

Comparison Between Follicular Antimüllerian hormone levels Between Women's With polycystic Ovarian Syndrome And Normal Ovulatory Women Undergoing Intracytoplasmic Sperm Injection (ICSI) Techniques.

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Background: AMH is a glycoprotein of the transforming growth factor- β (TGF- β) superfamily; it has an important role in regulation of the ovarian follicle development. AMH Produced by granulosa cells of growing follicles after birth. Polycystic ovary syndrome (PCOS) is the most common cause of menstrual dysfunction, infertility, and hyperandrogenism, and its prevalence is about 5-10% in female population of reproductive-age women. Follicular fluid (FF) surrounding the oocyte may play an important role in determining oocyte quality and the future potential to achieve fertilization and development of embryo and it provides a very good environment for the development of the oocytes.

Objective: Compare the level of follicular fluid of anti-müllerian hormone (AMH) in women with PCOS and normal ovulatory women that undergoing intra cytoplasmic sperm injection (ICSI) techniques. And in prediction of clinical outcome (pregnancy) of intra cytoplasmic sperm injection (ICSI) in women with PCOS by measuring the cut-off value of FF of AMH levels.

Subjects and Methods: This case-control study was carried out at College of Medicine, University of Baghdad and at Kamal Al-Samarai Hospital, Baghdad, Iraq, during the period from December 2014 to February 2016. The study involved 23 women with PCOS and 22 healthy women (male factor). Follicular fluid samples were collected from all women on day of oocyte pick up by U/S guided transvaginal technique and used for measurements of the concentrations of AMH. ELISA technique was used for hormone estimations.

Results: The results of this study revealed that follicular fluid (FF) concentrations of AMH were significantly increased in PCOS women when compared to healthy women ($P= 0.034$). The cut-off values of FF of AMH levels for prediction of pregnancy of ICSI in PCOS women were found to be 110.17 ng/ml. In healthy women with normal ovulatory cycle, the cut-off values of FF levels of AMH were found to be 85.46 ng/ml.

Conclusion: This study revealed the clinical importance of the measurements of FF levels of AMH in prediction of cases of PCOS in women's undergoing ICSI techniques.

Key Words: AMH, PCOS, follicular fluid, ICSI.

I. Introduction

Polycystic ovarian syndrome (PCOs) considered as the most common endocrine disorder in women in reproductive age with a prevalence of about 5-10% in female population and it is considered as the most common cause of chronic anovulation and hyperandrogenism in young women (1). PCOS is a diagnosis that is being made more commonly in adolescent girls; mirroring the increasing rate of obesity in this population (2). The exact mechanism affecting ovarian steroidogenesis in PCOS is still unclear. Insulin resistance is a common feature of PCOS that has been observed (3). Hyperinsulinemia has been recognized as a contributory factor for ovarian disruption due to increased production of ovarian androgens (4). Also obesity is commonly seen associated with this disorder; this further worsens the state of insulin resistance state in those patients. This heterogeneous nature of the disease has led to difficulty in building consensus for the standard diagnostic criteria (5). National Institute of Health (NIH) (1990) suggested the presence of oligomenorrhea with clinical or biochemical hyperandrogenemia are essential for the diagnosis (6). The Rotterdam Consensus (2003) expanded the diagnostic criteria for PCOS by adding the antral follicle count (AFC) on ultrasound as one of the diagnostic criteria and recommended that at least two out of the three criteria should be present (7). Finally, the Androgen Excess Society (2006) recommended that to define PCOS patients (the patient must have clinical and biochemical features of the disorder, these include: clinical and/or biochemical evidence of excess in androgen levels with either oligomenorrhea and/or polycystic ovaries on pelvic ultrasound) (8).

Excessive ovarian production of Anti-Müllerian Hormone (AMH), secreted by growing follicles, is now considered as an important feature of PCOS. Serum AMH is synthesized by small antral follicles of the ovaries, which are seen on ultrasound. Serum AMH could therefore be used as a representative for the AFC in the diagnosis of PCOS. Serum AMH has also demonstrated its utility in the treatment of infertility. But the absence of an international standard for serum AMH assay and the inability to define thresholds makes application of serum AMH more difficult (9).

