Anti-Mullerian hormone and insulin resistance in classic phenotype lean PCOS

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Abstract

Purpose This study is designed to explore the correlation between AMH levels and IR in normal weight PCOS women.

Materials and methods This prospective study was conducted on 55 patients, who were admitted to obstetrics and gynecology department of a university clinic. Study group was consisted of 34 patients diagnosed as polycystic ovary syndrome (PCOS) according to the Rotterdam Criteria, whereas control group was consisted of 21 healthy volunteers without any features of clinical or biochemical hyperandrogenism, who had regular menstrual cycles. BMI ≥ 25 kg/m^2 were considered overweight and obese and excluded. Blood samples were obtained during days 2–3 after spontaneous menses or progesterone-induced withdrawal bleeding after overnight fasting for at least 12 h. The weight, height, hip and waist circumferences of the patients were measured. Fasting insulin and glucose (FPG) levels were used for calculating different insulin resistance indexes (Homeostatic Model Assessment (HOMA-IR), Quantitative Insulin Sensitivity Check Index (QUICKI)).

Results No significant difference was found between PCOS and control groups regarding the mean age, BMI, waist to hip ratio (WHR), mean values of FPG, FPG/insulin ratio and HOMA B ($p > 0.05$). AMH values were significantly higher in PCOS cases when compared with controls (4.7 vs. 3.4 ng/mL) ($p < 0.05$). The mean values of HOMA-IR and QUICKI indexes were significantly higher among PCOS cases when compared with controls. E2 levels were significantly lower and Total-T were significantly higher in PCOS patients. When PCOS cases are categorized according to the existence of IR, no difference in Total-T and AMH levels between both groups. Although not statistically significant, a negative correlation of AMH with HOMA-IR and a positive correlation with QUICKI index were found. Among the hormone parameters, AMH was found to be positively correlated with Total-T ($r = 0.332, p = 0.013$).

Conclusion Although the relation between AMH and androgen production is supported by current evidence, the mechanism underlying the relation between AMH and insulin resistance is not clear yet.

Keywords Anti-Mullerian hormone · Polycystic ovary · Insulin resistance

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting 7–10 % of reproductive age women. Metabolic aspects play important roles in both the pathogenesis and the long-term sequelae of the condition. Insulin resistance (IR) and hyperinsulinemia is highly prevalent among PCOS women and affect 65–70 % of the cases [1]. Moreover, in the majority of women with PCOS (70–80 %), the presence of central obesity aggravates the metabolic comorbidities. The biologic actions of insulin on target tissues via binding to its own membrane associated heterotetromeric glycoprotein receptor, initiates the autophosphorylation of β-subunit on specific tyrosine residues and increases the tyrosine kinase activity. The activation of

insulin receptor initiates the phosphorylation of intracellular substrates known as insulin receptor substrates (IRS) [2–4]. These IRS act on regulation of mitogenic and metabolic actions of insulin. A postreceptor defect in tyrosine phosphorylation is the probable underlying cause for systemic resistance to insulin action in PCOS [2]. Although, the obesity is the major contributing factor for IR in PCOS, postreceptor mechanisms in insulin action might be the explanation for insulin-resistant normal weight PCOS.

Anti-Mullerian hormone (AMH) has gained attention during the recent years and is widely investigated in PCOS. AMH is a dimeric glycoprotein hormone, which is a member of transforming growth factor-β (TGF-β) superfamily, produced by granulosa cells of early developing preantral and antral follicles. AMH supresses follicle stimulating hormone (FSH) action on growing follicles by inhibiting aromatase and LH receptor expression until the time of follicle selection [5, 6]. AMH levels are positively correlated with antral follicle count [7]. In patients with PCOS, higher levels of circulating AMH has been documented but the underlying pathophysiology is still unresolved [5, 8, 9]. Besides, in recent years, the association of AMH and metabolic disturbances in PCOS has been a new area of research. In the literature, conflicting results exist about the relationship between AMH levels and IR [10–13]. Therefore, this study is designed to explore the correlation between AMH levels and IR in normal weight PCOS women.

