Correlation of Anti Mullerian Hormone (AMH) Level with Clinical & Pituitary Hormones (FSH, LH) in Infertile Sudanese Women with PCOA Case Control Study

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Citation

Abstract
Objectives: To correlate serum Anti-Mullerian hormone (AMH) level with clinical, & pituitary hormones (LH&FSH) in infertile women with PCOS. Materials and methods: This is prospective case control hospital base study, conducted at Prof. El-Sir Abo-Elhassan fertility center and Omdurman maternity hospital in Khartoum state, Sudan during the period from June 2015 to January 2016. Structured questionnaires were used to gather Socio demographic data from infertile women who had been diagnosed as PCOS according to ROTTERDAM criteria and hormonal levels were measured include FSH, LH, and AMH by using ELISA. Data was analyzed by SPSS. Result: Total of 138 infertile women, 69 with PCOS and 69 as control. Menstrual cycle irregularity was encountered in 65(94.2%), the majority complained of oligomenorrhea 59(85.5%), While in the control group 63(91.3%) had regular cycle. when correlate irregular menstrual pattern with AMH, P-value=0.026 which was significant (P>0.05). In the PCOS group obesity, Hirsute and acne account for 20.3%, 40(58%) and 33(47.8%), while, no woman in the control group complained of any. FSH level was normal in both groups, LH level was high in 10(14.5%) of the PCOS group while all the women in the control group had normal level. AMH level was high in 67(97.1%) of women diagnosed with PCOS while it was high in 3(4.3%) of the control group, when we correlate AMH with LH P value =0.03 (P >0.05). Conclusion: Infertile Sudanese women with PCOS usually presents mainly with oligomenorrhea, hirsute and acne. FSH level was normal in both groups, while AMH level is better diagnostic test than LH& FSH.

1. Introduction
Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders for premenopausal women with a prevalence rate of 4–12% [1]. PCOS is characterized by hyperandrogenism, irregular menses, chronic anovulation, infertility, and insulin resistance [2]. The aetiology of PCOS remains uncertain but intrinsic abnormalities in
the synthesis and secretion of androgens are a plausible basis for the syndrome, so extra-glandular synthesis of androgens, particularly in the adipose tissue, has been found to be involved in the path physiology of PCOS [3]. The distinctive feature is failure of follicular maturation, despite initial recruitment, resulting in anovulation and accumulation of pre-antral and small antral follicles, which contribute significantly to the production of AMH [4, 5]. AMH also inhibits aromatase activity, suggesting that AMH contributes to the severity of PCOS [6]. Regarding the investigations of PCOS physical assessment is important including clinical features of hyperandrogenism like acne and hirsutism, increase BMI, and acanthoses nigricans. Measurement of early follicular phase FSH, LH, and testosterone, serum AMH, ultrasound scan of pelvis, glucose tolerance test if BMI more than 35, and fasting lipid profile are the ideal investigations for such patients [2]. Anti-Mullerian Hormone (AMH) is a diametric glycoprotein a member of the transforming growth factor-ß super family. It has been mainly studied for its regulatory role in male sex differentiation. It is produced by the Sertoli cells of the fetal testis and induces the regression of the Mullerian ducts [2], [7]. In females AMH is produced exclusively by granulosa cells (GCs) of ovarian follicles only after birth [8], [9]. It is normally expressed at low levels in primary follicles, increases to maximal levels in large pre-antral and small antral follicles and then declines as the follicle grows [10]. AMH has an inhibitory effect on the primordial follicle recruitment as well as on the responsiveness of growing follicles to follicle-stimulating hormone (FSH) [11], [12]. This ovary-specific expression pattern in granulosa cells of growing non selected follicles makes AMH an ideal marker for “ovarian reserve” [13]. Several studies have reported a correlation between serum AMH levels and the number of small antral follicles [3], [4] [5], [6]. Women in whom AMH levels fall who have the best response to methods to induce ovulation. Normal AMH level ranging from 1.5 - 4.0 ng/ml. Over 4.0 ng/ml consider high level. Low normal range from 1.0 -1.5 ng/ml. Low level range from 0.5 -1.0 ng/ml and less than 0.5 ng/ml consider very low level [14].

2. Material & Methods

2.1. Population and Study Procedures

This is a hospital based prospective case control study conducted during the period from the 15th June 2015 to 28th January 2016. The study included 69 infertile women diagnosed as PCOS using Rotterdam 2003 definition The patients who have the following diseases were excluded from the study: hyperthyroidism, hypothryoidism, congenital adrenal hyperplasia (abnormal 17- hydroxyl progesterone level), pituitary insufficiency, & granulose cell tumor. The control groups were 69 infertile patients with regular cycle and they had normal ovaries.

