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Association of anti-Müllerian hormone and insulin resistance in adolescent girls with polycystic ovary syndrome

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Abstract

Introduction: Insulin resistance (IR) is confirmed as an important feature among polycystic ovary syndrome (PCOS) patients. Anti-Müllerian hormone (AMH), a vital marker of ovarian dysfunction, is proposed for inclusion in the diagnosis of PCOS in adolescents. We sought to investigate the relationship between the AMH level and IR in Chinese girls with PCOS.

Material and methods: 92 girls with PCOS aged 14–18 years were enrolled and divided into 2 subgroups: PCOS with IR group (n = 25) and PCOS without IR group (n = 67). A homeostasis model assessment-insulin resistance (HOMA-IR) value ≥ 2.5 was defined as IR. Clinical data and biochemical indexes were compared between the 2 groups. Multivariate logistic regression analysis and multivariate linear regression analysis were performed to determine which clinical variables were independently associated with IR and AMH level, respectively.

Results: PCOS girls with IR had higher levels of AMH than those of PCOS girls without IR ($p < 0.01$). Moreover, body mass index, triglyceride, and AMH values were shown to be independent risk factors for HOMA-IR after multivariate analysis. Meanwhile, age, insulin, and follicle-stimulating hormone levels were significantly related to AMH levels in those girls.

Conclusions: Our findings show that AMH is an independent determinant of IR in PCOS adolescents, and the fasting insulin level is closely associated with the AMH level, which indicates that the AMH pathway might play a role in the development of IR in PCOS adolescents. The interaction between AMH and IR in PCOS girls warrants further large-scale evaluation. **(Endokrynol Pol 2024; 75 (1): 83–88)**

Key words: *polycystic ovary syndrome; anti-Müllerian hormone; adolescents; insulin resistance*

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder characterized by hyperandrogenism and chronic anovulation, affecting 3–11% of adolescent girls worldwide, depending on the diagnostic criteria used and the population studied. A meta-analysis published in 2019 showed that the prevalence of PCOS in adolescents was 11.04% based on the Rotterdam criteria, 3.39% based on the National Institute of Health criteria, and 8.03% based on Androgen Excess and Polycystic Ovary Syndrome Society criteria [1]. Adolescence, as defined by the World Health Organization (WHO), is the period between 10 and 19 years of age that includes significant and critical changes in growth, development, and puberty [2].

A diagnosis of PCOS has life-long implications and is associated with increased risk of infertility, metabolic disorder, and cardiovascular diseases [3]. Although the exact aetiology of PCOS has not been elucidated yet, insulin resistance (IR) is confirmed as an important characteristic that aggravates the features of PCOS and serves as a key contributor to cardiometabolic outcomes by accumulating clinical data [4]. The gold standard of IR measurement is the hyperinsulinaemic-euglycemic clamp technique. This is a research technique with limited clinical applicability, while homeostasis model assessment (HOMA-IR), a non-invasive assessment tool of IR, is widely used in populations with endocrine disorder, including PCOS [5]. The WHO define IR as the highest quartile of HOMA-IR index in non-diabetic subjects, and the cut-off values reported for IR in the literature vary from 1.7 to 3.87 [5]. Most previous PCOS investigations adopted a cut-off value of more than 2.5 for IR [6, 7]. IR plays a vital role in the pathogenesis of PCOS, thus it is critical to study the factors closely associated with IR. Several studies have reported that adipocytokines [8], oxidative stress biomarkers [9], and hormones [10] are closely related to IR in women or girls with PCOS.