Materials and methods

The participants of this study ($n = 55$) consisted of patients who admitted to Obstetrics and Gynecology Department of a university clinic between January and September 2011. The study was approved by Ethics Committee and written informed consent was obtained from all participants. The diagnosis of PCOS was made as proposed at the Rotterdam Consensus Meeting [14]. Oligomenorrhea was defined as cycle intervals more than 35 days and amenorrhea as absence of menstruation for three consecutive months. Among the cases with PCOS, 34 with hyperandrogenemia (serum total testosterone ≥2.7 mmol/L) or clinical hyperandrogenism (Ferriman and Gallwey Score ≥8 and/or persistent acne) and oligo/anovulation (classic phenotype PCOS) constituted the study group. The controls ($n = 21$) were healthy volunteers without any features of clinical or biochemical hyperandrogenism who had regular menstrual cycles. Exclusion criteria were hyperprolactinemia, thyroid dysfunction, adrenal dysfunction, diabetes mellitus, and pregnancy. None of the patients had received any drugs known to interfere with hormone levels for at least 3 months before the study. All of the subjects were nonsmokers. Waist

circumference was measured at the narrowest level between the costal margin and the iliac crest and hip circumference was measured at the widest level over buttocks. Body mass index (BMI) was calculated as weight (kg)/height (m)^2. Subjects with BMI ≥ 25 kg/m^2 were considered overweight and obese and excluded.

Blood samples were obtained during days 2–3 after spontaneous menses, after overnight fasting for at least 12 h. In cases with oligoanovulation (luteal phase progesterone measurements <4 ng/mL) blood samples were taken after progesterone withdrawal bleeding. Levels of glucose, insulin, hormone profile [follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), total and free testosterone (Total-T and Free-T, respectively), dehydroepiandrosterone sulfate (DHEAS), 17 OH-progesterone (17OH-P) and thyroid-stimulating hormone (TSH)] were determined. Plasma glucose was determined with the glucose hexokinase method (Cobas Integra 400 Plus, Roche Diagnostics, Mannheim Germany). Fasting insulin and glucose (FPG) levels were used for calculating homeostatic model assessment (HOMA-IR) [15] (insulin × glycemia in μmol/L/22.5) and quantitative insulin sensitivity check index (QUICKI) [16] (1/log insulin + log glycemia in mg/dL). IR was defined as HOMA-IR > 2.1 and QUICKI < 0.357 [17]. Homeostatic model assessment was used to determine β-cell function (HOMA-B) calculated by the formula: (fasting insulin in μU/mL) × 3.33/(fasting glucose in mg/dL − 3.5) [18]. Serum levels of FSH, LH, E2, PRL, DHEAS, Total-T, insulin and TSH were measured with electrochemiluminescence assays (ELECYS 2010 HITACHI, Roche Diagnostic, Germany). Serum levels of 17 OH-P and Free-T were measured by radioimmunoassay. The intra- and interassay coefficients of variation (CV) were <1.9 and <4 %, respectively, for all assays performed.

For the detection of serum AMH levels remaining blood samples were immediately centrifuged, and serum was separated and frozen at −80 °C until assay. AMH concentrations were analyzed using a commercially available ELISA kit (Diagnostic System Laboratories, Beckman Couter, Webster, USA). The intra- and interassay coefficients of variation for AMH were 4.6 and 8 %, respectively.

Statistical analysis

Collected data were analysed by means of the Statistical Program for Social Science (SPSS) version 11.5 for Windows SPSS Inc. (Chicago, IL). Whether the distributions of continuous variables were normal or not was determined by the Shapiro–Wilk test. Descriptive statistics for continuous variables were shown as mean ± standard deviation or median (minimum–maximum). The mean differences between groups were compared by Student's t test.

Otherwise, Mann–Whitney U test was applied for the comparisons of the median values. Nominal data were analyzed by Pearson's χ^2 where appropriate. The correlation analysis was done using Pearson's correlation test. A $p < 0.05$ was considered statistically significant.

Results

The mean age and BMI of the participants were 26 years and 22 kg/m^2, respectively. The demographic characteristics of the cases and controls are shown in Table 1 and Fig. 1. No significant difference was found between PCOS and control groups regarding the mean age, BMI, waist to hip ratio (WHR) ($p > 0.05$). On admission, 82.4 % of the PCOS cases had oligomenorrhea and/or anovulation. Hirsutismus and acne was found in 44 and 38.2 % of the PCOS cases, respectively.

