The Relation between Anti-Müllerian Hormone with Antral Follicle Count and Ovarian Volume in Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women during reproductive age. Women with PCOS show raised Anti-Müllerian hormone (AMH) levels, due to increased number of small antral follicles and granulosa cells, and these may lead to anovulation. Measuring antral follicle count (AFC) and ovarian volume (OV) by ultrasound scanning gives insight to the ovarian function. This study is conducted to investigate the relation between AMH with AFC, OV and different biochemical parameters which determine PCOS. The study was carried out on thirty patients with PCOS as (group 1) compared with fifteen healthy women as control group (group 2). AMH, FBS, Insulin, HOMA/IR, Testosterone, cholesterol, Triglycerides (TG), body mass index (BMI) and hirsutism were measured for all women in both groups. The results revealed that the serum level of AMH is significantly higher in PCOS (11.90±4.23ng/ml) than control group (4.36±0.56ng/ml). As for AFC, there is high significant increase (26.10± 5.49 follicles) in PCOS group versus (9.07 ±1.83 follicles) in control group. Furthermore, the results revealed a highly significant difference in OV (11.20±2.78ml) in PCOS compared to (6.23±0.73ml) in control. There is a significant correlation between AMH, AFC and OV in PCOS. There is a high significant correlation between AMH, AFC, OV, BMI testosterone, HOMA/IR, HDL, cholesterol, and TG in both groups. It could be concluded that there is a tight positive correlation between AMH and AFC and OV.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women affecting 6–21% of reproductive aged women, which represents the main cause of infertility [1, 2]. According to the Rotterdam consensus, two out of three criteria are required for the diagnosis of this syndrome: oligo- or anovulation, clinical or biochemical hyperandrogenism and/or polycystic ovaries on ultrasound. It can also be associated with insulin resistance, obesity and altered gonadotrophin release [3].

The clinical implications of ovulatory dysfunction in PCOS include infertility, obesity, and increased prevalence of endometrial hyperplasia and cancer [4]. Altered quality of life is another consequence of PCOS due to infertility, obesity and hirsutism. However, in particular patients, altered menstrual cycles cause psychological disturbances which should be considered when deciding the treatment of a PCOS patient [5]. The wide range and frequency of symptoms made it difficult to establish a consistent clinical picture, which may change at different times of life [6].

Traditionally the absolute level of LH and FSH, as well as the LH/FSH ratio, may offer significant insight into the PCOS patient, but is not accurate. It is clear that AMH is a diagnostic marker of ovarian function and increased AMH levels correlated with PCOS [7]. The Anti-Müllerian

hormone (AMH) is produced by the granulosa cells in the premature ovarian follicles until they become sensitive to FSH. Its level is constant during the menstrual cycle as the primordial follicles, the dominant follicle and corpus luteum don't secrete AMH [8, 9]. The intra-follicular concentration of AMH depends on the follicle size [10]. AMH can be used as an indication for the remnant follicle pool because it is mainly produced by the small antral follicles [11]. AMH peaks when women are in their early twenties [12]. Later in life, it decreases until menopause which is no longer detectable [13].

Ovarian morphology must be assessed by transvaginal ultrasound which is accurate, although abdominal ultrasounds may be used in the case of young girls or in virgins with less accuracy of ovarian morphology assessment and calculation of the number of small follicles. Hyperandrogenism and insulin resistance in PCOS stop the follicle development and the resulting follicle arrest contributes to PCO morphology where small antral follicles are lined in ovarian periphery [14].

The present study was designed to clarify the correlation of AMH with AFC and OV in women with PCOS and in controls.

Patients and Methods

This case-control study was carried out on 30 PCOS patients and 15 healthy women in the middle age group. The study was conducted from June to December 2016 in Ain Shams Maternity Hospital. All participants gave written informed consent, and the procedures followed were in accordance with the ethical standards of the hospital and approved from the ethical committee of National Center for Radiation Research and Technology. The studied subjects were classified into two groups. **PCOS group:** thirty women with PCOS were characterized by (OA + HA + PCOM). Oligo-anovulation (OA) was defined as self-reported menstrual cycle length of $\geq$ 35 days or < 10 menstrual periods per year. Clinical hyperandrogenism (HA) was defined by scoring terminal hair growth on nine body sites from 0-4 at each site according to Ferriman-Gallwey (FG score), Hirsutism has been usually graded as mild up to a score of 15, moderate from 16 to 25, and severe above 25. Biochemical HA was defined as serum testosterone > 2.5 nmol/l.

