Serum anti-Mullerian hormone level as a marker of polycystic ovarian syndrome in Iraqi women

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ABSTRACT

Background: Polycystic ovarian syndrome is one of the common gynaecological diseases encountered nowadays in the gynaecological clinic. Many criteria and diagnostic test had been evolved to be used with different classifications methods.

Objectives: The present study aimed to measure the anti-mullerian hormone levels in serum of the women with Polycystic Ovary Syndrome and to test the possibility that if it can be used as a marker for diagnosis of polycystic ovary syndrome patients.

Methods: A cross sectional study that had been conducted at Kamal AL-Samaraee Hospital, AL-Suwayrah Hospital and Al-Elwiya Maternity Teaching Hospital during the period from July, 1st, 2013 – Jan. 1st, 2014. Where forty women with Polycystic ovarian syndrome (with mean body mass index of 33.25±6.79kg/m^2) were enrolled in the study group and being compared to apparently health women as a control group that were matched for age and their (mean body mass index was 27.63±3.51kg/m^2). Clinical history, biochemical and hormonal analysis were determined for both groups.

Results: The mean serum of anti-mullerian hormone showed statically significant difference (P = 0.0001) in poly-Cystic ovarian syndrome patients compare to the control group and when this hormone compared with other hormones that use for predicting the occurrence of PCOS as (LH , FSH , testosterone, prolactin and insulin), anti mullarian hormone showed the highest sensitivity and specificity as 82.10 % and 100 % respectively, with a cut off value of (>7.9) in Iraqi women.

Conclusions: Anti - mullerian hormone could be the best marker in comparison with other hormones used for the diagnosis of PCOS.

Keywords: Polycystic ovarian syndrome, anti-mullerian hormone, luteinizing hormone, follicular stimulating hormone.

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Polycystic ovary syndrome (PCOS) is a common hormonal disorder among women of reproductive age with a prevalence of (6.6-6.8) % [1]. The main symptoms being amenorrhea, hyperandrogenism, infertility, enlarged ovaries, weight gain or upper body obesity, insulin resistance , hyperinsulinemia and diabetes, hair loss from the scalp and hair growth on the face (Hirsutism), chest, back, abdomen, thumbs, acne with oily skin, caused by high androgen levels, acanthosis Nigerians (dark patches of skin, tan to dark brown) [2]. Anti-Mullerian hormone (AMH), a dimeric glycoprotein belonging to the transforming growth factor-beta super family [3]. In women, at the onset of puberty AMH, like inhibin B, is formed by the granulosa cells of the maturing ovarian follicle, but not by the primordial follicles and also not by the antral follicles under direct FSH regulation in the final regulator of folliculogenesis and of primordial follicular rupture . It reduces the rate of follicle conversion from the primordial to the growing stage and regulates follicle growth by inhibiting FSH-induced conversion from the early to the late stage [4]. This ovary-specific expression pattern in granulosa cells of growing non selected follicles makes AMH an ideal marker for "ovarian reserve" [5]. Although the ultimate pathogenesis of PCOS remains obscure, the distinctive feature is failure of follicular rmaturation, despite initial recruitment, resulting in anovulation and accumulation of preantral and small antral follicles, which contribute significantly to the production of AMH [6, 7]. AMH also inhibits aromatase activity, suggesting that AMH contributes to the severity of PCOS [8]. The aim of present study is to test the possibility that the AMH could be a marker for diagnosis of PCOS patients, and compares hormones as : AMH , fasting insulin , LH , FSH , Prolactin ,testosterone levels between PCOS and normo - ovulatory women , correlate the level of Anti – Mullerian Hormone with the biochemical features as hormones FSH , LH testosterone , prolactin and insulin resistance and to find a cut –off value for AMH in Iraqi women with PCOS.

Method. A cross sectional study that had been conducted during the period between 1st July 2013 to 1st January 2014. Forty women with PCOS (mean of body mass index (BMI) of 33.25±6.79kg/m^2) were enrolled in the study recruited from Kamal AL-Samaraee Hospital, AL-Suwayrah Hospital and Al-Elwiya Maternity Teaching Hospital. The diagnosis of PCOS was made according to the European society of human reproduction and embryology and American society for reproductive medicine criteria: PCOS is diagnosed if there are any two of the following: Presence of polycystic ovary on ultrasound examination, clinical or biochemical hyperandrogenemia and menstrual dysfunction with an ovulation. And exclusion criteria were patients on any type

of hormonal therapy, or with any other endocrine disorders or on metformin therapy. Forty healthy women; employees from the same hospitals staff who were matched for age, their mean BMI was 27.63±3.51kg/m^2, were recruited as a control. All subjects were studied during the early follicular phase (second to fifth day) of the menstrual cycle. 10 ml of venous blood were withdrawn after an overnight fasting from all subjects and allowed to clot in a plain sterile tube and then centrifuged. The separated serum was stored into aliquots at -20C for biochemical and hormonal determinations.

