Levels of Anti-Mullerian Hormone and Follicle Stimulating Hormone in Women with and without Polycystic Ovary Syndrome

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Abstract
Sixty women with age ranges from (18-40) years have been involved in this study, divided into two groups; the first group involved 30 women with polycystic ovary syndrome (PCOS) and the second group involved 30 women without PCOS. The descriptive data [age, weight, and height] and reproductive history [age at menarche, duration of menarche, age at marriage, duration of marriage, age at first childbirth and parity] have been recorded. Blood samples were collected to determine the levels of anti-mullerian hormone (AMH) and follicle stimulating hormone (FSH). The results showed a significant (p<0.05) increase in AMH levels in women with PCOS compared with women without PCOS, while there were non-significant (p>0.05) differences in FSH levels between women with PCOS and women without PCOS.

Regarding the correlation between AMH levels and other studied parameters, a non-significant correlation was found between AMH levels on one hand and age, the value of BMI, and duration of menarche on the other hand in women with PCOS, while a significant negative correlation was found in women without PCOS. A significant positive correlation was observed between AMH levels and duration of marriage in women with PCOS, while a significant negative correlation was found in women without PCOS. A significant negative correlation was found between AMH levels and age at first child birth in women with PCOS, while a non-significant correlation was found in women without PCOS.

Concerning the correlation between FSH levels and other studied parameters, a non-significant correlation was found between FSH levels on one hand and age, duration of menarche and duration of marriage on the other hand in women with PCOS, while a significant positive correlation was found in women without PCOS. In conclusion, the measurement of serum AMH could be a useful tool for diagnosing PCOS and for assessing ovarian ageing.

Keywords: Anti-mullerian hormone, Follicle stimulating hormone, Polycystic ovary syndrome.
Introduction

Anti-Müllerian hormone (AMH), also known as Müller inhibiting factor or Müller inhibiting substance, is a glycoprotein formed from two identical subunits. This hormone is part of the growth factor family [1]. The granulosa cells within the preantral and small antral follicles in the ovaries are the sole source of AMH in humans [2]. Its production begins when follicles are recruited from the primordial pool to become primary follicles, and it ends when they reach the final size and differentiation state available for selection by follicle-stimulating hormone (FSH) [3]. Since AMH is secreted exclusively in the gonads, its serum concentrations in females are thought to reflect the size of the ovarian follicle pool [4].

Follicle stimulating hormone is a hormone that is synthesized and secreted by gonadotropes in the anterior pituitary gland. Structurally, FSH is a glycoprotein of two subunits α (92 amino acids) and β (111 amino acids) [5]. Follicle stimulating hormone is involved in the regulation of ovarian function during the menstrual cycle. It stimulates the growth and recruitment of immature follicles in the ovary. Receptors for FSH are present in the ovary and it regulates its function by supporting the production of sex steroid hormones, oestrogens and progestins, as well as folliculogenesis [6].

Polycystic ovary syndrome (PCOS) is a common hormonal disorder among women of reproductive age with a prevalence of 6.6–6.8% [7]. Symptoms are presented in varying degrees and include hyperandrogenism, acne, obesity, insulin resistance, and anovulation [8]. Although the ultimate pathogenesis of PCOS remains obscure, the distinctive feature is failure of follicular maturation, despite initial recruitment, resulting in anovulation and accumulation of preantral and small antral follicles, which contribute significantly to the production of AMH [9]. Levels of AMH reflect the number of developing follicles; their measurement may be used as a marker of ovarian follicle impairment in PCOS. Serum values of AMH could be a precise, subsidiary diagnostic marker of the syndrome, particularly in cases in which the transvaginal ultrasound examination is not feasible [10].

The present study aims to estimate the levels of AMH and FSH in women with and without PCOS, and to investigate the correlation between the levels of these hormones and the other studied parameter [age, body mass index (BMI), age at menarche, duration of menarche, age at marriage, duration of marriage, age at first childbirth and parity].

