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Polikistik Overli Hastalarda Metformin Tedavisinin Antimüllerian Hormon Düzeylerine Etkisi

Effect of Metformin Therapy on Anti Mullerian Hormone Levels in Patients with Polycystic Ovary Syndrome

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Özet

Amaç: Polikistik over sendromu, üretken çağdaki kadınlarda sık görülen bir klinik durumdur. Hiperinsülinemi ve insülin direnci, hastalığın patofizyolojisinde önemli bir antite olarak karşımıza çıkar. Çalışmamızın amacı; antimüllerian hormon (AMH) düzeylerinin metformin tedavisi sonrası değişimini incelemekti.

Yöntem: Rotterdam kriterlerine göre Polikistik over tanısı alan 84 hasta ve 28 sağlıklı gönüllü çalışmaya alındı. Hastaların yarısı (n=42) 1,7 gr/gün metformin tedavisi alırken diğer 42 hasta tedavisiz izlendi. 6 aylık perioddan sonra hastaların laboratuar testleri karşılaştırıldı.

Bulgular: AMH düzeyleri tedavi öncesi benzerdi. Metformin tedavisi sonrası tedavi alan PCOS hastaları ve kontrol grubu AMH düzeyleri 6 aylık period sonrasında benzer düzeylerde bulundu. Tedavi edilmeyenlere göre 1.7 gr/gün metformin alan hastalarda AMH düzeyleri belirgin düşük olarak izlendi ($p<0.001$).

Sonuç: AMH düzeyleri antral follikül volümü ve serum testosteron düzeyleri ile korelasyon gösteren bir parametredir. AMH ölçümünün, ovaryan yaşlanmayı değerlendirmede, polikistik over tanısında ve tedavi takibinde yeri olduğu araştırılmıştır. AMH düzeylerinin metformin tedabisi sonrası düşmesi, metforminin PCOS hastalarında kullanımını destekleyen bir veridir.

Anahtar Kelimeler: Polikistik over sendromu, metformin, antimüllerian hormon.

Abstract

Objective: The polycystic ovary syndrome (PCOS) is a disorder of reproductive-aged women. The objective of this prospective randomised study was to observe antimullarian hormone (AMH) levels in patients with PCOS after metformin therapy.

Method: A total of eighty- four(84) consequtive patients diagnosed according to the criteria proposed in Rotterdam and twenty(28) healthy volunteers were included in the study. Half of patients (42) were treated with 1. 7 gr/ day metformin for six months. The other patients (n=42) were observed without therapy for six months and all laboratory tests were compared with control group after 6-month-period

Results: AMH levels were similar in two patient groups before treatment. After metformin therapy, AMH was measured significantly higher in PCOS- untreated group and PCOS+ metformin group before treatment($p<0.001$); but no difference is observed after 6- month treatment with controls. There was no significant relationship between AMH level and ovarial stromal thickness or follicle number; whereas, a positive correlation was found with ovarian volume ($p<0,05$).

Conclusion: Since serum AMH levels correlate with antral follicle volume and serum testosterone levels, AMH measurement could be used as a tool to evaluate ovarian ageing, to diagnose polycystic ovaries and follow efficacy of treatment. Serum AMH levels significantly decrease after metformin treatment, probably due to decrease in hyperinsulinemia.

Keywords: Polycystic ovary syndrome, metformin, anti mullerian hormone.

Introduction

The polycystic ovary syndrome(PCOS) is a disorder affecting 4% to 12% of reproductive aged women (1). It is represented with heterogeneous features such as oligo-anovulation, the presence of hyperandrogenism with ovulatory dysfunction and/or polycystic ovarian morphology.

Although the etiology of PCOS is not clearly established; increased androgen synthesis and secretion by ovarian theca cells (2), and hyperinsulinemia/insulin resistance have been held as primary mechanisms (3, 4).

Metformin is an agent widely used in diabetes mellitus to increase the sensitivity to endogenous insulin. Metformin treatment is

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Table 1. Comparison of demographic features between patient and control groups.

