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The Relevance Of Antimüllerian Hormone Level With Insulin Resistance In Polycystic Ovary Syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the most complex common and heterogeneous endocrine disorders of women in the reproductive age group, PCOS frequently is associated with insulin resistance (IR).Anti-Müllerian hormone (AMH) is a member of the transforming growth factor b family of growth and differentiation factors.

Keywords: Polycystic ovary syndrome (PCOS),Anti-Müllerian hormone (AMH) and insulin resistance (IR).

INTRODUCTION

PCOS has been controversial and still remains unclear due to the syndrome heterotrophic nature [1]. PCOS is associated with short-term reproductive and long –term metabolic dysfunction. It is characterize by hormonal abnormalities, ovulatory dysfunction and polycystic ovaries [2]. Hyperandrogenaemia is associated with clinical manifestations such as hirsutism, acne, male-pattern baldness, irregular menses and infertility. PCOS is characterized by an increased number of small antral follicles with arrested developing and a hypertrophied theca cell layer [1,2]. Patient with PCOS may suffer metabolic syndrome, therefore at risk of developing T2DM which in turn, put them at increased risk of developing cardiovascular disease (CVD) [2]. Insulin resistance (IR) is a common pathologic state in which target cells fail to respond to ordinary levels of circulating insulin. It results in inability of insulin to provide normal glucose and lipid homeostasis. Hence, higher than normal concentration of are needed in order to maintain normoglycaemia [3]. The subnormal biological response could be due to the inability of plasma insulin to bind to its receptor binding defect [4].IR is associated with a number of diseases including obesity and metabolic chronic infection. The overall prevalence of IR is reported to be 10-25% [5]. PCOS can be considered a human model of insulin resistance, given that both lean and obese women presenting with PCOS are insulin resistant when compared with their non-hyper androgenic counterparts, although insulin resistance is not a universal finding in PCOS patients [6]. Furthermore, PCOS is frequently associated with obesity, the metabolic syndrome and disorders of glucose tolerance [6]. Considering the frequent clustering of obesity and insulin resistance-associated disorders in PCOS patients, the adipokines adiponectin and resistin, among other molecules and hormones secreted by adipose tissue, have been proposed to play a role in the pathogenesis of PCOS [7].

Serum adiponectin levels are decreased in PCOS patients [7-8], yet this result may be explained by the concurrence of obesity [11], insulin resistance [9,10] and/or impaired glucose tolerance [12] in these women. Similar considerations apply to the increase in serum resistin levels described in PCOS [12,8] and to the discrepant results found regarding the possible influence of common polymorphisms inthegenes encoding adiponectin and resistin in the pathogenesis of PCOS [13-14]. Metformin improves reproductive features and IR in PCOS but there are scant data on its effects on endothelial function and available data is contradictory. Although OCP also improves reproductive features in PCOS, it has adverse effects on IR as well as on other cardiovascular risk factors and appears to improve low-density lipoprotein cholesterol (LDL-C) and ADMA despite increased IR [15]. Antimüllerian hormone (AMH) is formed in blood of both sexes. Among physicians it is mostly familiar as a predictor for successful *in vitro* fertilization, but its examination has a wider utilization [16, 17].

AMH was initially thought to be produced solely by the fetal male during sexual differentiation to promote regression of the Mullerian ducts. Over the last decade, however, a new and interesting role has emerged for AMH in the ovary [17]. In hum an ovaries, AMH is produced by granulosa cells from 36 weeks of gestation until menopause, with the highest expression being in small antral follicles. AMH production gradually declines as follicles grow; once follicles reach a size at which they are dominant, it has largely disappeared. Its removal from these larger follicles appears to be an important requirement for dominant follicle selection and progression to ovulation as AMH has an inhibitory role in the ovary, reducing both primordial follicle initiation and follicle sensitivity to FSH by inhibition of aromatase. It is for this reason that AMH is a focus of interest in polycystic ovary syndrome (PCOS) [18]. Understanding the reason for the raised AMH in PCOS may give clues as to the mechanism of an ovulation. AMH appears to have a major inhibitory role during folliculogenesis, which may contribute to an ovulation in PCOS [19].

