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Assessment of Anti Müllerian Hormone among Women with Polycystic Ovarian Syndrome in Khartoum State

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Abstract

Polycystic ovary syndrome (PCOS) is the most common cause of chronic anovulation and hyperandrogenism in young women .POCS is a common endocrinopathy that accompanied with long term complications. The early diagnosis of this syndrome can prevent it.

The study aim to assess serum levels of Anti Müllerian hormone among female with polycystic ovarian syndrome (PCOS). Sixty clinically diagnosed polycystic ovarian syndrome (PCOS), were chosen randomly from Reproductive Health Care Centre in Khartoum State as case group and thirty apparently healthy individuals as control group. Serum AMH and fertility hormones were measured by using ELISA technique. Results were analyzed using Statistical Package for Social Science (SPSS), computer programmed version 20. The mean serum levels of AMH was significantly increased in PCOS female patients when compare with control, (Mean± SD: 7.6±2.3 ng/ml versus 4.6±0.7 ng/ml), respectively with (P=< 0.001). There was a significant week positive correlation between Anti-Müllerian hormone levels and Luteinizing hormone levels (r=0.239; P= 0.023), and there was negative correlation between Anti -Müllerian hormone levels and Body Mass Index (r= -0.267; P= 0.039).

The serum Anti Müllerian Hormone (AMH) measurement was significantly higher in PCOS patients, which could use for early marker diagnosis of PCOS patients.

Keywords: Anti Müllerian Hormone, PCOS, Luteinizing hormone, BMI

Introduction

Polycystic Ovary Syndrome (PCOS) is the one most common cause of infertility due to an ovulation and it is often diagnosed for the first time in the fertility clinics. (1) Increase in the burden of PCOS has led to a considerable amount of research into its risk factors, pathophysiology and the management (2). Although PCOS is the most frequent endocrine disorder in women of reproductive age but its diagnosis remains one of

the most challenging issues in endocrinology, gynecology, and reproductive medicine. (3) It has been observed that for many years, different combinations of clinical, biological the diagnosis of PCOS on ultrasound (4). In the ovary, AMH has an inhibitory effect on primordial follicle recruitment as well as on the responsiveness of growing follicles to Follicle-Stimulating Hormone (FSH). The ovary-specific expression pattern in granulosa cells of growing non selected follicles makes. (5). The features of PCOS, including menstrual dysfunction, infertility and hirsutism have been described in medical records for more than 2,000 years (6). Since the 1980's, researchers expanded on these observations to report an association between hyperinsulinemia and hyperandrogenism bringing to light possible a etiologies and a complicated metabolic and reproductive condition with psychosocial and economic consequences across the lifespan (7).

. many women with PCOS have an increased risk of insulin resistance which, with the prevalence of obesity, is a powerful risk factor for progression to type 2 diabetes. They also have an increased long-term risk of endometrial hyperplasia /cancer (8).

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein, (9).clinical application of AMH measurement in the prediction of quantitative and qualitative ovarian response in assisted reproductive technologies (ART) (9).

AMH is produced by granulosa cells which they are selected for dominance by the action of pituitary FSH(10). Recent studies show that in adult rat ovaries FSH and estradiol may down-regulate AMH expression. AMH exerts its biological effects through a trans-membrane serine/threonine kinase type II receptor (AMHRII), which is specifically expressed in the gonads and in the mesenchyme cells adjacent to the Mullerian ducts (11). Studies show that AMH alters the expression of several hundred genes. The overall effects of AMH exposure was to decrease the expression of stimulatory factors, increase the expression of inhibitory factors and regulate cellular pathways that result in the inhibition of primordial follicle development (12) The recent study which showed that polymorphisms in the gene for AMH or AMH receptor type II seem to be related to follicular phase estradiol levels, suggesting a role for AMH in the FSH-induced steroidogenesis in the human ovary (13).

The reduction in ovarian reserve is a physiological process occurring in the late reproductive period and consistently associated with a decrease in AMH levels (14). The strong correlation existing between AMH levels and the resting pool of follicles has recently been highlighted by some papers showing that AMH measurement may be used to predict the occurrence of menopause (15) In women, AMH levels seem to be unmodified under conditions in which endogenous gonadotrophin release is substantially diminished, such as during pregnancy, GnRH agonist treatment and short-term oral contraceptive administration, indicating that non-cyclic FSH independent .(9)**.**

2-Materials and Methods

2-1. Study subject

A quantitative method was used to evaluate the serum AMH and glucose

levels among female with polycystic ovarian syndrome during the period from

November 2017 to March 2018 , this cross sectional case control study was conducted in Khartoum state, the capital of Sudan.