*Control group***:**
 fifteen women with regular menstrual cycles, no clinical or biochemical hyperandrogenism and normal ovaries on ultrasound. Subjects with history of ovarian surgery or treated with cytotoxic drugs or pelvic radiation, received hormonal therapy within three months before the beginning of the study, or had another endocrinological disorder were excluded.

Diagnosis of PCOS was based on Rotterdam criteria with two of 1-irregular menstrual cycles (oligoanovulation) OA ($\geq$35 days), 2-clinical (hirsutism, acne) or biochemical hyperandrogenism (HA) (increase in at least one circulating ovarian androgen) and 3-PCOM on ultrasound and with exclusion of related disorders [15]. The existing Rotterdam guidelines suggest that PCOM is indicated by the presence of at least 12 follicles measuring 2 to 9 mm in the whole ovary or by the finding of increased ovarian size (>10 mL) [16].

Patients and controls were subjected to: **1-** Complete history taking to clarify irregularity of menstrual cycles. **ll-** Clinical examination was conducted for all participants to detect the relevant data as the degree of hirsutism, weight and height. **lll-** Ultrasonography: AFC and OV were diagnosed by ultrasound which was performed with General Electric Logiq Book XP with vaginal probe 7.5 MHz (General Electric Medical Systems). Ovarian size was measured in three dimensions, and the volume was subsequently calculated by the formula: (height $\times$ width $\times$ depth $\times$ 0.5). All visible follicles of 2–9 mm in diameter in both ovaries were included in the AFC. All ultrasound examinations were performed by the same investigator. **IV**-Laboratory investigations: for all subjects, blood was withdrawn by syringe; serum was separated after clotting by centrifugation; placed in aliquots and stored at -20°C till the assay of the hormones. Hormones were measured during the follicular phase in controls and if possible in PCOS. In those with irregular cycles, this was not always possible; however, AMH does not vary considerably across the menstrual cycle [17]. AMH was analyzed using an ELISA kit (MBS 702605, My Biosource Company, USA) in a single batch from frozen serum.

Testosterone was measured on Beckman Coulter Unicel DXI 800 analyzer (Beckman Coulter Diagnostics Australia, Gladesville, Australia) using an automated competitive binding

immunoenzymatic assay. Fasting plasma glucose and fasting insulin, cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured using an enzymatic colorimetric method (BIOLABO REAGENTS SA 02160, Maizy, France).

Fasting glucose and insulin were measured to calculate homoeostatic model of insulin resistance (HOMA-IR) [18]. HOMA-IR was calculated as fasting serum insulin (mIU/l) × fasting blood glucose (FBG) (mmol/l)/22·5].

Statistical analysis

Results were presented as mean ± SD. Differences between the two groups (PCOS and control) were determined using an independent test. Relationships between variables were assessed using two-tailed Pearson's correlation coefficient (r). All statistical analysis were performed using SPSS for Windows 20.0 software (SPSS Inc, version 20.0 software, Chicago, IL, USA) with statistical significance accepted when $P < 0.05$.

Results

The results are demonstrated in Tables and Figures as follows: Table (1) shows the statistically significant differences between control and PCOS groups as regards different measured parameters. Table (2) shows a correlation between AMH and all parameters studied in PCOS group. There is a high significant direct correlation between AMH, AFC and OV, but non-significant correlation between AMH, age, BMI, hirsutism, testosterone, FBS, Insulin, HOMA/IR, cholesterol, HDL and TG. Table (3) shows that in both groups there is high significant direct correlation between AMH and Testosterone, HOMA/IR, BMI, cholesterol , HDL with p= .000 and significant correlation with TG. Table (4) shows high significant direct correlation of AMH with AFC and OV in both groups with p= 0.000 in both groups.