MATERIALS AND METHODS

Patients: Sixty female patients with PCOS attending Fertility Center in Al -Sader Medical City in Najaf province from January 2013 to April 2013. The patients ages ranged between (18-45) years. Any acute disease and chronic diseases other than PCOS such were excluded from the study.

Controls: A healthy subject group of 30 female was included in the study as a control group. None of these subjects had obvious systemic diseases.

Blood samples: Venous fasting blood samples (5ml) were collected from both patients b and healthy control group in plain tubes containing no anticoagulant. Disposable syringes and needles were used for blood collection. After allowing the blood to clot at room temperature for about 15 min, blood samples were centrifuged at 3000 xg for 15 min. Resulted serum was separated in two aliquots into plain tubes, where stored in -20^{o} C until to be assayed .

Determination of body mass index (BMI): The body mass index was calculated using the formula [20]
BMI = weight (Kg)/ height (m^2)

Determination of fasting glucose and triglyceride: Serum glucose and triglyceride were measured spectrophotometrically by enzymatic reactions using ready for use kits, supplied by Biolabo, France [21].

Determination of serum insulin levels: Serum insulin was determined by enzyme linked immune sorbent assay (ELISA) based on the sandwich principle (DRG kit, Germany) [22].

Determination of insulin resistance: Insulin resistance was measured by Homeostasis model assessment
HOMA-IR = [Glucose (mmol/l) × Insulin (µIU/ml)] / 22.5 [23].

Determination of serum AMH levels: AMH ELISA Kit enzyme linked immunosorbent assay (ELISA) based on the sandwich principle (Ansh lab, Germany).

RESULTS AND DISCUSSION

Host information data of the enrolled patients and control groups: The characteristics of the study groups are presented in table 1 which consists of the data of both patients with polycystic ovary syndrome and the control group. They include the number of women, age, weigh, height, BMI, the number of patients with hirsutism and those without hirsutism, those with primary infertility (PIF) and those with secondary infertility (SIF) and the menstruation pattern (regular and irregular). It is clear that the two groups are approximately well matched, thus results obtained could be considered creditable.

Table 1. Host information of the enrolled patients and the control group

Parameters	PCOS Patients (n= 60)	Control (n= 30)
	Mean ± SD	Mean ± SD
Age (y)	26.65 ± 5.19	26.35± 5.01
Weight (kg)	75.83±19.57	66.80±12.59
Height (m)	1.59± 0.07	1.59 ± 0.06
BMI (kg/m2)	30.04 ± 7.29	26.35 ± 5.01
With Hirsutism	45	-
Without Hirsutism	15	-
Primary infertility	37	-
Secondary infertility	23	-
Menstruation Pattern(regular)	23	-
Menstruation Pattern(irregular)	37	-

PCOS: Polycystic ovary syndrome

Biochemical characteristics of patients and the control group: Levels of biochemical parameters of the 90 recruited women are summarized in table 2. Significant elevations of the concentrations of fasting glucose ($p<0.001$), triglycerides ($p<0.001$), insulin ($p<0.001$), and antimullerian hormone ($p<0.001$) were obtained in the group of polycystic ovary syndrome patients group when compared with those of the control group.

Table 2. Biochemical characteristics of the enrolled patients and the control women

Parameters	PCOS Patients (n= 60)	Control Group (n= 30)	P- Value
	Mean ± SD	Mean ± SD	
FBS (mg/dl)	108.86 ± 19.94	78.42±9.60	0.001
TG (mg/dl)	125.35 ± 49.04	98.50±20.40	0.001
Insulin (µIU/ml)	18.23 ±7.81	6.80±2.42	0.001
AMH (ng/dl)	9.69 ± 8.41	4.18 ± 2.49	0.001

FBS: fasting blood sugar, **TG:** Triglyceride, **AMH:** Antimüllerian hormone.