2-2. Study population and Sample size

Sudanese infertile women which came to Reproductive Health Care Center, which will diagnosis as PCOS (based on Rotterdam criteria consensus) were considered as case group(N=60). Uniparous or multiparous females in reproductive age group with no complaints of infertility) was consider as control (N=30).

2-3. Inclusion criteria

Inclusion criteria in case group was PCOS diagnosis, 18<age<40, and presence of both ovaries. PCOS was ascertained, using the Rotterdam consensus statements, as the presence of two of the following three criteria: PCO morphology (more than 12 follicle with size 2-9 mm or ovarian volume more than 10 ml in one ovary), clinical or biochemical hyperandrogenism (hirsutism), and oligomenorrhea (cycle length >35 days) or amenorrhea.

2-4. Exclusion criteria

The criteria of exclusion based on excluding any menopausal and women

were history of ovarian surgery.

2-5. Ethical consideration

The study was revised and ethically approved by the ethical committee of the

Faculty of Medical Laboratory sciences, Azaim Alazhari university. Before the specimen was collected, the donors knew that this specimen was collected for research purpose.

2-6. Data collection

Data were collected using a questionnaire, which was designed to collect and maintain all valuable information concerning each case examined, Body mass index was measured as weight in kilogram per square of height in meter (Kg/m2). The patients were categorized as follows: Severely thin <16.9, underweight 17–18.4, desirable weight 18.5– 24.9, over weight 25–29.9 and obese when BMI was >30 Kg/m (16).

2-7. Sample collection and processing

venous blood were collected from 2 to 4 days of cycle from each participant in case group. The sample collected under aseptic conditions and placed in plain containers and centrifuged for 5 minutes at 3000 RPM to obtain serum then samples were kept at -80C until the time of analysis.

2-8. Methodology

Whole blood samples were collected from 90 of women who enrolled in this study, they were sorted to two groups, case group involved infertile women diagnosed with PCOS 60 (67%), the control group involved 30(33%) of the whole subjects. Serum samples assessed with Human products (Germany) and automated processing on clinical chemistry analyzers. They were assessed for blood glucose. Hormonal assessment with enzyme linked immune sorbent assay (ELISA) for AMH, FSH and LH. Various antigen-antibody combinations are used, always including an enzyme-labeled antigen or antibody, and enzyme activity is measured calorimetrically. The enzyme activity is measured using a substrate that changes color when modified by the enzyme. Light absorption of the product formed after substrate addition is measured and converted to numeric values. A target antibody is immobilized on the surface of microplate wells and incubated with an enzyme-labeled antibody to the target protein (or a specific antigen to the target antibody). After washing, the activity of the microplate well-bound enzyme is measured.

2-9. Statistical analysis

Data was analyzed to obtain means standard deviation and correlation of the sampling using statistical package for social science (SPSS) computer Programmed version 20, t test and person correlation were used for comparison correlation between variable.

3. Results

A total of 90 females (60 patients and 30 healthy controls) were included in the study. The results of serum Anti-Müllerian hormone levels and biochemical tests in patients with polycystic ovarian syndrome are given in tables and figures:

Table (3.1): Means of variables among POCS patients compared with control group.

Variable	POCS N=60 Mean ±SD	Control N=30 Mean ±SD	*P*
Anti-Müllerian hormone ng/ml	7.6±2.3	4.6±0.7	<0.001*
Follicle-stimulating hormone ng/ml	10.6±6.9	9.50±8.50	0.233
Luteinizing hormone ng/ml	23.2±15.9	6.7±5.2	0.000*
Random blood glucose mg/dl	97.5±36.8	105±35	0.124
Body Mass Index	24.3±4.8	21.7±3.2	< 0.001*

.. P value ≥0.05 are considered significant. SD: Standard Deviation.*
Independent sample T test was used for comparison.

Table (3-1): Illustrate the mean concentration of Anti-Müllerian hormone levels were significantly increased in PCOS patients compared with control group. (Mean ±SD: 7.6±2.3 ng/ml versus 4.6±0.7 ng/ml), respectively with (*P. value* = <0.001), the means of the variables in POCS group compare with control group the mean of the Luteinizing hormone level (LH) was (23.25±15.943mIμ/ml) which is considered to be high (P. value = <0.001). The mean of the Follicle-stimulating hormone level (FSH) was (10.58±6.935mIμ/ml) which is considered to be normal (P. value =0.233).