Table (1): Mean± Standard deviation of clinical, biochemical, hormonal and ultrasonographic parameters in both groups

Parameters	Control (n=15) Mean ± SD	PCOS (n=30) Mean ± SD	*P-values*
Age(years)	28.20±4.31	27.80±4.10	NS
BMI (kg/m²)	27.44±1.73	34.78±3.43	<0.0001
Hirsutism	7.73±1.66	26.49±3.6	<0.0001
FBG(mmol/l)	5.08±0.99	5.98±1.16	<0.05
Insulin(mIU/l)	13.02±2.44	23.21±5.91	<0.0001
HOMA /IR	2.74± 0.42	6.21 ± 2.49	<0.0001
Cholesterol(mg/dl)	167.66±17.19	217.74±20.96	<0.0001
TG (mg/dl)	84.00±8.83	98.76±13.97	<0.001
HDL(mg/dl)	55.80±4.53	39.45±7.25	<0.0001
Testosterone(ng/ml)	0.301± 0.13	0.856± 0.30	< 0.0001
AMH (ng/ml)	4.36±0.56	11.90±4.23	< 0.0001
AFC (follicle)	9.06±1.83	26.10±5.49	<0.0001
OV (ml)	6.23±0.73	11.30±2.78	<0.0001

Table (2): Correlation between AMH and all parameters studied in PCOS group shows a highly significant direct correlation between AMH, AFC and OV but non-significant correlation between AMH and BMI, Hirsutism, Testosterone, FBS, Insulin, HOMA/IR, Cholesterol, HDL, and TG

Pearson correlation	Age	BMI	Hirsutism
AMH *r* =	-0.017	-0.126	0.092
p =	0.972	0.507	0.629
Pearson correlation	AFC	OV	Testosterone
AMH *r* =	0.879	0.853	0.063
p=	0.000*	0.000*	0.740
Pearson correlation	FBS	Insulin	HOMA/IR
AMH *r* =	0.177	0.211	0.265
p=	0.350	0.262	0.157
Pearson correlation	Cholesterol	HDL	TG
AMH *r* =	0.101	-0.137	-0.112
p=	0.595	0.470	0.557

Table (3): Correlation between AMH and Testosterone, HOMA/IR, BMI, cholesterol , HDL and TG in both groups

Parameter	Pearson Correlation	AMH	HOMA/IR	BMI	Testosterone	Cholesterol	HDL	TG
AMH	r= P=	1	.581** .000	.494** .001	.552** .000	.594** .000	-.608-** .000	.286 .050
HOMA/IR	r= P=	.581** .000	1	.592** .000	.384** .009	.649** .000	-.442-** .002	.199 .189
BMI	r= P=	.494** .001	.592** .000	1	.578** .000	.631** .000	-.561-** .000	.448** .002
Testosterone	r= P=	.552** .000	.384** .009	.578** .000	1	.376* .011	-.584-** .000	.417** .004
Cholesterol	r= P=	.594** .000	.649** .000	.631** .000	.376* .011	1	-.494-** .001	.233 .124
HDL	r= P=	-.608-** .000	-.442-** .002	-.561-** .000	-.584-** .000	-.494-** .001	1	-.500-** .000
TG	r= P=	.286 .050	.199 .189	.448** .002	.417** .004	.233 .124	-.500-** .000	1

Table (4) Correlation of AMH with AFC and OV in both groups which show a highly significant direct correlation

Pearson Correlation		AMH	AFC	Ov. Vol
AMH	$r =$	1	.902**	.951**
	$P=$		.000	.000
AFC	$r =$	.902**	1	.903**
	$P=$	.000		.000
Ov. Vol	$r=$	.951**	.903**	1
	$P=$	.000	.000	

Figure (1) shows a highly significant direct correlation between AMH and AFC in PCOS group p<0.001. Figure (2) shows a highly significant direct correlation between AMH and Ovarian volume in PCOS group p<0.001. Figure

