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Potential Effects of Obesity on Anti-Müllerian Hormone in Polycystic Ovary Syndrome

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common endocrine abnormality with its known effects on reproduction. It is usually associated with infertility. Early detection is crucial the use of Antimullerian hormone (AMH) as indicator of ovarian function could be a reliable indicator. The association between PCOS and other metabolism-related disorders (e.g., obesity) is not sufficiently studied.

Aim of the work: we carried out this study aiming to investigate the possible association between AMH, ovarian reserve, AFC with BMI in women with PCOS. Generally, we seek the link between obesity and PCOS.

Methods: The study included 240 women with confirmed diagnosis of PCOS. They were categorized according to body mass index (BMI) into three groups the first for normal BMI (group A; n=100); the second for overweight women (Group B; n=65) and the third for obese women (group C; n=75). All were clinically (by history and examination) and radiologically (pelvic ultrasound) evaluated and then AMH, beside others were measured in plasma and data was compared between groups.

Results: Study groups were comparable regarding their age and incidence of hirsutism, ovarian volume. Hirsutism for example reported in 16%, 15.4% and 25.3% of normal weight, overweight and obese groups, respectively. Otherwise, waist circumference, hip circumference and waist/hip ratio were significantly increased progressively with increasing BMI. Acne also increased in C and B than in normal A group (41.3%, 21.5% vs 16.0%, respectively). By ultrasound, most follicles were peripheral (69.0%, 73.8% and 72.0% in groups A, B and C respectively). Furthermore, AMH and the antral follicular count (AFC) showed progressive increase in B and C groups than normal weight group (5.49±1.20, 18.61±6.40; 8.13±1.88, 23.73±5.94 versus 2.97±0.66 and 14.96±2.88, respectively). Finally, AMH was significantly and proportionately correlated with BMI, waist/hip ratio, ovarian volume and AFC. The correlation with BMI, W/H ratio and AFC was powerful ($r > 0.7$). In addition, there was moderate, significant and statistically significant correlation between AFC and each of BMI, W/H ratio and mean ovarian volume. However, the correlation between AFC and age was non-significant.

Conclusion: AMH showed significant increase with obesity, with positive correlation with BMI and ANF. Thus, BMI can play a crucial role in the development of PCOS.

Keywords: Polycystic Ovary; Body Mass Index; Hyperandrogenism; Infertility.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 6-10% of women in their fertile period. It is associated with infertility as the incidence of PCOS is reported in 30-40% of infertile women, which increased to 70-80.0% of anovulatory infertile women. In Egypt the prevalence is around 35.0% of overall infertile women [1-4].

PCOS is characterized by irregular menstruation, disturbances in glucose metabolism with development of insulin resistance (IR), oxidative stress, hormonal disturbances, androgen excess and polycystic ovaries [5,6].

Insulin resistances seem to be a major pathophysiological mechanism in development of polycystic ovaries and may be responsible for reproductive anomalies associated with PCOS [7,8].

In addition, abnormal lipids (dyslipidemia) are common findings in PCOS. This lipid profile changes in PCOS include decreased HDL-C (high density lipoprotein cholesterol), elevated triglycerides (TGs), and increased levels of LDL-C (low density lipoprotein cholesterol) [9,10].

Anti-Mullerian hormone (AMH) has been introduced as a normal indicator of ovarian function. It is a member of glycoprotein family known as transforming growth factor-β superfamily. The physiological function of anti-Mullerian hormones includes its important role in excessive early follicular growth and the maturation of the dominant follicle (i.e, promotion of follicular arrest). Increased AMH is associated with excessive increase in the antral follicular count (AFC). However, its association with discrimination of PCOS phenotypes and metabolic disturbances [11-13].

Obesity is a disease defined by excessive storage of triglycerides in fatty cells, which increased in number and size. Body mass index more than 30 kg/m^2 is the cutoff value for diagnosis of obesity. In addition, body mass index (BMI) was used to categorize obesity into different grades. Overweight and obesity have impacts on the general health as well as reproductive function [14,15].

In infertile women seeking pregnancy by natural methods, obesity has a significant adverse effect on conception and outcome pregnancy. Increased BMI was associated with reduction in fertility, increased miscarriage rate, when compared to normal-weight females [16,17]. Thus, reduction of weight before conception may be associated with improvement of fertility and pregnancy outcome. Poor outcome was also noticed in obese women submitted to assisted reproductive technologies (ARTs) [18,19]. However, the exact mechanism linking obesity to infertility and poor outcome of pregnancy or ARTs is not yet fully elucidated. Obesity contributes or oligo or anovulation, menstrual disturbances, reduced rate of conception, and poor response to different treatment modalities for infertility. Different indicators of ovarian reserve are used to investigate women regarding their reproductive outcome. These indicators include, but are not limited to, baseline follicle stimulating hormone (FSH, Estradiol2 (E2), Inhibin B, Antral follicle count (AFC), ovarian volume and antimullerian hormone (AMH) [20].