The mean of the random blood glucose level (RBG) was (97.58±36.825mg/dl) which is considered to be normal (P.value =0.124). The mean of the Body Mass Index (BMI) was (28.35±5.494) which is considered to be among overweight(P. value =<0.001).

Table 3-2: The correlation (*P* value , *r* value) of Anti-Müllerian hormone levels with variables in POCS group.

Variable	*r*	*P*
AMH with Body Mass Index	-0.267	0.039*
AMH with glucose	-0.021	0.876
AMH with Luteinizing hormone	0.239	0.023*
AMH with Follicle-stimulating hormone	0.035	0.788
AMH with age	0.055	0.608

*.Correlation is significant at the 0.05 level (2-tailed).

Table (3-2). Illustrate correlation between AMH and other variables .There was a significant week positive correlation between Anti-Mullerian hormone levels and Luteinizing hormone levels (*r*=0.239; *P*= 0.023).

There was a significant weak negative correlation between Anti-Müllerian hormone levels and Body Mass Index (*r*=0.267*; P*= 0.039*)*

There was no correlation between AMH and age (*r*=0.055; *P*= 0.608), Follicle-stimulating hormone levels (*r*=0.035; *P*= 0.788), random blood glucose levels

(*r*=-0.021; *P*= 0.876).

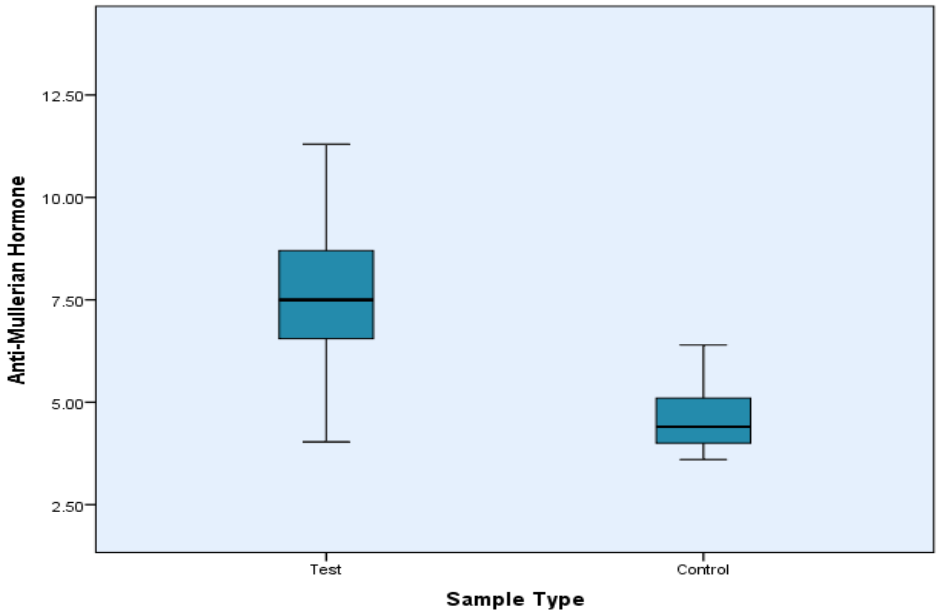

Figure 3-1: Comparison between test and control groups according to the means of Anti-Müllerian hormone levels. (*P*= 0.000)

Figure (3-1) : Show the difference in the means of Anti-Müllerian hormone levels between test and control groups with (P= 0.000).