(3) shows ROC curve for AMH shows area under the curve=1, cut off value for AMH which diagnose PCOS is 5.80 with sensitivity=100% and specificity 100%. Figure (4) shows AFC and OV in Ultrasound images.
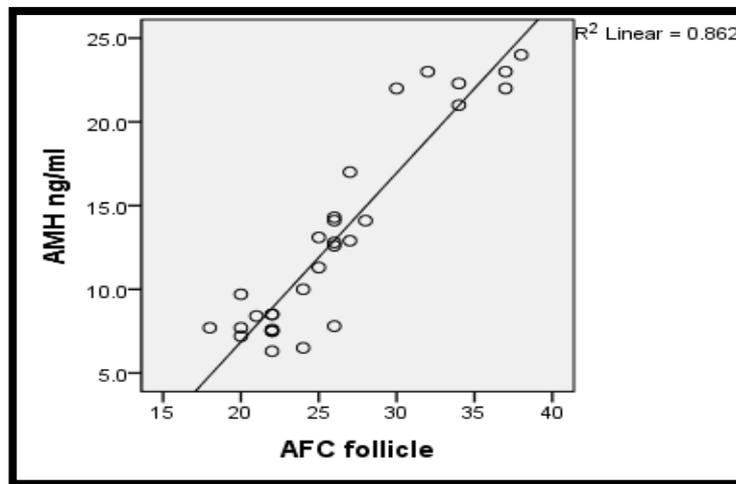

Figure (1): Correlation between AMH and AFC in PCOS group which show direct significant correlation with $r^2 = 0.862$ and p<0.001
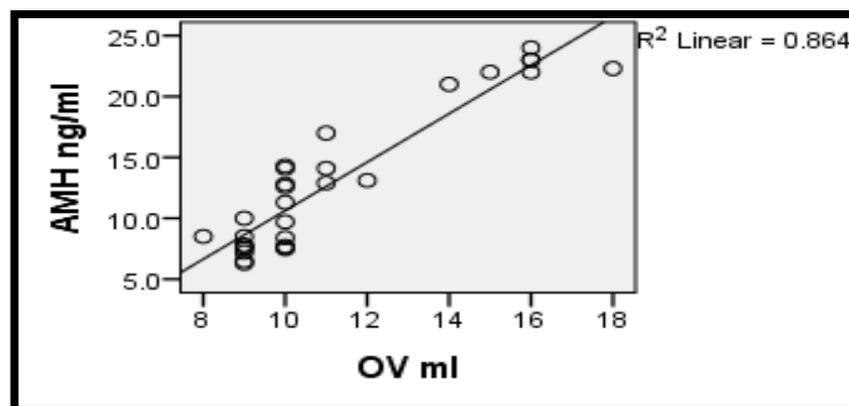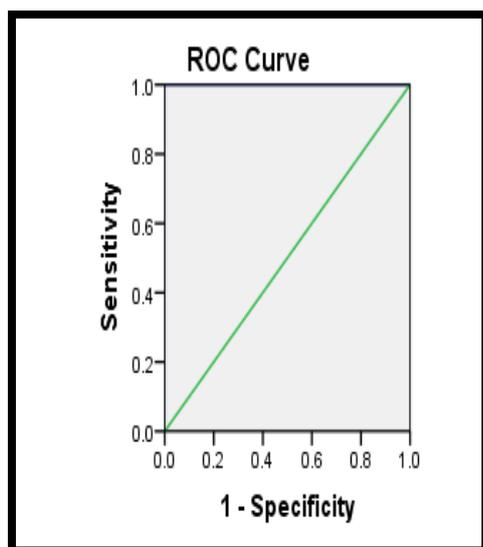

Figure (2): Correlation between AMH and Ovarian volume in PCOS group which show direct significant correlation with $r^2 =0.0864$ and p<0.001

positive if greater than or equal to	sensitivity	1 - specificity
4.5500	1.000	.400
4.6500	1.000	.267
5.0000	1.000	.067
5.8000	1.000	.000
6.4000	.967	.000
6.8500	.933	.000
7.3500	.900	.000
7.5500	.833	.000
7.6500	.800	.000
7.7500	.733	.000

Figure (3): ROC curve for AMH shows area under the curve=1, cut off value for AMH which diagnose PCOS is 5.80 with sensitivity=100% and specificity 100%