Regarding the mean values of FPG, FPG/insulin ratio and HOMA B, no significant difference was found between

Table 1 Demographic characteristics and insulin resistance parameters of the participants

Parameter	Controls ($n = 21$)	PCOS group ($n = 34$)	p values
Age (years) Mean ± SD	26.0 ± 2.5	26.0 ± 2.8	0.981
BMI (kg/m^2) Mean ± SD	22.0 ± 1.1	22.1 ± 1.9	0.816
WHR Mean ± SD	0.72 ± 0.06	0.74 ± 0.07	0.145
FPG (mg/dL) Mean ± SD	85.8 ± 7.7	88.3 ± 6.2	0.188
Insulin (μm/mL) Median (min–max)	7.9 (2.5–11.5)	9.3 (3.9–57.4)	0.052
FPG/I Median (min–max)	11.4 (7.5–35.2)	9.1 (1.7–22.3)	0.058
HOMA B Median (min–max)	123.5 (37.5–408.7)	142.9 (60.7–574.0)	0.239
QUICKI Mean ± SD	0.36 ± 0.03	0.34 ± 0.03	0.024*
HOMA-IR Median (min–max)	1.8 (0.5–2.4)	2.0 (0.8–14.0)	0.025*

BMI body mass index, *WHR* waist hip ratio, *FPG* fasting insulin and glucose, *FPG/I* fasting plasma glucose/insulin, *HOMA-B* homeostatic model assessment of steady state beta cell function, *QUICKI* quantitative insulin sensitivity check index, *HOMA-IR* homeostatic model assessment of tissue insulin sensitivity
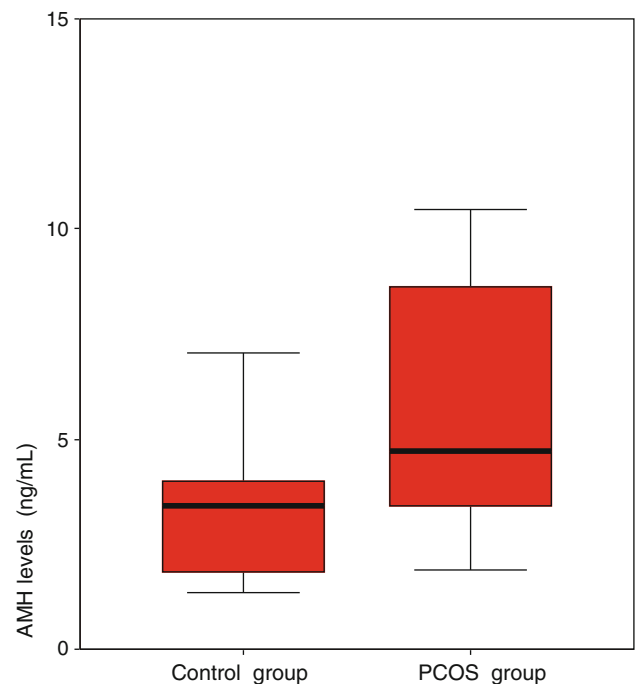
* Statistically significant

Fig. 1 AMH values of control and PCOS cases

PCOS and control groups ($p > 0.05$) (Table 1). The mean values of HOMA-IR and QUICKI indexes were significantly different among PCOS cases when compared with controls (Table 1). When PCOS patients were categorized according to the existence of IR, 15 out of 34 PCOS cases (44.1 %) were found to have IR (defined as HOMA-IR > 2.1). When IR is defined as QUICKI < 0.357, 70 % of PCOS women were found to have IR.

Among the hormone parameters (FSH, LH, E2, PRL, Total-T, Free-T, 17OH-P, DHEAS), E2 levels were significantly lower and Total-T were significantly higher in PCOS patients when compared with controls (Table 2). In addition, AMH values were significantly higher in PCOS cases when compared with controls (4.7 vs. 3.4 ng/mL) ($p < 0.05$). When PCOS cases were categorized according to the existence of IR, no difference in Total-T and AMH levels were found between HOMA-IR > 2.1 and HOMA < 2.1 groups (0.5 vs. 0.6 nmol/L for Total-T and 5.4 vs. 4.4 ng/mL for AMH in HOMA < 2.1 and HOMA > 2.1 groups, respectively). According to QUICKI index, PCOS cases with or without IR (QUICKI < 0.357 and QUICKI > 0.357 groups) also had no significant difference in total Total-T and AMH levels (0.5 vs. 0.6 nmol/L for Total-T and 4.9 vs. 4.4 ng/mL for AMH in QUICKI < 0.357 and QUICKI > 0.357 groups, respectively). According to Pearson's correlation analysis, the baseline characteristics (age, BMI, WHR) were not correlated with AMH levels ($p > 0.05$) (Table 3). Although