Materials and Methods

Subjects

The current study has extended from March, 2014 to September, 2015. Sixty women have been involved in this study, divided into two groups; the first group involved 30 women with PCOS during their attendance to the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies and the other group involved 30 women without PCOS.
and the second group involved 30 women without PCOS. The diagnosis of PCOS was made according to the ESHRE/ASRM criteria for the PCOS diagnosis [8].

The participants were handed questionnaire asking for descriptive data [age, weight, and height]. Body mass index was calculated as weight (kg) divided by the square of height (m²) [11]. Also, reproductive history was reported which involved [age at menarche, duration of menarche, menstrual cycle status, age at marriage, duration of marriage, age at first childbirth and parity].

**Collection of Blood Samples**

Blood samples (5 ml) were withdrawn from the participants by vein puncture during the early follicular phase (cycle day 2-4). Blood samples were kept at room temperature for about half an hour then the sera were separated by centrifugation for 15 minutes at 3000 rpm and stored at -20°C until used.

**Hormonal Assay**

Levels of AMH and FSH were measured in the serum of the participants by commercially available kits using enzyme-linked immunosorbsorbent assay (ELISA) according to [12,13]. Kit of AMH provided from diagnostic systems (CUSABIO, China), and kit of FSH provided from Human company/Germany.

**Statistical Analysis**

The Statistical analysis was done using statistical package for social sciences (SPSS), version 18, and computer software. Data were expressed as mean ± standard deviation (SD). Student’s t-test was used to compare the differences of the studied parameters between women with and without PCOS. Linear correlation coefficients and their significance were determined to study the relationship between studied variables. The level of significance was determined at P<0.05 [14].

**Results**

Descriptive and reproductive data were recorded for the participants as shown in Table-1. Non-significant (p>0.05) differences were noticed in mean value of age between women with PCOS and without PCOS. The mean value of BMI in women with PCOS (32.27 ± 4.95 kg/m²) was significantly (p<0.05) higher than that in women without PCOS (26.12± 3.24 kg/m²). Non-significant (p>0.05) differences were found in mean value of the reproductive data between women with and without PCOS except age at first childbirth which was significantly (p<0.05) higher in women with PCOS (26.44 ± 4.82 year) than that in women without PCOS (22.48 ± 3.43 year).

**Table 1-** Descriptive and reproductive data of women with and without PCOS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Women with PCOS (No. 30) (Means ± SD)</th>
<th>Women without PCOS (No. 30) (Means ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>28.94± 8.24</td>
<td>28.13± 8.72</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td>32.27± 4.95</td>
<td>26.12± 3.24</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td></td>
<td>12.80± 1.24</td>
<td>12.43± 1.56</td>
</tr>
<tr>
<td>Duration of menarche (years)</td>
<td></td>
<td>13.43± 4.67</td>
<td>14.23± 3.48</td>
</tr>
<tr>
<td>Age of marriage (years)</td>
<td></td>
<td>20.93± 3.82</td>
<td>20.75± 4.72</td>
</tr>
<tr>
<td>Duration of marriage (years)</td>
<td></td>
<td>7.36± 4.58</td>
<td>8.12± 5.23</td>
</tr>
<tr>
<td>Age at first childbirth (years)</td>
<td></td>
<td>26.44± 4.82</td>
<td>22.48± 3.43</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td>2.21± 1.82</td>
<td>2.82± 1.58</td>
</tr>
</tbody>
</table>

*Means in rows carrying similar small letters indicate a non-significant difference (p>0.05).*

*Means in rows carrying different small letters indicate a significant difference (p<0.05).*

The results in Table-2 show levels of AMH and FSH in women with and without PCOS. There was a significant (p<0.05) increase in AMH levels in women with PCOS (4.03± 1.707 ng/ml) compared with women without PCOS (1.654 ± 1.122 ng/ml), while there were non-significant (p>0.05) differences in FSH levels between women with PCOS (6.884 ± 2.912 IU/l) and women without PCOS (6.524 ± 2.231 IU/l).
en AMH levels and age of marriage in women with PCOS (r = 0.258, p>0.05); while a significant negative correlation was found in women without PCOS (r = 0.439, p<0.05). A non-significant correlation was found between the FSH levels and age of menarche in women with PCOS (r = 0.339, p>0.05). The results revealed a non-significant correlation (p>0.05) between AMH levels and age of menarche in the two groups of the present study. On the other hand, a non-significant correlation was found between AMH levels and duration of menarche in women with PCOS (r = 0.262, p>0.05); while a significant negative correlation was found in women without PCOS (r = -0.396, p<0.05).