Variables	Control	PCOS – untreated	Metformin PCOS	p
Age	26,0±4,5	25,3±6,7	25,1±7,1	0,941
Height	1,64±0,05	1,64±0,06	1,63±0,06	0,931
BMI	20,6±2,5	26,5±6,8	29,3±6,5	<0,001
Waist cir	74 (65-79)	87,5 (67-137)	92 (70-123)	<0,001
F.Gallwey	0 (0-0)	3 (0-15)	3 (0-12)	<0,001
Acne	0 (%0)	8 (%38,1)	10 (%43,5)	0,003

BMI: Body mass index, Waist cir: waist circumference F.Gallwey: Ferriman Gallwey score
PCOS: Polycystic ovary syndrome

effective in treatment of PCOS for providing insulin sensitivity, adjustment of metabolic and endocrine impairments and restoring normal endogenous ovulatory function with ensurement from hyperstimulation and multiple pregnancies (5).

Anti- mullerian hormone (AMH) is a member of the transforming growth factor-beta (TGF-β) superfamily. It is secreted by Sertoli cells for testicular differentiation until puberty. AMH is also expressed in the developing ovarian follicles. This hormone is not expressed in atretic follicles and theca cells (6). After puberty, AMH level diclines in circulation and becomes undetermined after menopause. It is also proven that AMH level is not effected from the fluctuations of reproductive hormones, and throughout the menstrual cycle. These observations leads to the hypothesis that there may be regulatory effect of AMH on ovarian activity (7). AMH levels in women with PCOS were found to be two or three times increased (8).

Therefore, the objective of the current report was to study some hormones such as FSH, LH, testosterone and AMH levels in healthy, PCOS without any treatment and PCOS groups before and after metformin therapy to observe the effect of metformin treatment on serum AMH level.

Materials and Methods

The study was conducted between June 2012 and February 2013 at Turgut Ozal University Faculty of Medicine, Ankara, Turkey. 84 patients followed by Endocrinology or Gynecology policlinics between 17- 35 age with the diagnosis of polycystic ovarian syndrome and 28 age-matched controls were included. The control group was consisted of individuals who had normal ovulatory function and biochemical parameters. Forty-two of patients were observed without any treatment and 42 of them were followed with metformin treatment for six months. All patients were selected randomly.

PCOS diagnosis was based on 2003-Rotterdam criteria. The diagnosis of polycystic ovary was confirmed by ultrasound. Ovaries bigger than 10 cm^3 and had 2- 9 mm follicles more than 10 at the periphery are accepted as polycystic. Ovarian volume calculation is made on image(0.5X heightX weightX thickness).

Exclusion parameters were hypertension, diabetes, abnormal liver function test, renal disease, endocrine anomalies such as hypo\ hyperthyroidism, hyperprolactinmia, Cushing syndrome, congenital adrenal hyperplasia; any previous therapy with glucocorticoids, thyroid preparations, agents for ovulation induction, antiandrogenics, treatment of oral contraceptive within 6 months, history of abortus and ovarian surgery. 24 patients diagnosed with PCOS and treated with ethynil estradiol and cyproterone acetate were excluded from the study.

Informed consent was obtained from each patient and our study protocol was suitable to the ethical guidelines of the Declaration of Helsinki as reflected in a prior approval of

instution's human research committee. The local ethic committee approved the study.

The BMI was calculated as weight in kg/height in meters squared for all participants(BMI=kg/m2). Waist circumferences of all groups are measured; narrowest diameter between chest and iliac crests was evaluated. Subjects with waist circumference more than 88 cm were regarded as android obese. Modified Ferriman- Gallwey scala used to evaluate hirsutism.

Subjects were classified as three groups; 1)Healthy control group 2)Untreated PCOS patient 3)PCOS patients treated with metformin hydrochloride 1700 mg/ day (Glucophage capsule 850 mg 2x1).