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Anti-Müllerian hormone (AMH) is produced predominantly by the ovarian granulose cells of preantral and antral follicles. Substantial epidemiological evidence has confirmed its role as an important marker of ovarian dysfunction. AMH concentration was shown to be 2- or 3-fold higher in women with PCOS than that of women with normal ovaries [11]. Clinical researchers from different countries investigated the correlation between AMH levels and IR in PCOS women, but they obtained inconsistent results. Wiweko et al. [12] revealed a positive correlation between them in PCOS women aged 18–35 years. In contrast, a significant negative correlation was observed in another study enrolling PCOS women aged 18–40 years [13]. Meanwhile, several other investigations found no correlation [6, 7, 14]. As for the value of AMH in adolescents with PCOS, many research have proposed that it could be a valuable marker for diagnosis as well as for the assessment of treatment efficacy [15]. However, data regarding the association between AMH and IR analysis in girls with PCOS is limited, especially in China. In this respect, we designed this study to determine whether the AMH level was independently correlated to IR in Chinese girls with PCOS, which would be helpful to explore the underlying mechanisms of IR via the hormone pathway. Moreover, the clinical treatment of female PCOS requires long-term follow-up, which necessitates serial stratified management. Comprehensive evaluation of AMH, IR, and their interaction starting from adolescence might be a feasible and individualized strategy for PCOS patients.

Material and methods

Study participants

This study was approved by the Worldpath Clinic International research Ethics Committee. Written consent was obtained from each participant before any information was collected. During the period from January 2019 to December 2022, 92 girls aged 14–18 years, who were diagnosed as PCOS according to the international evidence-based guidelines [16], were enrolled. The criteria included the following: (1) irregular menstrual cycles defined according to years post-menarche; > 90 days for any one cycle (> 1 year post-menarche), cycles < 21 or > 45 days (> 1 to < 3 years post-menarche); cycles < 21 or > 35 days (> 3 years post-menarche) and primary amenorrhea by age 15 or > 3 years post-thelarche. Irregular menstrual cycles (< 1 year post-menarche) represent normal pubertal transition; (2) Hyperandrogenism defined as hirsutism, severe acne, and/or biochemical hyperandrogenaemia confirmed using validated high-quality assays [16]. Participants were excluded if they had any of the following conditions: acute or chronic infectious diseases, pregnancy, malignancy, liver or renal failure, and taking any drug that could influence the hormonal parameters or IR (i.e. metformin [17], insulin sensitizer) within the past 12 weeks.

Sample size

The sample size was determined based on preliminary published clinical studies evaluating the relation of AMH and HOMA-IR level in PCOS patients. The sample size was calculated with the formula: $N = [(Z_{\alpha} + Z_{\beta})/C]^2 + 3$, described by Hulley et al. [18]. Assuming 2-tailed α value = 0.05 and $\beta = 0.2$, the minimum total sample size suggested was 79 for patients with PCOS.

Clinical data collection

Clinical data, including medical history, oral supplement habit, and exercise habit (more than 3 times/week and more than 45 minutes/time was defined as regular exercise) were collected from each subject. Body mass index (BMI) was calculated as the ratio of weight divided by the height squared (kg/m^2).

Biochemical measurements

Blood samples were collected in similar conditions from each participant on the third day of their menstrual cycle and after 8 hours of fasting. Fasting blood glucose (FBG), insulin (IN), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), creatinine (Cr), follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (TT), dehydroepiandrosterone sulphate (DHEAS), prolactin (PRL), and AMH levels were measured using standard laboratory methods. The HOMA-IR index was determined using the following formula:

$$HOMA\text{-}IR = [FBG(mmoL/L) \times IN(mU/L)]/22.5.$$

In our study, HOMA-IR values greater than 2.5 were defined as IR, which was the same as in previous studies [6–8]. Ninety-two girls with PCOS were divided into 2 subgroups: a PCOS with IR group (n = 25) and a PCOS without IR group (n = 67).

Statistical analysis

Data were expressed as mean± SD for numeric variables and as a number (percentage) for categorical variables. Non-normally distributed data were normalized using the natural logarithm. Differences between 2 groups were assessed with 2-sided Fisher exact tests, chi-square tests for categorical variables, and Student's t-tests for continuous variables. Multivariate logistic regression analysis was performed to determine which clinical variables were independently associated with HOMA-IR, and the results were reported as odds ratios (ORs) and 95% confidence intervals (95% CIs). Meanwhile, multiple linear regression analysis was used to examine the effect of clinical variables on AMH levels, and the results were reported as β coefficients and 95% CIs. All statistical analyses were performed using the SPSS version 21.0 software package (SPSS Inc., Chicago, United States). p-values < 0.05 were considered significant statistically.