The results showed a non-significant correlation (p>0.05) between AMH levels and age of marriage in the women with and without PCOS. A significant positive correlation was found between AMH levels and duration of marriage in women with PCOS (r = 0.457, p<0.05), while a significant negative correlation was found in women without PCOS (r = -0.343, p<0.05). Regarding the age at first child birth, the results indicated that a significant negative correlation was found between AMH levels and this parameter in women with PCOS (r = -0.530, p<0.05), while a non-significant correlation was found in women without PCOS (r = -0.181, p>0.05). A non-significant correlation (p>0.05) was found between AMH levels and parity in women with and without PCOS.

Table 2- Levels of AMH and FSH in women with and without PCOS

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Groups (Means ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH (ng/ml)</td>
<td>Women with PCOS (No. 30) 4.036 ± 1.707</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>6.884 ± 2.912</td>
</tr>
</tbody>
</table>

▲Means in rows carrying similar small letters indicate a non-significant difference (p>0.05).
▲Means in rows carrying different small letters indicate a significant difference (p<0.05).

The correlation between AMH levels and the values of other study parameters is shown in Table-3. This table indicated that a non-significant correlation was found between AMH levels and age in women with PCOS (r = 0.258, p>0.05); while a significant negative correlation was found in women without PCOS (r = -0.433, p<0.05). A non-significant correlation was found between the AMH levels and the value of BMI in women with PCOS (r = 0.051, p>0.05); while a significant negative correlation was found in women without PCOS (r = -0.311, p<0.05). The results revealed a non-significant correlation (p>0.05) between AMH levels and age of menarche in the two groups of the present study. On the other hand, a non-significant correlation was found between AMH levels and duration of menarche in women with PCOS (r = 0.262, p>0.05); while a significant negative correlation was found in women without PCOS (r = -0.396, p<0.05).

The results showed a non-significant correlation (p>0.05) between AMH levels and age of marriage in the women with and without PCOS. A significant positive correlation was found between AMH levels and duration of marriage in women with PCOS (r = 0.457, p<0.05), while a significant negative correlation was found in women without PCOS (r = -0.343, p<0.05). Regarding the age at first child birth, the results indicated that a significant negative correlation was found between AMH levels and this parameter in women with PCOS (r = -0.530, p<0.05), while a non-significant correlation was found in women without PCOS (r = -0.181, p>0.05). A non-significant correlation (p>0.05) was found between AMH levels and parity in women with and without PCOS.

Table 3- Correlation coefficient between AMH levels and other parameters in women with and without PCOS.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups (Means ± SD)</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Women with PCOS (No. 30) 0.258 NS</td>
<td>Women without PCOS (No. 30) -0.433 *</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.051 NS</td>
<td>-0.311 *</td>
</tr>
<tr>
<td>Age of menarche (years)</td>
<td>0.048 NS</td>
<td>0.113 NS</td>
</tr>
<tr>
<td>Duration of menarche (years)</td>
<td>0.262 NS</td>
<td>-0.396 *</td>
</tr>
<tr>
<td>Age of marriage (years)</td>
<td>-0.139 NS</td>
<td>-0.252 NS</td>
</tr>
<tr>
<td>Duration of marriage (years)</td>
<td>0.457 *</td>
<td>-0.343 *</td>
</tr>
<tr>
<td>Age at first child birth (years)</td>
<td>-0.530 *</td>
<td>-0.181 NS</td>
</tr>
<tr>
<td>Parity</td>
<td>-0.068 NS</td>
<td>-0.140 NS</td>
</tr>
</tbody>
</table>

* Significant differences (p<0.05).
NS: Non-significant.