Previous studies reported possible association between AMH and obesity. But, with conflicting results [21, 22].

We hypothesize that, the AMH linked to body mass index in PCOS can be a good indicator of ovarian reserve and antral follicles count. Thus, we carried out this study aiming to investigate the possible association between AMH, ovarian reserve, AFC with BMI in women with PCOS. Generally, we seek the link between obesity and PCOS.

PATIENTS AND METHODES

This was a prospective comparative study. It was carried out in Damietta Specialized Hospital and Al-Azhar University hospital (New Damietta). It was completed during the duration between the first of March 2019 and the end of August 2020. At first 300 women with confirmed diagnosis of PCOS were screened for eligibility criteria and were asked for participation in the study. Sixty of them were excluded due to different causes (mainly refusal to participate or withdrawn after inclusion according their well). Thus, the final analysis was performed for 240 women.

The diagnosis of PCOS was based on the Rotterdam 2003 criteria. (Clinical hyperandrogenism (Ferriman-Gallwey Score ≥ 8) or biochemical hyperandrogenism (elevated total/free testosterone); oligomenorrhea (less than 6-9 menses per year) or oligo-ovulation; polycystic ovaries on ultrasound (≥ 12 antral follicles in one ovary or ovarian volume ≥ 10 cm^3). At least two of these criteria must be detected to diagnose PCOS. Then patients were categorized according to their BMI into three groups. The first was for women with normal weight (BMI < 25kg/m^2) (100 women). The second for overweight women (BMI between 25-3025kg/m^2) (65 women) and the third group for obese women (BMI >30 kg/m^2) (n=75).

The inclusion criteria were women during their reproductive age (18-35 years), with confirmed diagnosis of PCOS according to previous criteria and they provided consent to participate. On the other side, exclusion criteria were pregnant women and breast feeding.

Methods:

All eligible women were clinically evaluated by complete history taking, clinical and radiological examination. In clinical examination, the general condition was evaluated, and special characters (acne and hirsutism) were recognized and documented. Acne was defined by a history of persistent acne (presence of acne on the most days for 3 years or more), recent acne treatment and presence of more than 10 inflammatory acne lesions). In addition, hirsutism was evaluated by Ferriman-Gallwey (FG) scoring system (score < 8 is indicator of hirsutism). The FG score is used to quantify hirsutism in women. The method was initially published in 1961 by Ferriman and Gallwey. Then modified to reduce body areas for assessment of hair growth from 11 to 9 [23,24]. These areas include upper lip, chin, chest, upper and lower back (2 areas), upper and lower abdomen (two areas), upper arms and thighs. The forearm and legs were in the original form but removed in the modified score. In the modified method, hair growth is rated from no growth of terminal hair (stage 0) to extensive hair growth (grade 4) in each of the nine locations. A patient's score may therefore range from a minimum score of 0 to a maximum score of 36. A score of 8 or higher is set as indicator of androgen excess (hirsutism).

The body mass index was calculated by the equation BMI= weight in kg /height in m^2). Other measures include waist/hip ratio and presence of central adiposity. The pelvis was examined for the presence of adnexal masses. Then pelvic ultrasound was performed to investigate ovarian criteria of PCOS (12 or more follicles measuring 2-9 mm and/or an increased ovarian volume of >10cm^3). It was performed in the early follicular phase (day 1-3) in lithotomy position as described elsewhere. Number and site of antral follicles was counted in the longest section of the ovary, and for better results, it was counted in the whole ovary by taking a 2D sweep across whole ovary. This method is very feasible and reliable when number of follicles is much more than in PCOS.

Finally, laboratory estimation of serum levels of AMH and testosterone were performed.

Statistical analysis of data: The collected data were organized in an excel sheet, then tabulated and statistically analyzed using a software computer package (the statistical package of social science (SPSS), version 18 (IBM ®SPSS Inc, USA), running on IBM compatible personal computer (PC). Qualitative data were summarized by the relative frequency and percentage and compared by the appropriate test (Chi square (X^2) or Mann Whitney test). Quantitative data represented by the arithmetic mean and standard deviation (SD), minimum and maximum (difference is the range); and for comparison between groups, the one-way analysis of variance (ANOVA) test was used. P value < 0.05 was considered significant.