Protocol**;** Clinical and BMI, (calculated as kg/m^2) were determined in all the subjects. AMH (CUSABIO-China), insulin (Demedetec Company, Germany) were determined by ELISA methods. Total testosterone (TT) was measured by an automated quantitative enzyme immunoassay on the VIDAS instrument, BioMerieux, France using the Enzyme Linked Fluorescent Assay (ELFA). Serum fasting plasma glucose level was measured by enzymatic method supplied by Bio Labo Diagnostics, France. Insulin resistance was calculated by using homeostasis model assessment (HOMA-IR) score that employs the formula: fasting insulin concentration (uIU/ml) × glucose (mmol/l) / 22.5 [9]. Measurement of glucose level by mg/dl was multiplied by 0.555 to get result by mmol/l to calculate HOMA-IR.

Statistical analysis performed by using SPSS version 21for Windows (Statistical Package for Social Science). Descriptive analysis was used to show the mean and standard deviation of the variables. The significant difference between mean values was estimated by the Student t-test. The point of statistical significance was noted when probability was $p<0.05$. Correlation analysis was used to test the linear relationship between parameters. Receiver operator curve (ROC) analysis was also employed. The area under the ROC curve gives an idea about the usefulness of a tested parameter in differentiating between two groups (one of which is a control group). In this context, the ROC analysis helps in comparing selected parameters to the rest of them. The closer the area to one (ideal test) the more useful it is in discrimination.

Results. Table 1, shows statistically significant difference in the mean serum levels of AMH, LH, LH/FSH, testosterone, prolactin between PCOS patients when compared to control group, while FSH level fails to show such difference. Table 2 shows that the mean serum levels of insulin and HOMA-IR have statistically significant difference between PCOS patients and control group, while FBS fails to show that.

The present study found that serum AMH level in patient group showed a positive significance correlation coefficient with LH (r = 0.33 P = 0.01), LH / FSH (r = 0.39 P = 0.007), testosterone (r = 0.52 P = 0.005), and negative significance correlation with FSH (r = -0.28 P = 0.05). But there is no significant correlation coefficient with other studied markers, as shown in table 3.

Cut-off values according to sensitivity and specificity could be calculated as shown in table 4. The result according to Sensitivity 82.10 % and Specificity 100 % shows that AMH could be the best marker as comparisons with other hormones (LH, FSH, LH:FSH ratio, testosterone, prolactin, insulin).

Table 1: Hormonal profile in PCOS compared to control.

Characteristic	PCOS (No=40)	Control (No=40)	P value
AMH (ng/ml) Mean± SD	13.55±8.95	6.61±0.94	0.0001
LH (IU/L) Mean± SD	5.98±3.88	4.09±1.93	0.008
FSH(IU/L) Mean± SD	5.04±1.33	4.67± 1.77	NS
LH:FSH ratio Mean± SD	1.22±0.83	0.73±0.23	0.001
Testosterone(nmol/L) Mean± SD	1.30±0.43	0.41± 0.16	0.001
Prolactin (ng/ml) Mean± SD	17.46±12.95	7.94±3.79	0.001

Table 2: Insulin and biochemical marker in PCOS patients compared to control.

Characteristic	PCOS (No=40)	Control (No=40)	P value
Fasting serum Insulin (μU/ml) Mean± SD	34.69±17.49	15.46±6.03	0.0001
Fasting plasma glucose (mmol/L) Mean± SD	4.49±0.78	4.36±0.60	NS
HOMA (insulin resistance) Mean± SD	6.94±3.72	2.97±1.16	0.0001

Table 3: Correlation coefficient of AMH in the PCOS patients.

	PCOS (No= 40)		
Parameters	r	p	Sig.
LH	0.33	0.01	S
LH:FSH	0.39	0.007	S
FSH	-0.28	0.05	S
Testosterone	0.52	0.005	S

Table 4: The discriminatory ability of the tests for diagnosis of PCOS.