Increased AMH levels correlated with PCOS severity and are associated with greater ovarian stimulation and higher clinical pregnancy rates following assisted reproductive technology. These data characterize a population of women with elevated AMH levels, demonstrating that the vast majority of women with AMH >10 ng/mL have PCOS (10). It has been found that higher AMH levels in PCOS women compared with normo-ovulatory controls matched for age and BMI. However, these levels decreased at a higher rate over time in PCOS patients, narrowing the difference. The estimated reproductive lifespan of PCOS cases was extended by 2 years on average compared with normal ovulatory women (11).

II. Subjects And Methods:

This study involved 65 women aged nearly 15-42 year and classified into two groups; 33 women with PCOS and 32 women with normal ovulation [male factor infertility]. Both groups of women will undergo hormonal stimulation according to ICSI technique at Kamal AL –Samarai Hospital and Baghdad IVF center. The index of obesity [BMI], history of cycle regularity, hirsutism, acne, number of children was recorded. Women who have had chronic endocrine disorder were excluded from the study.

Blood sample collected from peripheral vein of each woman on the day of the oocyte pick-up. Follicular fluid was taken from each woman from the pool of ovarian follicles guided by transvaginal ultrasound technique. AMH level measured in follicular fluid using ELISA technique. The FF levels of AMH in PCOS women and women's with normal ovulation were measure and compared during ICSI technique. The differences in the mean (\pm SE) values of hormonal study were calculated.

III. Statistical Analysis

In our study the data of the 65 patients were analyzed by the statistical package for social science (SPSS) software version 17.

Descriptive statistics were presented as mean, standard error, frequencies and percentage. The comparisons of follicular fluid AMH were tested using paired t test. The comparison between PCOs with nonPCOs was done using students's t-test. In each group, the student t-test was used to compare the parameters of the pregnant versus thenon pregnant patients. ROC curve for the follicular fluid AMH were used to express the sensitivity and specificity index of the follicular fluid AMH. A level of significance (p value) of ≤ 0.05 was considered significant.

IV. Results

The clinical characteristics of the studied groups are shown by table (1). The mean values of age of PCOs women's (30.43 ± 1.28) did not differ significantly from that of control group women's (30.40 ± 1.25). The mean values of BMI did not differ significantly between the PCOs and control groups women's (26.2 ± 0.414 , 25.2 ± 0.598 , respectively). The female age of both study groups was ranged between 19- 42 years. The BMI of both studied groups was ranged between 35.35- 49.39.

Table (1): General demographic data among studied groups

Parameter	PCOs M \pm SE	Non PCOs M \pm SE	P Value
Age	30.43 \pm 1.28	30.40 \pm 1.25	0.831
BMI	26.2 \pm 0.414	25.2 \pm 0.598	0.173

PCOs: Polycystic ovary syndrome patients. M \pm SE: Mean \pm Standard Error. BMI: Body Mass Index.

Table (2) revealed that the mean \pm standard values of follicular fluid (FF) AMH level of PCOs women's (45.03 ± 6.63) was significantly higher than that of non PCOs women's (26.52 ± 5.17 , $P= 0.034$).

Table (2): The values of follicular fluid level of AMH in PCOs and non PCOs groups.

Parameter	PCOs M±SE	Non PCOs M±SE	P Value
AMH (ng/ml)	45.03± 6.63	26.52± 5.17	0.034*

* Significant P value(P ≤ 0.05).

Table (3) showed the cutoff values of the follicular fluid AMH concentration for prediction of pregnancy outcome by using the percentiles 90 and 95 in PCOs, non PCOs and all patients in the study. This study may help in prediction of clinical outcome (pregnancy). AMH level were directly correlated with clinical pregnancy outcome. Using non pregnant (PCOs and non PCOs) in general to introduce a cutoff level for prediction of pregnancy outcome.

Table (3): Cutoff values of the follicular fluid AMH concentration for prediction of pregnancy outcome

Parameter	AMHf(ng/ml)	
PCOs (non pregnant)	Percentiles 90	110.17
	Percentiles 95	111.70
Non PCOs (non pregnant)	Percentiles 90	85.46
	Percentiles 95	97.16
All cases (non pregnant)	Percentiles 90	99.51
	Percentiles 95	110.25

Figure (1) shows the ROC curve for the follicular fluid AMH. This figure revealed that the sensitivity and specificity index of follicular fluid AMH (70.3%).