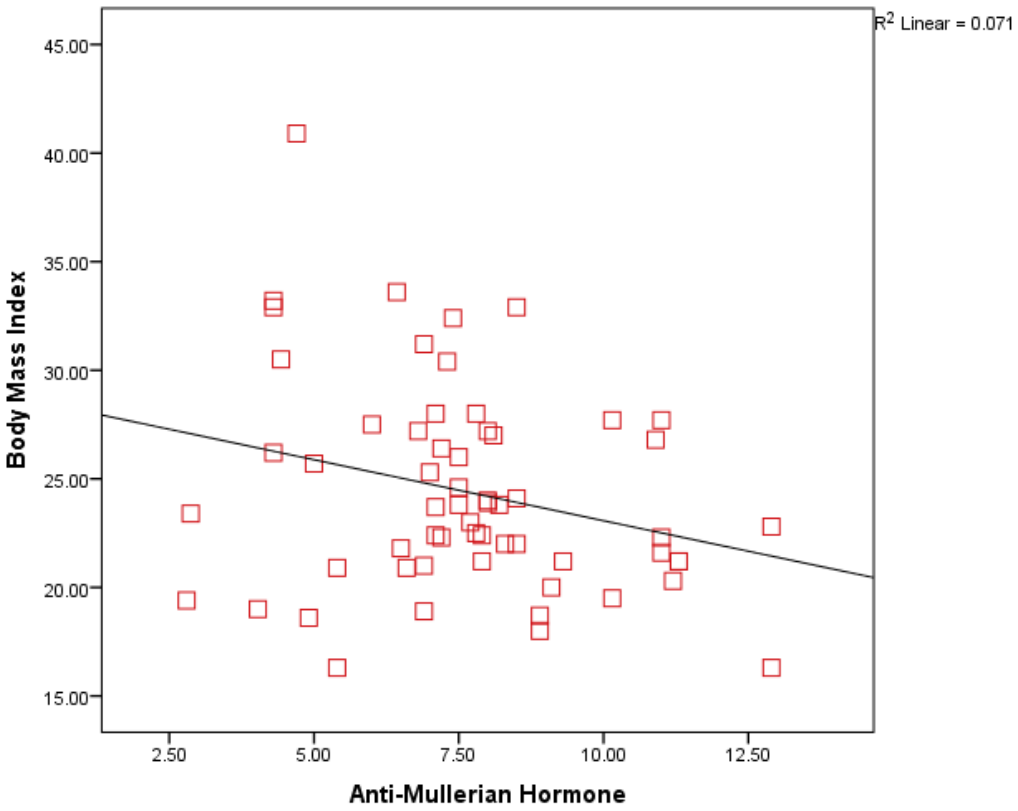

Figure 3.2: Correlation between Anti-Müllerian hormone levels and Body Mass Index. (r=0.267; P= 0.039) , (R^2=0.071).

Figure. (3-2): Show weak negative correlation between Anti-Müllerian hormone levels and Body Mass Index(r=0.267; P= 0.039) , (R^2=0.071).