Figure (4): AFC and OV in PCOS
Antral follicle count and ovarian volume in PCOS patient

Discussion

Polycystic ovary syndrome is a collection of signs and symptoms varying from mild to severe and affecting the reproductive, endocrine and metabolic functions [19]. PCOS is one of the leading causes of female sub-fertility [20] and the most frequent endocrine problem in women of reproductive age [21]. Despite many decades of extensive research, the exact etiology and pathogenesis of this complex disorder remain still hidden. AMH is a promising marker as its level is constant throughout the menstrual cycle and not influenced by fluctuations in other reproductive hormones [22].

The present study was carried out to examine the relation between serum levels of AMH with Antral follicle count and ovarian volume in PCOS patients and comparing them with healthy women. The foregoing results of this study confirmed that fasting Blood glucose in PCOS group (5.98 ±1.16mmol/L) is significantly higher than in control group (5.09±0.99mmol/L). There is

significantly high insulin level (23.21 ± 5.91 mIu/L) in PCOS than in control group (13.02 ± 2.44 mIu/L).

This study confirmed that HOMA/IR is (6.21 ± 2.49) in PCOS group which is significantly higher compared to (2.74 ± 0.42) in control group. The results of the present study show highly significant direct correlation between HOMA/IR and cholesterol, which is consistent with the results of Lee et al. who showed a significant correlation between HOMA-IR and TG, LDL and HDL cholesterol [23]. In our study the cutoff value for HOMA/IR ≥ 3.22 with sensitivity 87.3% and specificity 87.3% which is consistent with a recent research by Alebić et al. who investigated whether insulin resistance in PCOS is exclusively associated with BMI and suggested specific HOMA-IR cutoff value ≥3.15 for PCOS [24].

Insulin resistance (IR) is the most common etiological factor in women with PCOS and that (IR) causes the generation of increased oxidative stress and diminished antioxidant status [25]. As for regards hirsutism, there is a significantly higher Ferriman–Gallwey (mFG) score in PCOS (26.49±3.6) than that in the control (7.73±1.66) with a cutoff value of ≥15.7 with a 100% sensitivity and 100% specificity. Some authors [26] have reviewed many published results and some of them reported a cutoff value of mFG score of ≥11 for white Middle Eastern women [27]. Androgen excess disorders may be present with normal body hair; hence the absence of hirsutism does not exclude the consideration of PCOS in which other features of androgen excess, such as acne, alopecia, infertility or menstrual dysfunction are present [28].

The mean cholesterol level is significantly higher in the PCOS group (217.74± 20.96 mg/dl) than that in the control group (167.66 ±17.19 mg/dl). The mean triglycerides level shows a significant increase in PCOS (98.76± 13.97 mg/dl) versus (84.00± 8.83 mg/dl) in control group. In this study the mean HDL level is significantly lower in the PCOS group (39.45± 7.25 mg/dl) which indicates high risk level in this group compared with control group (55.80± 4.53 m(g/dl) which indicates a low risk level of HDL. This is consistent with the results of Gambineri et al. who stated that low HDL-cholesterol is the criterion which best explains the high prevalence of the metabolic syndrome in PCOS subjects and is influenced by hyperinsulinemia, rather than by hyperandrogenemia [29]. In the current study the BMI show a significant increase in PCOS (34.78±3.43 Kg/m²) compared with control (27.44±1.73 Kg/m²) and is significantly directly correlated with AMH in all subjects.

Moran et al. found that exercise decreases AMH in overweight women with polycystic ovary syndrome [30].