Approximately hormonal profile were indicated to be altered in PCOS patients when they were compared with those of the control group. To understand the underlying causes involved in such alterations in patients with PCOS we have to speculate on the etiology of the disease. Several mechanisms are believed to direct the development of PCOS, i.e., genetic factors, insulin resistance, hyperinsulinaemia, and hyperandrogenaemia [24].Increased insulin resistance is a prominent feature of polycystic ovary syndrome (PCOS). Though insulin resistance is amplified by increasing obesity, women with PCOS are more insulin resistant than can be accounted for by their obesity alone. Ovarian sensitivity to insulin maybe enhanced in

PCOS and measures that reduce insulin metabolism ameliorate androgen secretion in women with the syndrome [25].

PCOS is considered to be a syndrome of preserved [26], if not increased, ovarian sensitivity to insulin with systemic resistance to insulin action. Moreover, ovarian antral follicle counts and ovarian volume correlate positively with endogenous and exogenous hyperinsulinemia, as reported in women with PCOS and with type 1 diabetes mellitus [27]. There are several factors that may be involved in the rise of AMH secretion in women with PCOS. AMH is produced exclusively in the gonads from the granulosa cells and is involved in the regulation of follicular growth and development [16]. AMH is undetectable in women who have undergone bilateral oophorectomy or in those with menopause [28].

AMH is expressed predominantly in the small antral follicles and is not expressed in atretic follicles or theca cells. Increased serum AMH concentrations in PCOS could be explained by the increased number of small ovarian follicles responsible for AMH secretion [29]. Therefore, AMH measurements may represent both a quantitative and qualitative marker of granulosa cell activity and the ovarian follicle pool. Thus, factors that may disturb granulosa cell function may affect the production of AMH [30]. Changes of AMH levels in the present investigation appears to be in consistence with those reported previously at which AMH derangement was related to the severity of the syndrome, since levels have been observed to be higher in insulin-resistant PCOS women than in patients with normal insulin sensitivity [31].

Evaluation of insulin resistance in polycystic ovary syndrome patients: Insulin resistance was evaluated by using four methods; Homeostasis Model Assessments (HOMA), Quantitative Insulin Sensitivity Check (QUICKI), McAuley's (McA) and Fasting Insulin level (FI). Results stated that out of the 60 investigated patients54 (90%), 54 (90%), 40 (66.67%) and 46 (76.67%) patients were found to be insulin resistant when they were evaluated by HOMA, QUIKI, MCA and FI methods respectively (Fig 1).

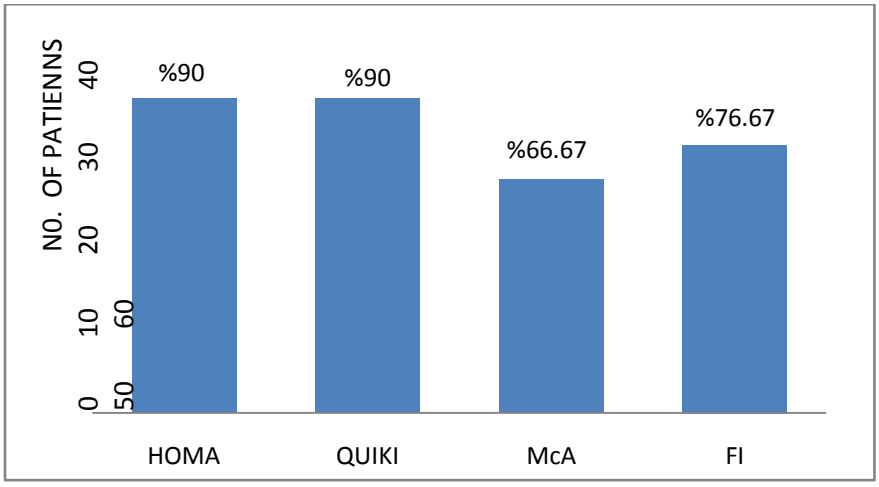

Fig 1. Results of estimation of insulin resistance (IR) in patients with PCOS by HOMA, QUIKI, McA , and FI methods.