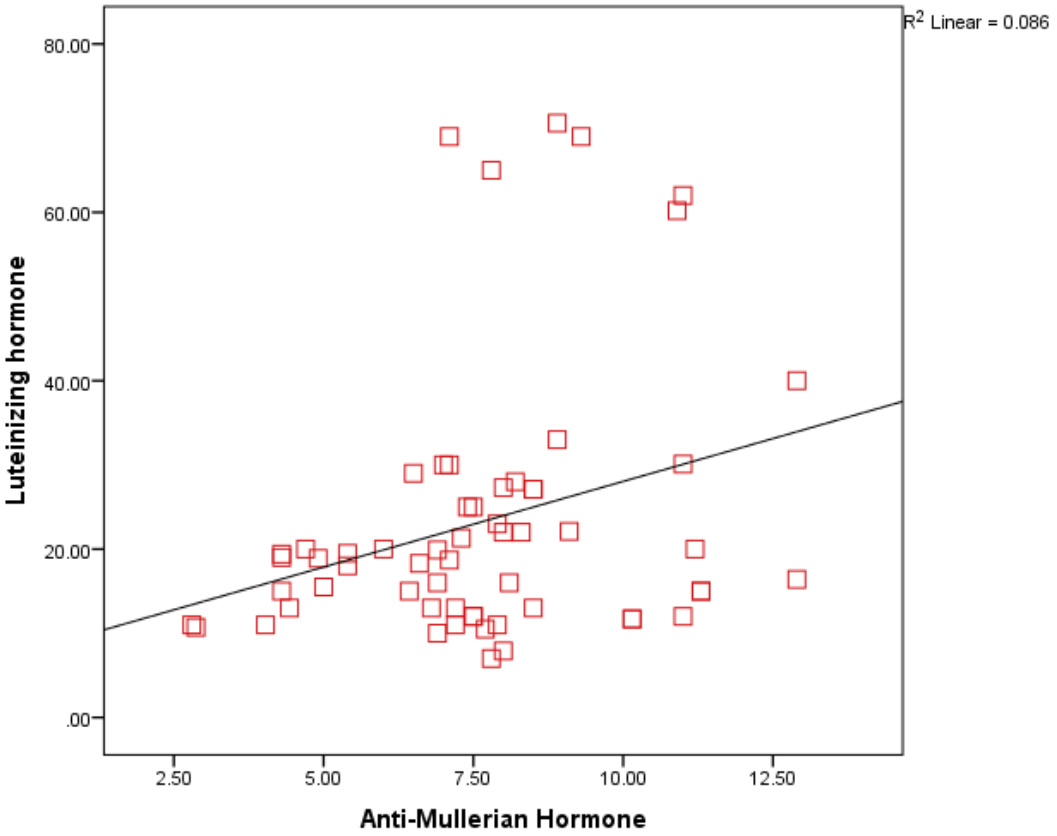

Figure3.3: Correlation between Anti-Müllerian hormone levels and luteinizing hormone (*r*=0.239; *P*= 0.023), (R^2=0.086).

Figure. (3-3): Show weak positive correlation between Anti-Müllerian hormone levels and luteinizing hormone (*r*=0.239; *P*= 0.023), (R^2=0.086).

Discussion

The polycystic ovary syndrome has reproductive, metabolic and psychological features and is the most common cause of an ovulatory infertility(17). Once the diagnosis of PCOS is made, additional evaluation is suggested including a cardio metabolic risk assessment, as well as screening for mood disorder and sleep apnea, screening for diabetes mellitus and fertility assessment of ovulatory status (18). AMH an ideal marker for the size of the ovarian follicle pool and also a prognostic factor for fertility potential (5). AMH has an important role in the development and maturation of follicles (19). Several studies have suggested that AMH serum levels may be a marker for polycystic ovary syndrome (PCOS). As the diagnostic criteria for ultrasound findings is the presence of more than 12 follicles with a diameter of 2–9 mm or when the ovarian volume is more than 10 cm3 (20), it may correlate with the level of serum AMH. The level of AMH circulating in the blood is not affected by the menstrual cycle nor altered during the use of oral contraceptives, therefore it can be used as a potential biological marker for PCOS (21).

The present study demonstrated that there is positive correlation between AMH level and PCOS, there have been several clinical studies that have confirmed the increased levels of serum AMH levels i.e. two to three times higher in PCOS compared with the levels in women with normal ovaries (22-23). Another study reported significantly elevated levels of AMH in normogonadotrophic, normoestrogenic, an-ovulatory patients compared with controls. Moreover, female sufferings with PCO have higher levels of AMH whether obese or lean as compared to a female with no PCOS. This has been reported in a study that recruited sample from a community that included both lean and overweight women. Elevated circulating AMH levels were found among PCOS females versus

non PCOS women, regardless of Insulin resistance and adiposity status (24). Similarly another study suggested that non obese adolescents with Polycystic Ovary Syndrome (PCOS) had higher levels of Anti Müllerian Hormone (AMH) as compared to the controls (25).

Furthermore, AMH levels are positively correlated with individual features of PCOS, including LH concentrations, testosterone, mean ovarian volume and the number of ovarian follicles as reported by Laven et al (26).

In this study the negative correlation was obtained in between serum AMH levels and Body Mass Index (BMI). The study conducted by Maya Kriseman *et al*., showed that inversely correlated with AMH in women with PCOS (27). Furthermore, AMH levels are positively correlated between AMH level and LH concentration this agree with previous study conducted by Tomer Singer *et al*, showed that In conditions of increased LH and normal-to-low FSH, such as PCOS, AMH serum levels are increased and tend to be associated to serum LH, while in conditions of increased FSH such as premature ovarian failure, AMH serum levels are decreased and tend to be associated to serum FSH (28). Samantha Cassar et al reported that AMH levels were increased in community recruited lean and overweight women diagnosed with PCOS based on the Rotterdam criteria, compared with controls. Increased AMH was related to hyperandrogenism rather than to insulin resistance , obesity or gonadotrophins. This highlights the direct interaction with androgens and potential indirect role of insulin resistance in the pathophysiology of reproductive dysfunction in PCOS (24).The severity of PCO morphology on ultrasound is a key correlate of increased AMH in PCOS(29).

Conclusion:

Our data is consistent with previous studies reporting higher AMH in PCOS compared with controls. Thus, the serum AMH measurement can be used as an early marker of Polycystic Ovary Syndrome.

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