Test	Sensitivity%	Specificity%
AMH (ng/ml)	82.10	100
LH (IU/L)	61.5	67.5
FSH(IU/L)	56.5	65.00
LH:FSH ratio	46.20	92.50
Testosterone(nmol/L)	28.21	95.00
Prolactin (ng/ml)	51.30	95.00

Discussion. Polycystic ovary syndrome is associated with an increase prevalence of AMH. The present study show high significant differences (P=0.0001) between PCOS patients and control groups, Anti-Mullerian hormone 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). Serum levels are doubled, and granulosa cell production is greatly increased 10. When testing the sensitivity and specificity of this hormone as a marker for detection of PCOS, it shows that it has the highest detection rate, with sensitivity of 82.10 % and specificity of 100 % when compared with other hormones (LH, FSH, testosterone, prolactin, and insulin). The only paper that did test the sensitivity and specificity of this hormone it did that in comparison with polycystic ovarian morphology and it showed that when replacing the morphology with AMH, the specificity and sensitivity for identifying PCOS were 97.1 and 94.6% and 97.2 and 95.5% respectively depending on the criteria use for the diagnosis [11]. Also, we did conclude a cut off point for AMH which is (>7.9) in the Iraqi patients women.

In the present study there was a significant positive correlation between AMH and LH, LH: FSH, Testosterone, while a significant negative correlation was found between AMH and FSH. Which is in line with the finding that serum AMH levels reflect the number of small antral follicles demonstrated in several studies[12-15,16-23]. Our findings regarding LH and LH/FSH are comparable with the results of previous studies [14, 19, 23]. The results of the present study revealed no significant correlations between AMH and age, BMI, and fasting insulin. This is in agreement with Pigny et al. [15]. A significant positive correlation was found between AMH and serum testosterone in the PCOS group exclusively. These findings are in accordance with the results of previous studies [14, 15, 18, 19, 22], and add to the existing evidence for small ovarian follicles in the production of both AMH and androgens. Pigny et al.[15] suggested that the increase in AMH serum levels in PCOS is a consequence of androgen induced excess in small antral follicles and that each follicle produces normal amount of AMH. However, Pallet et al. [21] found that raised serum AMH in PCOS is a reflection of both an increase in production per cell and the increase in follicle number since they used cells from size matched follicles in patients and controls plated at the same density. It could be also speculated that since AMH inhibits FSH induce aromatase activity in human granulosa cells [24], it may also be responsible for the reduced aromatase activity in PCOS granulosa cells, [25] and contributes to the elevated androgen levels. Moreover, Crisosto et al. [26] proposed that AMH expression is modulated by androgens in bovine granulose cells from small follicles; suggesting that androgens, by inhibiting AMH expression, may promote follicle recruitment, increasing the early growing follicular pool.

In conclusion, there was a significant increment in AMH level in PCOS women compared with control subjects as well as AMH could reflects the severity of PCOS and is a better marker for the diagnosis of PCOS than others hormones because of the highest sensitivity and specificity as could be used in the future as the only test to confirm the presence of the disease.