They were matched for age, and ethnicity, however the weight (BMI) of the study group was noted to be higher than the (BMI) of the control group. The patients were usually referred to Dr Elsir Abo-elhassan Infertility Center & Omdurman Maternity Hospital fertility centre in Khartoum State by gynecologists from our own hospital, other local hospitals, Family Planning Clinics and the private sector.

Every patient was counseled and gave an informed consent prior to participating in the study, which had been approved by the Ethics Committee, Sudan Medical Specialization Board. Full history concerning their age, type and duration of their infertility, menstrual pattern, acne and hirsute were reported then their weight and height were recorded and the body mass index (BMI) was calculated.

2.2. Laboratory Methods

Blood sample was obtained from all women, put on a specific containers and sent to the lab for measurement of serum levels of anti mullein hormone (AMH), luteinizing hormone (LH) and follicle stimulating hormone(FSH) all in the early follicular phase, day 3 or 4 of natural cycle or progesterin induced withdrawal bleeding in polycystic ovary syndrome.

The AMH Gen II ELISA is an enzymatic ally amplified two-site immunoassay. In the assay, calibration, controls and sample are incubated in micro titration wells which have been coated withanti-AMH antibody. After incubation and washing, anti-AMH detection antibody labeled with biotin is added to each well. After a second incubation and washing step, strepativ in - horseradish peroxidase (HRP) is added to the wells. After a third incubation and washing step, the substrate was determined by dual wavelength absorbance measurement at 450 nm and between 600 and 630 nm. The absorbance measured is directly proportional to the concentration on AMH in the samples. A set of AMH calibrators is used to plot a calibration curve of absorbance versus AMH concentration. The AMH concentration in the samples can then be calculated from this calibration curve.

The data was analyzed using statistical Package for the Social Science (SPSS) and summarized using the percentage γ2 was used as test of significance (two tail) at(P≤0.05 is significant)

3. Results

Sixty nine infertile Sudanese women with PCOS were included & studied, their age was ranged between 16 to 45 years and 50(86.9%) had an age range of 16-36 years while the mean age at diagnosis was 24.5±5(Table 1). The majority of study group had higher body weight &BMI (29.9± 6.0). 14 (20.3%) of the 69 women was obese (BMI>30) compared with the control (average BMI: 21.7 ± 3.0) Concerning socio demographic data showed that both PCOs & control groups almost more that 50% are housewife secondary school educators & they reside in the
rural area. The clinical features of PCOS among the study population were that the majority of the women in the PCOS group had irregular cycle 65 (94.2%) in the form of Oligomenorrhea which was the main complain 59 (85.5%), amenorrhea was in 6 (8.7%) and menorrhagia was in 4 (5.8%), which were demonstrated in figure 1. In the other hand, the majority of the control group their cycle were regular 63 (91.3%). Forty (58%) women in the PCOS group had hirsute, while no woman in the control group had it. Nearly half of the women in the PCOS group 33 (47.8%) had acne, while no woman in the control group had it, shown in Table 2. So the classical triad in the control group are almost absent because they had normal ovaries and so their androgen level is normal. In regards to the hormonal profile, blood test for LH, FSH and AMH were obtained for these women. FSH level was found to be normal in the study group as well as a control group level of FSH 30 IU/L or less. 59 (85.5%) of the women with PCOS group had normal LH levels (15 IU/L or less), while 10 (14.5%) had high LH levels. The entire control group had normal LH levels. Sixty-seven (97.1%) of women on PCOS group had high AMH level (1.5 ng/ml-4.0 ng/ml) while it was only high in 3 (4.3%) in the control group, while in the remaining were normal as shown in figure 2. The AMH level was correlated to menstrual cycle pattern, & LH, which was found to be significant with P-value (0.026), (0.030) respectively, regarding correlation with hirsute it was insignificant result with P-value (0.064).

### Table 1. Age distribution.

<table>
<thead>
<tr>
<th>PCO group</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 - 26 years</td>
<td>27</td>
<td>39.1%</td>
</tr>
<tr>
<td>27 - 36 years</td>
<td>33</td>
<td>47.8%</td>
</tr>
<tr>
<td>37 - 45 years</td>
<td>9</td>
<td>13.0%</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>100%</td>
</tr>
</tbody>
</table>

The mean age was 24.5 ± 5.