Table 4 shows the correlation between FSH levels and the values of other studied parameters. This table indicated that a non-significant correlation was found between FSH levels and age in women with PCOS (r = -0.178, p>0.05), while a significant positive correlation was found in women without PCOS (r = 0.346, p<0.05). A non-significant correlation (p>0.05) was found between the FSH levels and the value of BMI in women with and without PCOS. The results showed a non-significant correlation (p>0.05) between the FSH levels and age of menarche in women with and without PCOS. Also, a non-significant correlation was found between FSH levels and duration of menarche in women with PCOS (r = -0.179, p>0.05), while a significant positive correlation was found in women without PCOS (r = 0.339, p>0.05).

The results revealed a non-significant correlation between FSH levels and age of marriage in the two studied groups. Also, a non-significant correlation was found between FSH levels and duration of marriage in women with PCOS (r = -0.121, p>0.05), while a significant positive correlation was found in women without PCOS (r = 0.439, p<0.05). Regarding the age at first child birth and parity, the
results indicated that a non-significant correlation (p>0.05) was found between FSH levels and these two parameters in women with and without PCOS.

**Table 4- Correlation coefficient between FSH levels and other parameters in women with and without PCOS.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Parameters</th>
<th>Groups</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Women with PCOS (No. 30)</td>
<td>Women without PCOS (No. 30)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>-0.178 NS</td>
<td>0.346 *</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td>-0.153 NS</td>
<td>0.072 NS</td>
</tr>
<tr>
<td>Age of menarche (years)</td>
<td></td>
<td>-0.041 NS</td>
<td>-0.139 NS</td>
</tr>
<tr>
<td>Duration of menarche (years)</td>
<td></td>
<td>-0.179 NS</td>
<td>0.339 *</td>
</tr>
<tr>
<td>Age of marriage (years)</td>
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<td>-0.100 NS</td>
<td>-0.001 NS</td>
</tr>
<tr>
<td>Duration of marriage (years)</td>
<td></td>
<td>-0.121 NS</td>
<td>0.439 *</td>
</tr>
<tr>
<td>Age at first child birth (years)</td>
<td></td>
<td>0.010 NS</td>
<td>0.168 NS</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td>-0.142 NS</td>
<td>0.080 NS</td>
</tr>
</tbody>
</table>

* Significant differences (p<0.05).
NS: Non-significant.

**Discussion**

The results of the present study revealed that there were no significant differences in age between women with PCOS and without PCOS. This finding is similar to that reported by previous studies in Iraq [15,16]. A significant increase in mean of BMI was found in women with PCOS when compared with women without PCOS. This finding is consistent with previous studies [17,16] that found a greater BMI in women with PCOS than in women without PCOS. Obesity or overweight have negative impact on the consequences of PCOS [17]. In contrast, other previous study [18] didn't report any differences in BMI value between women with PCOS and without PCOS. Obesity is one of the clinical characteristics of the PCOS, however not all obese women have PCOS and not all PCOS women are obese [19]. Regarding the other reproductive data of the participants, there were unchanged among the studied groups except age at first childbirth which was increased significantly in women with PCOS than in women without PCOS. This means that the women with PCOS have problems in getting their first child in contrast to the women without PCOS that getting their first child in smaller age than PCOS women.

The results of the current study showed that the AMH levels in women with PCOS were higher significantly than in women without PCOS, nearly three times higher. This result is in agreement with previous studies [20,15]. There are two hypotheses to explain this finding; one is that the follicles are transformed into cysts when they are at the preantral or antral stage, and remain at this stage and continue to secrete the hormone, and the other is that granulosa cells secrete a greater concentration of AMH, detectable at the follicular level [20].

Regarding the levels of FSH, non-significant differences were found between the two studied groups. Similar results were reported by other researchers [16]. Unlike serum FSH, AMH levels fluctuated very little during menstrual cycle and therefore can be taken at any times during the menstrual cycle [21]. Follicle stimulating hormone mostly reflects the last two weeks of follicular maturation when follicles become gonadotropin sensitive, while AMH is mostly representative of the young, post-primordial to pre-antral follicle pool going through earlier stages of folliculogenesis [22].