References

1. Evanthia Diamanti-Kandarakis, Charikleia D Christakou, Eleni Kandaraki1 and Frangiskos N Economou. Metformin: an old medication of new fashion: evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome: ***European Journal of Endocrinology*** 2010; 162 (2):193-212.
2. Teede H, Deeks A and Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. ***BMC Medicine*** 2010; 841:243-249.
3. Karkanaki A, Vosnakis Ch, Panidis D. The clinical significance of anti-Müllerian hormone evaluation in gynecological endocrinology. ***Hormones*** 2011; 10 (2):95-103.
4. Ivana Zec , Dubravka Tislaric-Medenjak, Zeljka Bukovec Megla, Ivana Kucak. Anti-Müllerian hormone: A unique biochemical marker of gonadal development and fertility in humans. ***Biochemia Medica*** 2011; 21(3): 219-30.
5. Van Rooij IA, Broekmans FJ, Te Velde ER, Fauser BC, Bancsi LF, de Jong FH, et al. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. ***Hum Reprod*** 2002; 17:3065-71.
6. Laven JS, Mulders AG, Visser JA, Themmen AP, De Jong FH, Fauser BC. Anti-Mullerian hormone serum concentrations in normo-ovulatory and anovulatory women of reproductive age. ***J Clin Endocrinol Metab.*** 2004; 89: 318-23.
7. Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, et al. Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. ***J Clin Endocrinol Metab.*** *2003*; 88:5957-62.
8. Clemente N di, Ghaffari S, Pepinsky RB, Pieau C, Josso N, Cate RL, et al. A quantitative and interspecific test for biological activity of anti-mullerian hormone: the fetal ovary aromatase assay. ***Development*** 1992; 114:721-7.
9. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. ***Diabetologia*** *1985*; 28: 177-182.
10. Pellatt L, Rice S, Mason HD; ***Anti-Mullerian hormone and polycystic ovary syndrome***: amountain too high 2010;139 (5):825-33.
11. Eilertsen TB, Vanky E, Carlsen SM. Anti-Mullerian hormone in the diagnosis of Polycystic Ovary Syndrome: can morphologic description be replaced? ***Hum Reprod*** 2012 Aug; 27(8): 2494-502.
12. Annemarie De Vet A, Laven J S E, Jong F H de, Themmen A P N, Fauser B C G M. Anti-Mullerian hormone serum levels: a putative marker for ovarian aging**.** ***Fertile Steril*** *2002*; 77(2): 357-62.
13. Fanchin R, Schonauer LM, Righini C, Frydman N, Frydman R,Taieb J.; Serum anti-Mullerian hormone dynamics during controlled ovarian hyperstimulation. ***Hum Reprod*** 2003; 18:328-32.
14. Laven JS, Mulders AG, Visser JA, Themmen AP, De Jong FH, Fauser BC. Anti-Mullerian hormone serum concentrations in normo-ovulatory and anovulatory women of reproductive age. ***JClin Endocrinol Metab*** 2004; 89: 318-23.
15. Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, et al. Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome:

relationship to the ovarian follicle excess and to the follicular arrest. ***J Clin Endocrinol Metab*** *2003*; 88: 5957-62.
16. Cook CL, Siow Y, Brenner AG, Fallat ME. Relationship between serum mullerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. ***Fertil Steril*** *2002*; 77:141-6.
17. Mulders AG, Laven JS, Eijkemans MJ, de Jong FH, Themmen AP, Fauser BC.;(Changes in anti-Mullerian hormone serum concentrations over time suggest delayed ovarian ageing in normogonadotrophic anovulatory infertility. ***Hum Reprod*** *2004*; 19: 2036-42.
18. Piltonen T, Morine-Papunen L, Koivunen R, Perhenntupa A,Ruokonen A, Tapanainen JS.; Serum antimullerian hormone levels remain high until late reproductive age and decrease during metformin therapy in women with PCOS. ***Hum Reprod*** 2005; 20:1820-6.
19. Eldar-Geva T, Margalioth EJ, Gal M, Ben-Chetrit A, Algur N,Zylber-Haran E, et al. Serum anti-Mullerian hormone levels during controlled ovarian hyperstimulation in women with polycystic ovaries with and without hyperandrogenism . ***Hum Reprod*** 2005, 20:1814-9.
20. Pigny P, Jonard S, Robert Y, Dewailly D.;Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. ***J Clin Endocrinol Metab*** *2006*; 91:941-5.
21. Pallet L , Hanna L , Brincat M , Galea R, Brain H, Whitehead S , et al. Granulosa cell production of antimullerian hormone is increased in polycystic ovaries. ***J Clin Endocrinol Metab*** 2007; 92:240-5.
22. Chen MJ, Yang WS, Chen CL, Wu MY, Yang YS, Ho HN. The relationship between anti-Mullerian hormone, androgen and insulin resistance on the number of antral follicles in women with polycystic ovary syndrome. ***Hum Reprod*** 2008; 23:952-7.
23. Nardo LG, Yates AP, Roberts SA, Pemberton P, Laing I. The relationships between AMH, androgens, insulin resistance and basal ovarian follicular status in non-obese subfertile women with and without polycystic ovary syndrome. ***Hum Reprod*** *2009*; 24:2917-23.
24. Grossman MP, Nakajima ST, Fallat ME, Siow Y.; Mullerian inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. ***Fertil Steril*** 2008 ,89:1364-70.
25. Visser JA, de Jong FH, Laven JSE, Themmen APN. Anti-mullerian hormone: a new marker for ovarian function. ***Reproduction*** *2006;* 131:1-9.
26. Crisosto N, Sir-Petermann T, Greiner M, Maliqueo M, Moreno M, Aedo P, Lara HE, et al. Testosterone-induced down regulation of anti-Mullerian hormone expression in granulosa cells from small bovine follicles. ***Endocrine*** *2009*; 36: 339-45.