The estimation of insulin resistance is a promising approach in the management of diseases related to the disorders of carbohydrate metabolism in particular T2DM. To a great extent the etiology of PCOS is complex and not clear [32]. However, it is generally recognized that insulin resistance is a key component in the pathogenesis of the disorder. Hence the measurement of insulin resistance in patients with PCOS may add benefits in the management of the disease [33].

The euglycemic insulin clamp and the intravenous glucose tolerance tests are gold standard methods for measurement of insulin resistance in research, but they are cumbersome in clinical practice and are

difficult to perform in population-based research studies. Therefore, indirect indices: McAuley's index McA, FI, HOMA and QUICKI were used for the assessment of IR in the study [34]. The homeostatic model assessment (HOMA) method of insulin resistance is a simple and less expensive method and therefore widely used in epidemiological studies [35]. Homeostasis model assessment, based on fasting glucose and insulin, is the oldest and most widely used and published test. For this reason alone, it offers some advantage over other methods in that it permits comparison among studies that use this metric. HOMA has the additional advantage of providing an assessment of β-cell function in addition to insulin sensitivity.

Estimation of levels of biochemical parameters in insulin resistant and insulin sensitive patients: Recruited patients with polycystic ovary syndrome patients (60) were classified into 2 groups, with respect to the response to insulin action as measured by the HOMA method. The insulin resistant patients (IRP) contained 54 patients, while the group of insulin sensitive patients (ISP) consisted of 6 patients. Results pointed out significant elevations of FIN ($p<0.001$), and AMH ($p<0.01$) concentrations in insulin resistant patients when compared with those of insulin sensitive patients (Table 3).The linear regression analysis exhibited significant ($r=0.86$, $p<0.00$) positive correlations of FIN levels with HOMA values (Table 4). However AMH levels did not show significant correlations with levels of investigated parameters.

Table 3. Levels of biochemical parameters in insulin resistant and insulin sensitive patients and

Parameters	Group	Mean ± SD	Min-Max	P-Value
FIN (μIU/ml)	IRP ISP	21.29 ±10.50 8.13 ± 2.40	12.15-58.36 3.17-11.97	0.001
AMH(ng/ml)	IRP ISP	10.24 ± 9.24 7.85 ± 4.47	0.62-36.54 2.04-18.44	0.05 0.01

Table 4 .Results of univariate analysis of HOMA values in insulin resistant patients with investigated parameters

Parameters	R	P
FIN (μIU/ml)	0.861	0.000
AMH(ng/ml)	-0.067	0.633

FIN: Fasting insulin, **AMH**: Antimullerian hormone.

In the current study several metabolic and hormonal changes are clear in insulin resistant patients with PCOS, as significant changes of the levels of the investigated parameters were obtained. Causes of these changes are strongly related to the impaired involvement of insulin in metabolic events in PCOS patients. Insulin resistance is frequently brought on by obesity or being overweight which results in reduction of insulin receptors and impaired post-insulin binding signaling transduction mechanisms [36]. In women with PCOS, it was suggested that IR and hyperinsulinaemia may represent two distinct features of the insulin disorders of the syndrome: the former appears to be more dependent on obesity, whereas the latter appears to be a primary feature of PCOS [37].Insulin resistance and the consequent development of hyperinsulinaemia appear to be central in the pathophysiologic mechanism that links PCOS to its concurrent metabolic derangements [38].

Hyperandrogenemia and ovulatory disturbances are commonly encountered in the syndromes of extreme insulin resistance when they occur in premenopausal women [39].Obesity and IR may enhance the follicular excess through the dysregulation of AMH or through the pathway of hyperandrogenaemia [30]. This hypothesis may be supported by the improvement of the ovulatory function of women with PCOS when adequate body weight and IR were improved.

CONCLUSIONS

1-AMH may be involved in the pathogenesis of PCOS.
2-Most of patients with PCOS are presented with insulin resistance.
3-Changes of AMH levels are independent directly on insulin resistance.
4-Anthropometric parameter direct the change of AMH levels in PCOS.

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