Blood samples were collected from patients and control group after overnight fasting. All of patients were oligomenorrheic and at the follicular phase when blood for hormone test were taken. Complete blood count, fasting blood glucose, follicle stimulating hormone(FSH), luteinising hormone (LH), estradiol (E2), prolactin(PRL), total testosteronee, dehydro-epiandrostenedion(DHEAS),17-OHP(17 hidroxy progesterone), insulin and anti-mullerian hormone(AMH) were studied. Progesterone, FSH, LH, E2, PRL, DHEAS testosterone were studied via electrochemilucent method(Roche Diagnostics ELECSYS 2010). HOMA-IR index is assesed for every subject by using fasting insulinXfasting glucose(mmol per liter)/ 22. 5 formule.

Serum AMH levels were determined by using enzyme-linked immunsorbent assay kit (Diagnostic Systems Laboratories, Beckman Coulter Company, Webster, USA). Values are presented as nanograms per mililitre. The intra-assay and inter-assay coefficients of variation (CV) for MIS/AMH were assessed, and the values of the CVs ranged from 2. 4 to 6. 7 %. The lowest detectable level of MIS/AMH is 0.006 ng/mL.

All analyses were performed by using statistical package of social programs(SPSS) for Windows, version 13. 0 packed programs (SPSS Inc. , Chicago, USA). The relationship among variables was analysed using Spearman's correlation test. Parameters were normally distributed. Significance of difference between the groups in terms of average variance (One-Way ANOVA) and significance of difference in terms of median values was determined by Kruskal-Wallis test. Data were announced as mean value ± Standard Deviation(SD). P value less than 0.05 was accepted as statistically significant.

Table 2. Comparison of antimullerian hormone levels between patient and control groups after 6 month follow-up

Groups	AMH
Control	3,36 (0,22-5,96)
PCOS- untreated group	5,33 (1,38-13,70)
PCOS- metformin group	3,49 (0,91-11,20)
p	0,017

PCOS:Polycystic ovary disease AMH:Antimüllerian hormone

Results

A total of 84 consequtive patients diagnosed with PCOS according to 2003 Rotterdam criteria were included to this study. Results were compared with 28 healthy volunteers. Subjects were arranged in three groups: metformin-PCOS group (n=42), PCOS group without any treatment (n=42), healthy control group (n=28). Age distribution was similar between all groups. Demographic features of subjects are given at table 1.Mean body weight and BMI values were significantly higher in PCOS-untreated($p<0.001$) and PCOS-metformin groups compared to control group ($p=0.012$, $p<0.001$). Ferriman Gallwey scores and acne frequency were also much more in PCOS groups ($p<0.001$)

Serum insulin but not FSH, LH, E2, progesterone and thyroid hormone levels were significantly different in PCOS groups when compared to control subjects. HOMA indexes of patients with PCOS were similar at the begining of the period (p> 0. 5) but this value was lower at control group and PCOS+metformin groups after 6- month therapy ($p<0.01$). No statistically significant

change was observed between ACTH, cortisole and 17 OH PRG levels(p>0.05).

Total testosterone and DHEAS levels of PCOS group without treatment were significantly higher when compared to healthy subjects (p=0.02, p=0.047).

Baseline AMH levels was 3.36(range 0.22- 5.96) at control group and 5.33(1.38- 13.70) at PCOS-untreated ones, respectively (p<0.001). There was not statistically significant difference between AMH levels of control and PCOS-metformin groups(p>0. 5).

No correlation was detected between AMH and any hormone levels, including FSH, LH,E2 and testosterone. But, ovarian volume was positively correlated with serum AMH level respected to Spearman's correlation test.

Discussion

PCOS is a heterogeneous syndrome characterized with laboratory and clinical features of hyperandrogenism, oligo\anovulation and increased follicle numbers and ovarian volume.

Obesity, a common component of PCOS, is a reason of impaired folliculogenesis. Obesity causes insulin resistance and hyperinsulinemia; leads to impairment in steroidogenesis, decreased sex hormone binding globuline(SHBG) production from liver and increased androgen synthesis from teca cells of ovary (9). Also insulin inhibits the function of aromatase enzyme; by this way contribute to hyperandrogenism (10). In our findings, insulin was decreased in patients with PCOS after therapy.