Table 2 Endocrine characteristics of the patients

Parameter	Control group (n = 21)	PCOS group (n = 34)	p values
FSH (mIU/mL) Mean ± SD	5.4 ± 1.4	5.6 ± 1.7	0.655
LH (mIU/mL) Median (min–max)	6.5 (1.1–11.5)	6.1 (1.7–23.8)	0.897
E2 (pmol/mL) Median (min–max)	59.7 (14–230)	33.5 (5–146)	**0.012***
PRL (ng/mL) Mean ± SD	14.1 ± 5.8	12.7 ± 5.7	0.390
Total-T (nmol/L) Median (min–max)	0.2 (0.1–0.6)	0.5 (0.03–1.3)	**<0.001***
Free-T (pg/mL) Median (min–max)	2.5 (1.0–4.2)	2.2 (0.8–4.6)	0.788
17 OH-P (ng/mL) Median (min–max)	1.6 (1.0–2.6)	1.6 (0.5–2.8)	0.728
DHEAS (μg/dL) Median (min–max)	268 (55–526)	229.5 (64–677)	0.494
TSH (μIu/mL) Median (min–max)	1.4 (0.4–10.4)	1.9 (0.5–5.3)	0.310
AMH (ng/mL) Median (min–max)	3.4 (1.4–7.0)	4.7 (1.9–26.1)	**0.003***

FSH follicle-stimulating hormone, *LH* luteinizing hormone, *E2* estradiol, *PRL* prolactin, *Total-T* total testosterone, *Free-T* free testosterone, *17OH-P* 17 OH-progesterone, *DHEAS* dehydroepiandrosterone sulfate, *TSH* thyroid-stimulating hormone, *AMH* anti-Mullerian hormone

* Statistically significant

Table 3 Correlation analysis of the variables with AMH values

Variables	Correlation coefficient	p values
Age (years)	0.021	0.878
BMI (kg/m^2)	−0.163	0.235
WHR	−0.107	0.435
Insulin (μm/mL)	−0.055	0.691
QUICKI	0.068	0.623
HOMA-IR	−0.010	0.943
Total T (nmol/L)	0.332	**0.013***
Free T (pg/mL)	−0.058	0.672
17 OH-P (ng/mL)	−0.103	0.455
DHEAS (μg/dL)	−0.124	0.366
E2 (pmol/ml)	−0.176	0.200

BMI body mass index, *WHR* waist hip ratio, *QUICKI* quantitative insulin sensitivity check index, *HOMA-IR* homeostatic model assessment of tissue insulin sensitivity, *Total-T* total testosterone, *Free-T* free testosterone, *17OH-P* 17 OH-progesterone, *DHEAS* dehydroepiandrosterone sulfate, *E2* estradiol

* Statistically significant

not statistically significant, a negative correlation of AMH with HOMA-IR and a positive correlation with QUICKI index were found (Table 3). Among the hormone parameters, AMH was positively correlated with Total-T ($r = 0.332$, $p = 0.013$) (Table 3).

Discussion

The results of this study demonstrated that AMH is positively correlated with Total-T levels in normal weight PCOS cases. In addition, a possible negative correlation of AMH with IR was found. In case of PCOS with hyperinsulinemia intact insulin-stimulated steroidogenesis and impaired glucose metabolism in granulosa luteal cells might be the possible explanation for the positive correlation between AMH and androgen levels but an inverse correlation with IR in our study. To document strong conclusions on this issue any existing correlation between AMH and BMI has to be clarified.

Our data support the previous reports documenting two-to threefold higher serum AMH levels in women with

PCOS as compared to controls [19–21]. As the highest expression of AMH was found in preantral and early antral follicles smaller than 4 mm in size [22], increased AMH levels in PCOS have been explained by the increased number of growing follicles in polycystic ovaries [21]. Further, in cultures from granulosa cells, AMH levels were 4- and 75-fold higher than normals in ovulatory and anovulatory PCOS cases, respectively [5]. The increased production of AMH per follicle due to intrinsic properties of granulosa cells might be the underlying cause for high AMH levels in PCOS [19]. AMH acts via suppression of FSH-induced aromatase activity which in turn results in decreased E2 levels. In our study, significantly higher levels of AMH and diminished E2 levels in PCOS cases is in agreement with the previous reports [23].