Results

Demographic, clinical, and biochemical characteristics of 92 PCOS girls are presented in Table 1. Twenty-five girls were placed into the IR group, and the prevalence of IR was 27.2% in our study subjects. BMI, IN, LDL-C, TG, and AMH were higher in the IR group than in the PCOS without IR group ($p < 0.05$), while FSH and LH levels were lower in the IR group than the other group ($p < 0.05$). The ratios of taking calcium supplements, probiotic supplements, and having an exercise habit were lower in the IR group ($p < 0.05$). As for age, FBG, TC, HDL-C, Cr, TT, DHEAS, and PRL, there were no significant differences between the 2 groups ($p > 0.05$).

Table 1. *Comparisons of clinical characteristics and biochemical parameters of polycystic ovary syndrome (PCOS) adolescents in 2 groups*

Variables	PCOS with IR (n = 25)	PCOS without IR (n = 67)	p-value
Age [years]	15.6 ± 1.2	16.0 ± 1.6	0.218
BMI [kg/m^2]	25.9 ± 1.7	23.2 ± 1.3	0.005
Vitamin D supplements habit (n, %)	3 (12%)	11 (16.4%)	0.017
Calcium supplements habit (n, %)	2 (8%)	5 (7.4%)	0.396
Probiotics supplements habit (n, %)	3 (12%)	9 (13.4%)	0.529
Regular exercise habit (n, %)	5 (20%)	21 (31.3%)	0.007
FBG [mmol/L]	5.0 ± 1.6	4.8 ± 1.4	0.083
IN [mU/L]	22.6 (17.1–26.9)	18.3 (14.1–21.6)	0.005
HOMA-IR	3.0 ± 0.3	2.2 ± 0.2	0.002
TC [mmol/L]	6.5 ± 1.3	6.3 ± 1.4	0.534
HDL-C [mmol/L]	0.9 ± 0.2	0.8 ± 0.3	0.243
LDL-C [mmol/L]	3.9 ± 0.9	3.2 ± 1.0	0.027
TG [mmol/L]	2.8 ± 0.3	2.3 ± 0.5	0.011
Cr [μmol/L]	70.5 ± 12.6	72.6 ± 13.1	0.626
FSH [mUI/mL]	5.0 ± 1.3	5.9 ± 1.5	0.008
LH [mUI/mL]	6.2(3.1-10.9)	7.8 (3.4–12.6)	0.013
TT [ng/dL]	32.1 (29.7-44.6)	31.8 (27.2–45.6)	0.301
DHEAS [μg/dL]	107.8 ± 31.2	119.6 ± 27.9	0.719
PRL [μg/L]	18.3 ± 4.1	17.9 ± 5.3	0.326
AMH [ng/mL]	11.6 ± 5.8	7.4 ± 4.1	0.003

BMI — body mass index; FBG — fasting blood glucose; LnIN — natural logarithm of insulin; HOMA-IR — homeostasis model assessment-insulin resistance; TC — total cholesterol; HDL-C — high-density lipoprotein cholesterol; LDL-C — low-density lipoprotein cholesterol; TG — triglyceride; Cr — creatine; FSH — follicle-stimulating hormone; LH — luteinizing hormone; TT — total testosterone; DHEAS — dehydroepiandrosterone sulphate; PRL — prolactin; AMH — anti-Müllerian hormone

Multivariate logistic regression analysis identified BMI (OR = 1.083, p < 0.01), TG (OR = 1.123, p < 0.05), and AMH (OR = 1.032, p < 0.05) as independent variables significantly associated with HOMA-IR (Tab. 2). The adjusted R^2 value was 0.28 for this model.

Furthermore, in multiple linear regression analysis, age (β = –0.26, p < 0.01), IN (β = 0.21, p < 0.05), and FSH (β = –0.05, p < 0.05) were independently associated with the level of AMH (Tab. 3). The adjusted R^2 value was 0.19 for this model.