### Table 2. Clinical presentations.

<table>
<thead>
<tr>
<th>Clinical presentations</th>
<th>PCO Frequency &amp; Percent</th>
<th>Control Frequency &amp; Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle pattern: a-regular</td>
<td>4 (5.8%)</td>
<td>63 (91.3%)</td>
</tr>
<tr>
<td>b-Irregular</td>
<td>65 (94.2%)</td>
<td>6 (8.7%)</td>
</tr>
<tr>
<td>Hirsute</td>
<td>40 (58%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Acne</td>
<td>33 (47.8%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

### Figure 1. Cyclical pattern of women in the PCO group.

### Figure 2. AMH level in both groups.

#### 4. Discussion

Polycystic ovary syndrome is one of the commonest endocrine disorders in the reproductive age. There was no big difference between study & control in the demographic data, educational level and socioeconomic status. Obesity (BMI > 30) was higher in the PCOS group (20.3% Vs. 4.3%). Gambineri et al mentioned in his study that approximately 50% of PCOS women are overweight or obese, which is similar to our study as, 33.3% of the women of PCOS were overweight (total of 53.6%) [15]. In regards to menstrual cycle, 94.2% of the women in the PCOS group had irregular cycle. Oligomenorrhea was the main complain (85.5%), the rest complained of amenorrhea and menorrhagia. In comparison to the control group, only 8.7% had irregular cycle the result was the same as Buggs C& Rosenfield R L. 2005 whom were found that two - thirds of PCOS women have anovulatory symptoms (manifested as amenorrhea, oligomenorrhea, dysfunctional uterine bleeding or unexplained infertility [16]. Hyperandrogenism is one of the common manifestations of PCOS; 58% of the women in the PCOS group had hirsutism and 47.8% of them had acne while none of the control group had hirsutism nor acne. It’s proven that substantial intra-ovarian androgen excess appears to stimulate excessive growth of small follicles; it also causes theca and stroma hyperplasia. Eighty percent or more of cases of PCOS appear to arise from dysregulation of ovarian androgen secretion [17].

Hormonal profile was done to both groups; LH, FSH and AMH and compared between both groups. LH level was abnormal in 14.5% of the PCOS group which is a small number, on the other hand all of the women in the control group had normal LH level. FSH level was normal in both groups. Wiser et al concluded that PCOS patients with LH/FSH ratio of more than 1.5 had higher basal testosterone, E2 and AFC but decreased pregnancy rate. This could be due to the deleterious effect of LH on folliculogenesis and endometrial receptivity [18]. Ninety – seven percent of the women in the PCOS group had high level of AMH, & 4.3% of the control group. S. Lliodromiti et al concluded in his study in 2014, that the serum AMH level had a sensitivity of 76.1% and a specificity of 74.6% and AMH serum levels were significantly higher in PCOS patients than in controls [19].
AMH levels were correlated with menstrual cycle pattern, hirsutism, hormonal profile and ultrasonographic findings in women diagnosed with PCOS. It appears that only two women of the women had oligomenorrhea had normal AMH levels the rest of women who complained of irregular menstrual cycle including amenorrhea, oligomenorrhea and menorrhagia had high levels of AMH, the results was significant, P-value 0.026 (P-value 0.05). In the other hand, 93.3% of the women who had hirsute, also had high levels of AMH (P-value 0.064). Tal et al showed in his research correlating AMH with PCOS, that AMH >10 ng/ml had greater prevalence of polycystic ovarian morphology and oligomenorrhea than women with lower levels [20].

All women had high level of LH had high levels of AMH, this is similar to the finding of Tal et al as he noticed that serum AMH correlated positively with luteinizing hormone [20]. While, 95.8% of the women who had normal LH level had high level of AMH. This results were highly significant as P-value 0.030, and prove that AMH level is a better diagnostic test for the presence of PCOS than LH level.

In regards to sonographic findings, apparently, 96.2% had large ovarian volume > 10 cm (P-value 0.091), number of ovarian follicles > 12 and the follicular diameter between 2 - 9 mm was prominent in women had high levels of AMH, accounting for 95.5% and 96%; these results were also significant P-value making 0.037 and 0.026 respectively. RCT done in 2015, raising the possibility of using AMH, level as an alternative test for PCOS. Studies were collected and reviewed from the year of 2000 to 2015; it concluded that AMH levels are at least 2 - 3 fold higher in the patients with polycystic ovary syndrome, which also corresponds to the increased number of AMH producing pre-antral and small antral follicles. Moreover, AMH levels have been found to be associated in direct proportion to the follicle numbers per ovary or antral follicular count, assessed by the Trans vaginal ultrasound scan. This conclusion was similar to the study results [21].

5. Conclusion

Infertile Sudanese obese women with PCOS usually presents with irregular menstrual cycle, especially oligomenorrhea with hirsute and acne which indicate hyperandrogenism, so the classical triad in the control group are almost absent because they had normal ovaries and their androgen level is normal. FSH was normal in both groups. AMH level appears to be a better diagnostic test than LH level as, only 14.5% of the women diagnosed with PCOS had abnormal levels of LH. While, 97% of them had high level of AMH so AMH is predictive and gold standard investigation in cases of PCOS. The recommendations are that any woman complains of irregular cycle, hirsute or acne, confirmation of PCOS should be done using ultrasound scan according to Rotterdam criteria in addition LH and FSH levels can be used as screening tests for the diagnosis of PCOS while AMH level is a good predictor for ovarian reserve in PCOS and should be part of PCOS investigation.

References