In the present study, the serum level of AMH in PCOS (11.90±4.23 ng/ml) is significantly higher than in control group (4.36±0.56ng/ml) and these results agreed with that of **Dewailly et al.** who found that elevated AMH values (>4.5 ng/ml) may be useful as a substitute for ovarian morphology when no accurate ovarian ultrasound is available [31]. In this study, the AMH mean level is elevated 2.72 fold in the PCOS patients in comparison with normal subjects. This is in agreement with the findings of previous researchers [7, 32] who reported that AMH serum levels are elevated 2- to 3- fold in women with the PCOS in comparison with normo-ovulatory women, consistent with the increased number of small antral follicles in the PCOS. Additionally, Piltonen et al. found that serum levels of anti-Müllerian hormone were higher in patients with PCOS than in normal women [33]. The present study showed that serum AMH cutoff level of 5.8 ng/ml can diagnose PCOS patient with sensitivity 100% and specificity 100%. Lin et al. obtained the cutoff AMH level of 7.3 ng/mL, giving 76 % specificity and 70 % sensitivity to predict PCOS [34]. On the other hand, Wiweko et al. [35] obtained a cutoff value of 4.45 ng/mL which is lower than the findings of Lin et al. [34]. In addition, measurement of serum AMH levels may also be used as an indicator of PCOS patients' response to therapeutic approaches [30] and evaluation after treatment with insulin sensitizers [33], and monitoring after laparoscopic ovarian drilling [36].

The results of this study demonstrated that AFC mean counted, in both ovaries in PCOS group (26.10±5.49 follicles), is significantly higher than that in the control group (9.06±1.83 follicles). These data are consistent with those of Chen et al. [37] who confirmed ≥12 as threshold for follicle number per ovary (FNPO) in the Chinese women, whereas Kosus et al. [38] proposed an FNPO threshold of 8 follicles per ovary for Turkish women. The 2003 Rotterdam consensus, the most common ultrasound definition employed to date, was based on the Balen et al. study and on expert

agreement [16]; Distinguishing PCOS cases from controls as judged by transvaginal Ultrasonography, with an FNPO threshold of ≥12 follicles measuring 2–9 mm in diameter (mean of both ovaries) were established by many authors [31, 39]. Moreover, Dewailly et al. [31] applied cluster analysis in order to exclude clinically normal women with PCOM prior to determining the threshold for FNPO. Their analysis would have yielded a cut-off value of 25 follicles.

These results are similar to the results obtained by Lujan et al. [39]. Moreover, Dewailly et al. added that the value of AMH in the diagnosis of PCOS may replace AFC in the future [40]. This is consistent with our study in which there is high significant correlation between AMH and AFC with r = 0.879 and P <0.0001 in PCOS group, depending on this powerful correlation we can rely on clinical picture plus serum AMH without AFC to diagnose PCOS.

As for the ovarian volume, there is a higher significant increase in the PCOS group reaching (11.30 ± 2.78 cm³) versus (6.23± 0.73 cm³) in the control group, and there is high significant correlation between AMH and OV with r = 0.853 and P <0.0001 in PCOS. In agreement with the present findings, a relevant study by Koutlaki et al. demonstrated that AMH had a statistically significant positive correlation with the ovarian volume with (r =0,623, P<0.01) [41]. In a recent study, the ovarian volume showed the strongest positive correlation (r=0.62) with the serum AMH level among related factors [42]. In the present study there is also a highly significant correlation between AFC and OV with (r = 0.861, P< 0.0001). This study shows a ratio of AMH/AFC as 0.46 in PCOS and 0.48 in controls which gives an estimation of AMH amount per each follicle. Hence, the number of the follicles from the serum level of AMH can be estimated. In agreement with our results, Laven et al. found significant relationships between serum AMH levels and increasing testosterone, increase in number of follicles and the ovarian volume on ultrasound examination [32]. The highly significant correlation between AMH and AFC in PCOS, in the present study, shows an agreement with the findings of Lin et al., who reported that if at some time, ultrasound scanning cannot provide accurate data; the levels of AMH may be used to replace the number of follicles as a diagnostic criterion [34]. Zhang et al., suggested that AMH may be used as a

marker in the diagnosis in PCOS women with oligomenorrhea [43].

Conclusion

Our results confirmed that AMH is high significantly correlated with AFC and with OV. The accuracy of the AMH as an easy diagnostic tool plus the presentable clinical symptoms for diagnosis of PCOS could replace all other diagnostic biochemical tests and ultrasound diagnosis especially in virgins as AMH measurement could predict PCOS morphology as there is a tight positive correlation between AMH and AFC and OV.

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