulin resistance were more than the control group, and although it decreased after the treatment, it didn't decrease to similar levels with the controls.

One of the controversial issues in the diagnosis and treatment of PCOS is the clinical signs of hyperandrogenism. Because of ethnic differences, epilation methods used before clinical evaluation, and subjective detection, diagnosis of hirsutism is difficult.[20] Researchers have focused on this subject and developed standardized scoring methods to use in clinical practice Although the number of studies on polycystic ovary syndrome in the adolescent age group is more limited compared to the adult age group, the percentage of hirsutism was reported to be 67% in an adolescent PCOS study by Orsino A. et al.[21] In this study, the frequency of hirsutism was found to vary between 88-92% in the measurements made according to the Ferriman-Gallwey scoring. In the literature, it was argued that metformin treatment was an effective way to treat hirsutism by increasing insulin sensitivity,[22] but it was found no significant change for hirsutism between the group before and after treatment. Acne, another finding of hyperandrogenism, is frequently observed in the adolescent age group, but it is accepted as a multifactorial, transient phenomenon.[23] Therefore, in the patient population of this study, the presence of acne at presentation was not evaluated within the diagnosis of hyperandrogenism.

Table 4. Comparison of fasting glucose, insulin and glucose/insulin ratio between groups

	IR (+) Group		IR (-) Group	P		
	Before treatment (a)	After treatment(b)	Control (c)	a-c	b-c	a-b
Fasting Glucose(mg/dL)	89.73±6.78	89.20±6.86	84.52±6.03	0.008	0.007	0.884
Fasting Insulin(mIU/mL)	19.21±9.68	13.14±6.30	8.46±3.07	< 0.001	0.004	0.003
Glucose /Insulin	5.36±0.05	8.35±1.12	11.75±0.72	<0.001	0.002	0.004

Table 5. Comparison of the effect of metformin treatment on insulin resistance, menstrual function, ovulation and hirsutism

		IR (+) Group		IR (-) Group	P		
		Before treatment (a)	After treatment (b)	Control (c)	a-c	b-c	a-b
IR (HOMA)	+	25 (100%)	17 (68%)	25 (100%)	<0.001	0.005	0.004
	-	0 (0%)	8 (32%)	0 (0%)			
IR (QUICKI)	+	25 (100%)	5 (20%)	25 (100%)	<0.001	0.039	0.050
	-	0 (0%)	20 (80%)	0 (0%)			
Ovulation	+	13(52%)	16 (64%)	15 (60%)	0.375	0.773	0.762
	-	12 (48%)	9 (36%)	10 (40%)			
Hirsutism	+	22 (88%)	23 (92%)	23 (92%)	1.000	1.000	1.000
	-	3 (12%)	2 (8%)	2 (8%)			
Menstruel irregulation	+	22 (88%)	12 (48%)	17 (68%)	0.091	0.156	0.003
	-	3 (12%)	13 (52%)	8 (32%)			

HOMA; Homeostasis model assessment, QUICKI; The quantitative insulin-sensitivity check index.

In the insulin-resistant group with the changes mentioned above, 17 OH Progesterone levels were significantly decreased to similar levels with the control group after treatment. While free testosterone levels were significantly higher in the resistant group than in the control group before treatment, this rate decreased after the treatment, but could not reach the control group. SHBG levels were increased with metformin treatment, thus free androgen indexes were decreased in the IR+ group ($p>0.05$). Finally, the average LH / FSH ratio decreased from 1.82 to 1.59 in the IR+ group, while it was 1.20 in controls. When these data were compared before and after treatment and the control group, no significant change was found. But in our opinion, it is an important finding as in the literature, the increased LH level is present only in 40-60% of adolescents with hyperandrogenism, thus the LH / FSH ratio has no significant value in the diagnosis of PCOS in adolescents.[24] The obtained data show that LH / FSH ratio may have predictive value for insulin resistance and IR+ PCOS patient's follow-up.

The most studied insulin-sensitizing agent in the treatment of insulin resistance in patients with PCOS is metformin. Metformin, an oral antidiabetic from the biguanides group, is the most commonly used and oldest oral antidiabetic in the world.[25] Positive effects on impaired glucose mechanisms were found in patients with PCOS treated with metformin. Minimum 12-weeks therapy of metformin is were suggested in the studies for improved insulin sensitivity.[26] In another study, 18 patients with PCOS aged between 15 and 18 were given metformin treatment for 6 months, and significant improvements in insulin resistance were reported.[27] In this study, a statistically significant decrease was found in insulin resistance in the IR+ group after 3 months of metformin treatment. Fasting insulin levels decreased from 19.21 to 13.14, both HOMA and QUICKI indexes were changed, such that 8 patients were recognized as non-resistant due to HOMA, this number was 20 when measurements were done with QUICKI.

When the effectiveness of the treatment was compared in terms of menstrual function and ovulation rates between the groups; there was a significant improvement in the treatment group (52% vs 64%, respectively), and even better results were obtained than the control group (60%) in ovulation rates according to progesterone levels between days 19-21. For menstrual irregularity which was subjectively questioned, significant improvement in the treatment group was observed at menstrual irregularity (12% vs 52%, $p<0.05$). These results led metformin to be associated with an improvement in ovulatory functions.

The main limitation of this study is the small sample size and non-randomization. With a large size of the sample, the study power would increase, and also more significant differences would be achieved if the groups were determined by randomization and the late results of the control group were included. On the other hand this study is considered to be the first prospective study to investigate the efficacy and safety of metformin in the adolescent population. Although side effects have not been documented there was no patient reported any repercussion.

CONCLUSION

In conclusion, this study revealed that 3 months of metformin treatment in adolescents with PCOS accompanied by insulin resistance reduced fasting plasma glucose and insulin levels resulting in decreased insulin resistance. As a secondary outcome, free androgen indexes statistically significantly decreased in accordance with decreased free testosterone levels and increased sex hormone-binding globulin levels. Although it was not possible to evaluate the alteration in clinical hirsutism status due to a short interval of treatment for about 12 weeks, it is possible to declare that metformin has favorable effects on hyperandrogenism.

Additionally, the treatment was found to statistically significantly reduce menstrual irregularity accompanied by increased ovulation rates which have been demonstrated by luteal phase progesterone levels. Hence, this study supports the conclusion that metformin improves menstrual and ovulatory functions, and by putting forward efficiency and safety of the treatment it encourages the development of fully powered trials in adolescent population.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Local Ethics Committee of the Fatih University with the approval number of 2013/100.1.

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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