Antimullerian hormone (AMH) is a hormone synthesized only from granulosa cells of preantral and antral follicles. Serum AMH levels are elevated in patients with PCOS when compared to healthy women (11- 13). Because AMH is only secreted in small maturing follicles, it may be an indicator of primordial folllicle volume (14). Similarly, we have observed positive correlation between ovarian volume and AMH level (p<0.05). AMH level may be compatible with number of antral follicles and confirmed as an important marker to detect early ovarian follicles (15) . In a study, serum AMH levels were measured two times in 1. 1- 7. 3 years and compared with other ovarian reserve markers. Reduction in AMH levels was observed correlative with serum follicle stimulating hormone level and number of antral follicles at ultrasonographic image (16).

In our report, serum AMH levels significantly higher in patients with PCOS than normo-ovulatory group(p=0.017). Also AMH level significantly higher at untreated-PCOS group when compared to controls(p<0.001) Determination of serum AMH level in oligoanovulatory women will support the clinician understanding ovulatory function in patients with PCOS. Elevated AMH levels prevent follicles to response stimulation of FSH and causes atresia of follicles. Granulosa cells are activated irregularly at PCOS with unknown reasons and this event corrupt folliculogenesis and results with ovulatory dysfunction. So, AMH levels can be used to assess ovulatory functions in this patient group (17).

Also, ovulatory function can differ between PCOS patients according to ovulation ability. A report has declared that AMH levels were increased at two or three times in ovulatory PCOS patients and seventy-five times increased in anovulatory patients when compared with controls. Production of AMH is not influenced from FSH and LH level at normal ovary but FSH can decrease AMH discharge at granulosa cells of polycystic ovary; on the contrary, LH increases its production. Decrease in AMH level is important at the determination of dominant follicle and follicular recruitment. When collated with normal ovarian pattern, AMH deteriorates maturation of follicles and causes ovulation disability (18).

Oral contraceptives are widely used at PCOS to control seborrhea, acne, hirsutism and to preserve endometrium from excess estrogen exposure. But there is limited data about changes in AMH level with different treatment modalities. A study about this subject have demonstrated oral contraceptives provided

decrease in AMH level but this change was statistically insignificant (19). Metformin is an agent increase the sensitivity to endogenous insulin. It is also reported that metformin is more effective at insulin resistance but oral contraceptives were more beneficial at amelioration of menstrual irregularity and hyperandrogenemia (20). Also, its effect is proven on serum AMH levels in patients with PCOS (21,22). In our study, Serum AMH levels were significantly higher in patients with PCOS when compared to healthy women. Age distribution was similar between groups. Patients observed with metformin treatment showed lower AMH levels when compared to untreated patient group. AMH was similar between control and metformin groups.

Not a few study implicating that IGF 1 levels are important at follicle maturation and folliculogenesis. Hyperinsulinemia cause increased bioactivity of IGF1 by inhibiting the synthesis of IGFBP. IGF1 stimulate production of ovarian androgen by autocrine and paracrine effects. Metformin diclines ovarian androgen production by the way of increasing IGFBP levels in serum (23). Also it is reported that metformin causes spontaneous ovulation without effecting BMI, also leads to decrease in total testosterone but not effecting SHBG, IGF-1 (24) . Our findings also in accord with these studies that testosterone levels were significantly lower in controls and PCOS metformin group. Even though all of these studies, Cochrane Data Base couldn't explain effect mechanism of metformin on PCOS (17).

No correlation was observed with other hormones such as PRL, FSH, LH, E2, PRG, insulin, TSH, fT3, fT4, total testosterone, DHEAS, ACTH, cortisole and 17OH progesterone levels and AMH ($p>0.05$). In a controlled study; there was inverse correlation between AMH and FSH levels at the early follicular phase of normoovulatory women. AMH level doesn't change througout the menstrual cycle. Also there are some reports confirming that FSH,E2 production doesn't influence AMH level immediately (25); correlated with our study.

Serum AMH levels significantly decrease after metformin treatment, probably due to decrease in hyperinsulinemia.

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