The current results showed non-significant correlation between AMH and age in women with PCOS. This result disagrees with [23] who reported that the AMH levels correlated well with age in women with PCOS; while a significant negative correlation was found in women without PCOS. This finding may be due to the fact that increase of age leading to decrease of follicles so the AMH levels decrease and also leading to ovarian impairment. This result agrees with [21] who reported a linear decline of AMH levels over time; this decrease is attributed to a decreasing number of follicles in the primordial pool. In women with PCOS, the correlation between levels of AMH and values of BMI was non-significant. This finding disagrees with that of [24] who reported a weak negative correlation between serum AMH levels and patients’ BMI. In agreement with previous studies [25], the present study revealed a significant negative correlation between levels of AMH and values of BMI in women without PCOS. The correlation between AMH and age of menarche in two studied groups was non-significant. This result is disagreement with results of [26] who showed a strong negative correlation
between the years since menarche and AMH in women with and without PCOS, indicating that the ovarian follicle pool decreases with the increase in the gynecological age. Also, [27] reported that lower AMH levels were associated with a higher age at menarche. Non-significant correlation was found between the levels of AMH and the duration of menarche in women with PCOS, this result can be due to that the women with PCOS have high AMH level so the increase of duration of menarche didn't effect on AMH levels so non-significant correlation between them; while a significant negative correlation was found in women without PCOS. This finding is logical, since increasing of duration of menarche is associated with increasing of the age of these women which had high levels of AMH. The correlation between AMH and age of marriage for women with and without PCOS was non-significant, so the age of marriage didn't effect on the levels of this hormone because of the women may be married in small age or old age. The correlation between AMH and duration of marriage was significantly positive in women with PCOS; this is because of in women with PCOS the level of AMH increase even when increasing age, so the AMH increase when duration of marriage increase; while a significant negative correlation was found in women without PCOS, this mean that the AMH levels decrease when the duration of marriage increase; this due to the fact that AMH level decrease when the women aging and the increase of duration of marriage reflect increase of age of women, so the levels of AMH decrease. The correlation between AMH levels and age at first child birth was significantly negative in women with PCOS; while it was non-significant in women without PCOS; this result is agreement with [27] who reported that there was no association between the age at first childbirth and AMH in women without PCOS. A non-significant correlation was found between AMH levels and parity in women with and without PCOS.

The results of this study revealed a non-significant correlation between FSH levels and the age in women with PCOS; while a significant positive correlation was found in women without PCOS. This result is agreement with a previous study [28] who reported a positive correlation of age with serum FSH levels, and disagreement with result of [29] who reported that FSH had a negative correlation with age. This difference could be a result of different age distributions in the studies. Non-significant correlation was observed between FSH and BMI in two studied groups. This result is disagreement with [28] who reported that the BMI had a moderate positive correlation with FSH. The results showed non-significant correlation between FSH and age of menarche in two studied groups. This result is disagreement with result of [30] who reported a significant positive correlation between FSH and age of menarche. The correlation between FSH levels and duration of menarche was non-significant in women with PCOS; while it was significantly positive in women without PCOS, this mean that the increase of duration of menarche reflect the increase of age of women so when the women ages the level of FSH increase. The results showed non-significant correlation between FSH levels and age of marriage in women with and without PCOS. Non-significant correlation was found between FSH levels and duration of marriage in women with PCOS; while there was a significant positive correlation in women without PCOS. This finding is also due to increase of duration of marriage reflect the increase of age and the FSH level increase when age increase. A non-significant correlation was found between FSH levels and age at first child birth in women with and without PCOS. The correlation between FSH levels and parity was non-significant in two studied groups. This result is agreement with result of [28] who reported no relation between FSH levels and parity in married women.

In conclusion, the measurement of serum AMH could be a useful tool for diagnosing PCOS and for assessing ovarian ageing.

References