Table 2. *Logistic regression analysis for homeostasis model assessment-insulin resistance (HOMA-IR) in adolescent polycystic ovary syndrome (PCOS)*

Variables	Odds ratio	95% CI	p-value
BMI	1.083	1.022-1.215	0.006
TC	1.123	1.054-1.361	0.017
AMH	1.032	1.019-1.085	0.018

BMI — body mass index; TG — triglyceride; APN — adiponectin; AMH — anti-Müllerian hormone

Discussion

IR, a key underlying endocrine dysfunction in adolescents with PCOS, could further increase androgen production, anovulation, and the risk of metabolic abnormalities [2, 3]. In our study, we found that PCOS girls with IR had higher levels of AMH than girls without IR. Moreover, BMI, TG, and AMH values were shown to be independent risk factors of HOMA-IR after multivariate analysis. On the other hand, age, IN, and FSH level were significantly related to the AMH

Table 3. *Multiple linear regression analysis of anti-Müllerian hormone (AMH) levels with clinical parameters as the dependent variable*

	β	95% CI	p-value
Age	–0.26	–0.45– –0.13	0.008
IN	0.21	0.16– 0.43	0.017
FSH	–0.05	–0.18– –0.26	0.019

AMH — anti-Müllerian hormone; IN — insulin; FSH — follicle-stimulating hormone

level in those girls. Our findings indicated that the AMH pathway might play a role in the development of IR in PCOS adolescents. The interaction between AMH and IR in PCOS adolescents deserves further large-scale evaluation.

It is known that IR is prevalent in PCOS females and critically involved in reproductive and metabolic complications of the syndrome [4]. PCOS girls with IR are at greater risk of developing cardiovascular diseases in later adulthood, making it important to explore its correlative factors. Meta-analysis has proven that HOMA-IR values were significantly higher in obese adolescents (12–18 years of age) than in non-obese ones [19]. In our study, we found that BMI was an independent risk factor of HOMA-IR after multivariate analysis. Moreover, several studies proposed that paediatricians should place a high premium on BMI as well as abdominal fat distribution [20]. This emphasis would be helpful for individuation management of IR-related disorders in PCOS.

Extensive research has revealed that lipid abnormalities, including elevated LDL-C and TG levels and reduced HDL-C levels, are often found in PCOS patients and bound up with hyperandrogenism, IR, oxidative stress, and infertility [21]. Park et al. observed 458 Korean PCOS women and concluded that TG was a useful surrogate marker for IR in those women [22]. They also found that the optimal cut-off point of TG ≥ 68.5 mg/dL was a marker for predicting IR in non-obese PCOS patients and TG ≥ 100.5 mg/dL in an obese group [22]. In addition, a retrospective study enrolling 507 PCOS women and 1246 age- and BMI-matched controls in our country showed that HOMA-IR correlated positively with TG, TC, and LDL-C but negatively with HDL-C. The TG level remained significantly correlated with HOMA-IR even after adjustment for BMI [23]. Nevertheless, fewer similar studies were carried out in PCOS adolescents. To our knowledge, the present study was the first to focus on lipid panels and IR in Chinese girls with PCOS, and TG was identified as a significant determinant of HOMA-IR, which was consistent with prior investigations in adult PCOS. This result indicated that decreasing hypertriglyceridaemia in girls with PCOS might improve their IR in clinical management to a certain extent.

A great deal of literature has documented elevated AMH concentrations in PCOS patients, which is probably related to the increased number of pre-antral and antral follicles or increased production of AMH by these follicles. Although the AMH level has not been included as diagnostic criteria in the guidelines for adolescents, a great deal of research has suggested that it could be a more specific marker for PCOS in adolescents, compared to antral follicle results by ultrasound, because small antral follicles are difficult to detect by ultrasound. AMH is undetectable in cord blood samples, but the level increases during infancy and then remains elevated until adolescence and early adult life. It starts to decline at the age of approximately 30 years and reaches undetectable levels approximately 5 years before the final menstrual period. A nationwide population-based study published in 2016 reported an age-specific reference range for serum AMH in healthy Chinese women throughout reproductive age to menopause [24]; however, homothetic research in Chinese girls with PCOS is sparse. Our sample size was small, but the regression analysis confirmed that age was a significant factor influencing the AMH level in those girls.