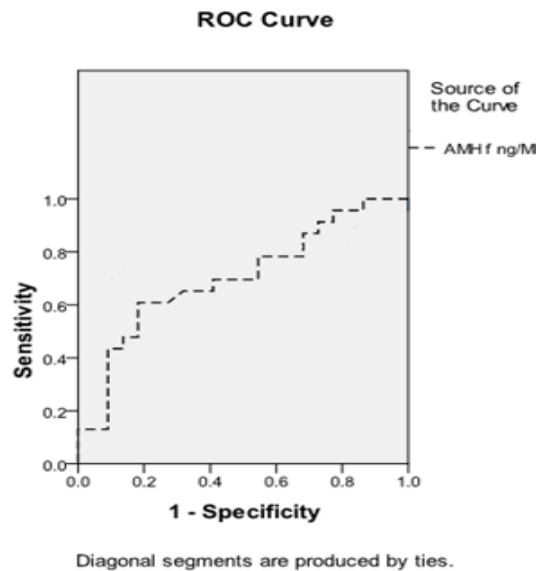


Figure (1): Receiver operating characteristic (ROC) curve analysis calculation of follicular fluid AMH

V. Discussion

In the present study female age of all study groups ranged between 19- 42 years. The mean values of age in the PCOs women's (30.43± 1.28) did not differ significantly from that of women's with normal ovulatory cycle (30.40± 1.25). This result is in agreement with result of Bukman&Heineman, that suggested that although the age factor is an important in cases of infertility, it was not very precise in predicting reproductive potential (19). The mean value of BMI was not differing significantly between the women's with PCOs (26.2±0.414) and control women's normal ovulatory cycle (25.2±0.598). Reduced fertility in the obese women (BMI ≥30) is usually related to many factors including the deviation in endocrine and metabolic functions that can affect the follicular growth, implantation and clinical pregnancy outcome (20).

Our study revealed that the follicular fluid (FF) AMH level in PCOs women's (45.03± 6.63) was significantly higher than in the non PCOs women's (26.52± 5.17, P= 0.034). This result in agreement with study

done by Cook et al, they notice that the PCOS women have a high level of AMH in the serum and follicular fluid than in other healthy controls women's (14). Moreover, it was founded that there was a significant correlation between the follicular fluid and serum concentrations of AMH in the PCOS group but not in controls (21). The ROC curve for follicular fluid AMH, this curves were constructed by computing the sensitivity and specificity of the follicular fluid AMH, in this study area under the curve consider high for FF AMH. Low level of anti-Müllerian hormone predicts a low response to ovarian hyperstimulation programs, while a greater level may predicts a high response (22). Subsequently, higher AMH levels are usually associated with greater chance of live birth after IVF, even after adjusting for age (23). Means that the AMH is a good predictor of ovarian response.

The sensitivity and specificity index of the follicular fluid AMH (70.3%). This result in agreement with study done by Alipour et al, that study the sensitivity of AMH and FSH serum level, the result of the study indicate that AMH has high sensitivity. AMH seems to be more useful in the early diagnosis of premature ovarian failure (24). The results done by Krawczuk-Rybak et al revealed that the AMH measurement can be used as a sensitive marker of a reduced ovarian reserve in young cancer survivors (25).

The main finding of this study is that the role of AMH to diagnose PCOS, especially when combined with the other diagnostic criteria of PCOS. So, AMH can be substituted for PCO treatment and is equally good in diagnosing PCOS (20). Data about our control group agree with previous reports from Fanchin et al. (21) who found a tight relationship between the AMH serum level and the FN at U/S in regularly menstruating infertile women studied at baseline before undergoing in vitro fertilization. Van Rooij et al. (16) also showed that the AMH level was a good predictor of the number of retrieved oocytes, as previously reported by Seifer et al. (17). However, our data have produced new information by showing that the 6- to 9-mm follicles do not contribute to the serum AMH level in normo-ovulatory women, whereas being overweight has a mild, although significant, negative influence on this parameter.

VI. Conclusion

This study revealed the clinical importance of the measurements of FF levels of AMH in prediction of cases of PCOS in women's undergoing ICSI techniques. Also our study revealed the clinical importance of the measurements of the FF levels of AMH in assessment of clinical outcome or pregnancy of ICSI in PCOS infertile women. The study revealed that the serum AMH is a good predictor of ovarian response. In our study we found that AMH directly correlated with the clinical pregnancy outcome. The obtained cut-off values using the non-pregnant (PCOs and non PCOs) in general of serum and FF concentrations of AMH are very important for prediction of the result of the ICSI technique.

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