The correlation of AMH levels with IR is still controversial. Few authors researched the relationship between AMH levels and IR documenting different results with positive [12] and negative [10] associations. The study of La Marca et al. [12] reported a positive correlation of AMH with IR in 14 cases of PCOS with a mean BMI of 25.1 kg/m^2. This study [12] does not report the number of cases with IR or the mean values of HOMA-IR. Therefore, strong conclusions cannot be drawn with this limited number of cases and data. In contrast, Chen et al. [10] analysed the relationship between IR and AMH levels in 99 PCOS women with a mean BMI of 23 kg/m^2 (ranged 16–37 kg/m^2). In their study [10], the mean HOMA index and AMH values were 1.87 and 94.67 nmol/L (13.3 ng/mL). The authors reported a negative correlation of AMH with BMI and IR ($r = -0.213$, $p = 0.035$ and $r = -0.220$ and $p = 0.030$, respectively). Our results from normal

weight PCOS (BMI 22 kg/m^2) patients is in accordance with Chen et al. [10]. In addition, in our study, no difference was found between AMH levels of PCOS cases with or without IR. Previously, an inverse relation of AMH and IR was also detected in non-PCOS normal weight women (BMI 22 kg/m^2) [11]. The authors suggested that induced oxidative injury on granulosa cells and perhaps vascular injury causing suboptimal functioning of granulosa cells is the reason for decreased AMH levels in IR [11].

The existing negative correlation between AMH levels and IR seems to be related with BMI rather than PCOS per se. However, the data in the literature are insufficient to clarify any existing correlation between AMH and BMI. Conflicting results from small population studies [10, 13, 24] disable us from drawing strong conclusions. In a study by Wetzka and colleaques, much higher levels of AMH were found in normal weight PCOS women (BMI 21.8 kg/m^2) when compared with obese PCOS cases (BMI 30.7 kg/m^2) (15.6 vs. 11.6 ng/mL, respectively) [24]. In contrast others report no correlation between AMH levels and BMI [13, 21, 24]. In our study, due to lack of different BMI groups, further analysis could not be performed to evaluate the relationship between AMH and BMI. Unfortunately, in the literature no data exist about the correlation of IR with AMH levels in different BMI PCOS groups.

Regarding the hypotheses that AMH levels might be correlated with IR, few authors researched the change in AMH values after treating PCOS for IR [26–29]. In a prospective pilot study, short course of low-dose metformin treatment did not alter the AMH levels despite a decrease in antral follicle count and improvement in IR after 1 week of metformin therapy (850 mg once daily) [26]. Others [27] documented a decrease in AMH values after at least 4 months use of metformin (500–850 mg three times a day). The authors [27] suggested that metformin therapy can cause more normal follicles to enter the following cohort by improving androgen level, IR and ovulation. However, the significant weight loss during the study period is not discussed by the authors as a possible confounding factor. We suggest that the reported decrease in AMH values in their study might be associated with this decline in BMI.

Unlike the above-discussed correlation of AMH with IR, the data in the literature about the correlation of AMH with androgen levels is more clear. Many authors documented a positive correlation between AMH and androgen levels [13, 24, 29]. Androgen excess is one of the typical features of PCOS. In PCOS, gonadotropin-independent follicular growth in preantral and antral follicles with hyperandrogenic intraovarian environment stimulates granulosa cell proliferation and inhibits apoptosis. Accumulation of small follicles and granulosa cell proliferation due to androgen excess is the probable explanation for the association between AMH (product of granulosa cells) and androgen levels [30].

Hyperinsulinemia is also another determining factor for hyperandrogenism. Insulin and LH act synergistically on theca cells to enhance androgen production [31]. In the presence of hyperinsulinemia in anovulatory PCOS, the current evidence supports that insulin stimulated steroidogenesis remains intact while glucose uptake and metabolism is impaired in granulosa luteal cells [32]. This might the possible explanation for the positive correlation between AMH and androgen levels, but an inverse correlation with IR in our study. Effects of insulin on granulosa and theca cells are mediated through phosphorylation of IRS proteins. Both cells have IRS isoforms, but concentrations and types of them are different. Insulin may have a positive effect on androgen production via this augmented IRS-1&2 isoforms in theca cells whereas the mechanism is not so clear for granulosa cells [2]. Impaired phosphorylation of IRS proteins may result in activation of different metabolic and steroidogenic signalling pathways in granulosa and theca cells. Further studies are needed to explore the role of insulin on granulosa cell activity.

In conclusion, serum levels of AMH is higher in women with PCOS as compared to controls. Although the current evidence support the association between AMH and androgen production, the relation between AMH and IR is not so clear yet. The association of AMH and BMI needs to be concluded in large population studies with or without PCOS. Thereafter, the correlation of AMH with IR can be resolved in PCOS.

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