Another factor closely associated with AMH concentration is the FSH level, in accordance with previous clinical observations in PCOS women, which demonstrated that the AMH level correlated inversely with the FSH value [25]. AMH plays a critical role in the regulation of folliculogenesis, inhibiting the initial requirement of the primordial pool and the responsiveness of the follicle to FSH [25]. The negative correlation between AMH and FSH might be explained partly by this mechanism.

Besides those positive findings, the most important point in the current study was that AMH was an independent determinant of HOMA-IR in PCOS girls. Because IR and AMH are 2 important aspects in clinical assessment of PCOS patients, accumulating research has been designed to explore their relationship. Evidence regarding the association between them in PCOS women is conflicting, and we believe different sample size, age range of enrolled PCOS participants, measurement of IR (HOMA-IR or hyperinsulinaemic-euglycemic clamp technique), and ethnicity are probably responsible for the inconsistent results. Nevertheless, we believe it is very meaningful to collect and analyse this information from girls with PCOS, because integrated and comprehensive evaluation is strongly recommended for PCOS patients, beginning from adolescence. Tokmak et al. found that serum AMH levels were higher in non-obese PCOS adolescents with IR than those of PCOS patients without IR, as well as healthy controls. There was a significant positive correlation between AMH levels and IR in non-obese adolescent females with PCOS [26]. Similarly, a study based in Turkey showed that AMH was one of the contributors to IR (defined by oral glucose tolerance test) in PCOS girls, and the effect was BMI-independent [27]. Our investigation was also supportive of AMH being an independent risk factor of IR, and the fasting insulin level was shown to be related significantly to the AMH value. The association of hyperinsulinaemia and PCOS

was first reported in the 1980s [28], and researchers put forward IN as a candidate contributing to elevated AMH in PCOS women [18, 29]. An *in vitro* experiment showed that the expression and secretion of AMH was suppressed by IN, similarly to FSH, and co-incubation with FSH and IN decreased AMH secretion significantly more than FSH alone [30]. Recently, it was reported that *in vitro* treatment of isolated islet cells with high concentration of AMH plus leptin enhanced insulin secretion significantly [31]. Combined with the finding that AMH was strongly positively correlated with HOMA-IR and insulin concentrations for the 1st and 2nd hours of glucose treatment after fasting among obese PCOS patients, Li et al. concluded that AMH may involve in the pathological process of pancreatic β-cells in obese PCOS women [31].

Despite these interesting findings, several limitations should be considered. First, our study was an observational, single-centre study in which only a small number of girls with PCOS were available for analysis. Secondly, our study was cross-sectional, and it would have been ideal to have obtained repeated determinations in the same patients over time to prove their relationship. Thirdly, although we considered the potential influence of some oral supplements on girls with PCOS, including vitamin D [32], calcium [32], and probiotics [33], we neglected to check some personal conditions that also might affect the subjects' IR, such as non-alcoholic fatty liver disease [34]. Further long-term prospective studies enrolling more subjects are needed to elucidate the crosstalk between AMH and IR in adolescents with PCOS.

Conclusion

Several studies have investigated the association of AMH with IR in women with PCOS, and we found that AMH levels were higher in adolescents with PCOS with IR than in PCOS adolescents without IR. In addition, it was shown that BMI, TG, and AMH were independent determinant factors of IR in girls with PCOS, and the fasting IN level was closely associated with the AMH level, which indicated that the AMH pathway might play a role in the IR of adolescents with PCOS. Serial studies enrolling more girls with PCOS are needed to confirm our notion and explore further the underlying mechanisms.

Availability of data and materials

The data used to support the findings of this study are included within the article. The data and materials in the current study are available from the corresponding author upon reasonable request.

Conflict interest

The authors declare no conflicts of interest.

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Authors' contributions

G.G. and H.Z. were responsible for clinical data collecting; H.Z. was responsible for data analysing and manuscript drafting; X.W. was responsible for study design and manuscript editing and final approval.

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