RESULTS

In the current work, study groups were comparable regarding patient age and incidence of hirsutism. However, waist circumference, hip circumference and waist/hip ratio were significantly increased progressively with increasing BMI. Acne also increased in groups C and B than in group-A (41.3%, 21.5% vs 16.0%, respectively) (Table 1).

The study groups showed non-significant differences between groups regarding ovarian volume (right, left and mean values). In addition, most follicles were peripheral (69.0%, 73.8% and 72.0% in groups A, B and C respectively). The difference between groups B and C were significantly indifferent. Furthermore, AMH and the antral follicular count (AFC) showed progressive increase in overweight and obese women than normal weight group (5.49±1.20, 18.61±6.40; 8.13±1.88, 23.73±5.94 versus 2.97±0.66 and 14.96±2.88, respectively) (Table 2).

AMH was significantly and positively (proportionately) correlated with body mass index (BMI), waist/hip ratio, ovarian volume and antral follicular count. The correlation with BMI, W/H ratio and AFC was powerful (r > 0.7), while it was moderate with other variables. However, no significant correlation was reported between AMH and patient age (Table 3).

In addition, there was moderate, significant and statistically significant correlation between AFC and each of BMI, W/H ratio and mean ovarian volume. However, the correlation between AFC and age was non-significant (Table 4).

Table (1): Demographic and clinical data of study groups

Variable	Group A (<25) (n= 100)	Group B (25-29) (n=65)	Group C (>30) (n=75)	Test	p
Age (years)	27.25±2.43	27.35±2.30	27.57±2.23	0.412	0.661
Waist Circumference	62.68±1.39	72.47±3.50	84.54±2.24	*1798.20*	*<0.001**
Hip circumference	84.73±3.44	86.04±3.53	91.77±3.11	*99.49*	*<0.001**
W/H ratio	0.74±0.03	0.85±0.04	0.92±0.04	*573.11*	*<0.001**
Acne (n,%)	17(17.0%)	14(21.5%)	31(41.3%)	*14.10*	*<0.001**
Hirsutism (n,%)	16(16.0%)	10(15.4%)	19(25.3%)	3.113	0.211

Table (2): Comparison between study groups regarding ovarian volume, site of follicle, AMH and AFC

Variable		Group A (<25) (n= 100)	Group B (25-29) (n=65)	Group C (>30) (n=75)	F	p
Right OV		12.41±1.09	12.42±0.93	12.63±0.96	1.20	0.303
Left OV		12.63±1.02	12.56±0.94	12.70±0.98	0.361	0.698
Mean OV		12.52±1.01	12.49±0.90	12.67±0.96	0.716	0.490
Site of follicle	Peripheral	69(69.0%)	48(73.8%)	54(72.0%)	0.482	0.768
	Mixed	31 (31.0%)	17(26.2%)	21(28.0%)		
AMH	Mean±SD	2.97±0.66	5.49±1.20	8.13±1.88	339.6	<0.001*
	Min.–Max.	1.8-4.7	3.6-9.3	4.5-12.3		
AFC	Mean±SD	14.96±2.88	18.61±6.40	23.73±5.94	64.47	<0.001*
	Min.–Max.	10.0 – 22.0	13-34	14-34		

Table (3): Correlation between AMH and other variables

	AMH	
	Pearson Correlation (r)	p
Age	0.026	0.694 (NS)
BMI	0.850	<0.001*
Waist hip ratio	0.736	<0.001*
Right ovarian volume	0.414	<0.001*
Left ovarian volume	0.393	<0.001*
Mean ovarian volume	0.412	<0.001*
AFC	0.818	<0.001*

Table (4): Correlation between AFC and other studied parameters

	AFC	
	Pearson Correlation (*r*)	p
Age	0.014	0.828
BMI	0.579	<0.001*
Waist hip ratio	0.504	<0.001*
Mean ovarian volume	0.404	<0.001*

DISCUSSION

The potential association between serum AMH and body mass index is poorly studied in patients with PCOS. Thus, the current work was designed to investigate such relationships. Included women were categorized into normal body mass index (group A), overweight (group B) and Obese (group C).

Previous studies had described increased prevalence of obesity in infertile women with or without PCOS [25]. These reflected the importance of investigating the relationship between obesity and PCOS. This explains the design and conduct of this study

The mean age in the study groups was 27.25±2.43, 27.35±2.30 and 27.57±2.23 years in groups A, B and C respectively, with no significant difference between groups. These results are comparable to **Cengiz *et al.*** [26] who reported non-significant differences between obese and non-obese women with PCOS as regard to their age.

Acne was significantly increased with obesity (progressive increase with BMI). However, the hirsutism showed non-significant difference between groups. In addition, the overall prevalence of hirsutism associated with PCO in this study was (18.8%). This value is low when compared to previous studies (39.0% and 29.0%) [27,28]. This lower incidence of hirsutism in the current work than previous studies may be attributed to the different selection and diagnostic criteria for hirsutism. However, others reported non-significant difference between obese and non-obese women with PCOS in the study of **Dos Reis *et al.*** [29]. These results are in line with the current work. In addition, **Legro *et al.*** [30] reported higher prevalence and severity of hirsutism with increased body weight. However, the difference between groups was not significant from the statistical point of view.

Results of the current work showed non-significant differences between study groups regarding ovarian volume (right, left or mean values). However, **Balen *et al.*** [31] reported that increased ovarian volume is a characteristic feature of PCOS, which was not reflected in the current work. Again, this may be different selection and diagnostic criteria.

Most of study females (about three fourths), had peripheral distribution of follicles in the ovary, with no significant difference between groups. These confirm the results of **Guraya *et al.*** [27], who reported peripheral distribution of follicles among 82.8 of women with PCOS

AMH was significantly increased in groups B and C than group A (i.e., there was progressive increase of AMH with increased BMI). These results are comparable to previous studies stated that, plasma AMH was significantly different between normal, overweight and obese adolescent females with PCOS. Interestingly, the same situation was reported for women without PCOS, reflecting the association between increased BMI and higher serum levels of AMH [32-34].

However, contradictory results are reported by **Cengiz *et al.*** [26] who did not find significant difference between normal and obese women with PCOS as regard to AMH levels. This can be explained by the different age groups, as those authors included adolescent girls.

On the extreme side, **Legro *et al.*** [30] reported significant reduction of AMH with increased body mass index, especially in severe obesity. This can be attributed to different selection criteria as they compared morbid obesity to non-obese PCOS. Their results are reported by other researchers [35].

To explain the ovulatory derangements in PCOS, **Jonard and DeWailly** [36] reported that, these changes are due to different etiologies (changes): 1) increased follicular growth in early phases, which lead to a larger than normal reserve of these follicles; 2) inability to select one follicle of this pool leading to what is known as follicular arrest.

AMH is known to as an inhibitor of the initial follicle recruitment [37] and causes follicular arrest [38]. In addition, AMH levels are increased in PCOS. Thus, it is postulated that its involvement in PCOS-associated anovulation depends only on the follicular arrest [36]. However, Jonard and DeWailly reported that the higher AMH production in PCOS is due to increased number of small follicles. However, this was not supported by other studies.

Searching correlation between AMH and other variables revealed significantly positive correlation with BMI, W/H ratio and ovarian volume. However, the correlation with age was statistically non-significant.

These results are contradictory to **Skalba *et al.*** [39] who cannot find any significant correlation between BMI and serum AMH in obese women. But they can find such correlation in women with normal weight PCOS. However, in the current work, we studied the correlation among all study females and do not calculate correlation separately for each group. However, the same authors reported a weak impact of body mass index on the AMH values in women in their reproductive age. It is important to confirm that, their patients were younger than women of the current work.

In the present study, antral follicular count (AFC), was significantly increased with higher BMI. This cannot be confirmed in the previous study **Legro *et al.*** [30]. Nonetheless they reported lower AFC in obese than non-obese women. This may be due to difference in selection criteria or methods used to estimate AFC.

In the present study, correlation between AFC was significantly positive with BMI, W/H ratio, mean ovarian volume and AMH. But it was not significant with patient age. These results are in line with previous studies reporting a direct and significant correlation between AFC and AMH. They explained higher values of AMH in PCOS by higher number of early antral follicles [40]. However, others stated that the increase in AMH is largely due to the increase of AMH by each follicle, not due to increased number of early antral follicles [41]. Anyway, the changes in antral follicles either in count or size and early maturation are responsible for increased AMH. Thus, AMH can be used as an indicator for ovarian aging due to its correlation with the number and early antral follicles [40].

Elmashad [42] also reported a significant positive correlation between AMH levels with ovarian volume and the AFC in PCOS, which was not surprising as ovarian volume reflects the number of small antral follicles present in PCOS, which are the only source of AMH. Furthermore, a significant correlation was reported between serum values of AMH in PCOS and hyperandrogenism in the current and previous studies [40,43].

In short, the current work showed increased levels of AMH in PCOS with obesity. These values positively correlated with BMI and AFC. Thus, we can say that body weight can play a role in pathogenesis and development of PCOS. However, the cause effect relationship needs further investigation in future studies.

Conflict of interest: None

Financial disclosure: None

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