Abstract

Background: Polycystic ovarian syndrome (PCOS) is the most common endocrine disorders affecting 4-8% of women of reproductive age. Studies on basal & stimulated serum cortisol concentration are conflicting, unchanged, decreased or increased levels are reported. Premature ovarian failure (POF) is defined by the association of amenorrhea, sex steroid deficiency and elevated levels of serum gonadotropins before the age of 40 years. Objective: the aim of this study is to: 1. compare serum cortisol levels of patients with PCOS & POF with controls. 2. Compare serum levels of FSH, LH, PRL, testosterone, T3,T4,TSH in patients with PCOS& POF with controls. 3. Find the number and % of PCOS & POF women having low serum T3 & to compare serum cortisol, gonadotropins, & testosterone in PCOS women having low thyroid hormones with the control subjects. Patients & Methods: Fifty seven women with PCOS, their mean age was 34.2 yr, thirty women with POF, their mean age was 32.7 yr, fifty eight women, their mean age was 32 yr as controls were included in the study from Al-Batoul Teaching Hospital in Mosul( May-July 2013). After obtaining a consent, all subjects were examined clinically, blood samples were obtained for the measurement of serum morning& afternoon cortisol, serum FSH, LH, PRL, testosterone, T3,T4,TSH using ELFA. Results: the mean serum morning cortisol concentration in women with PCOS& POF was within normal although they were significantly lower than in controls. Serum testosterone was significantly higher in PCOS women & lower in POF women as compared to controls. Serum FSH was significantly higher in POF women while serum prolactin was higher in PCOS& POF women as compared to controls. Thirteen PCOS women (56.52%), ten POF women (43.48%) have low thyroid function. A significant increase in serum LH, PRL, & testosterone while a decrease in serum FSH was noted in PCOS women with low thyroid function as compared to controls. Concerning serum cortisol, there was no difference between PCOS women & controls. Conclusion: Serum morning cortisol concentration was within normal in PCOS & POF women. PCOS women with low thyroid function have altered hormonal function & further studies might be needed to confirm the relationship between ovary & thyroid functions. Key Words: polycystic ovary syndrome, premature ovarian failure.
Introduction

Cortisol is a steroid hormone, more specifically glucocorticoid produced by the zona fasiculata of the adrenal cortex (1). In humans, the amount of cortisol present in the blood undergoes diurnal variation, the level peaks in early morning (approximately 8 am) and reaches its lowest level at about midnight 4. Am, or three to five hours after sleep (2).

One of cortisol’s more important functions is to act in synergy with thyroid hormone at the receptor-gene level. Cortisol makes thyroid work more efficiently (3).

Thyroid hormones have various effects on the reproductive system of the human female, alteration in thyroid function, particularly hypothyroidism, can cause ovulatory dysfunction, the later being the leading cause of impaired female fertility (4,5,6).

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder affecting 4-8% of women of reproductive age (7), metabolic syndrome & future risk of developing diabetes (8). PCOS is diagnosed when a patient has two of these three criteria: polycystic ovaries on ultrasound, oligo and/or an ovulation, and hyperandrogenism (8).

The pathogenesis of this syndrome, first described by Stein & Leventhal in 1935. The excessive adrenal gland derived androgen secretion has led to the hypothesis that the pituitary-adrenal system in PCOS is upregulated (9,10).

Studies on basal and stimulated serum cortisol concentration are conflicting, unchanged, decreased and increased levels are reported (11, 12, 13, 14, and 15).

It has been shown that chronic hyperandrogenism and hyperinsulinemia in PCOS affect the secretion of gonadotropins in favour of increased LH, which contributes to the mechanism of anovulation (16,17).

Premature ovarian failure (POF) is a mysterious disorder. It is defined by the association of amenorrhea, sex steroid deficiency and elevated menopausal levels of serum gonadotropins before the age of 40 years. It is not a rare condition; its incidence is estimated to be as great as 1 in 100 by the age of 40, and 1 in 1000 by the age of 20 years (18).

Although most cases of POF are idiopathic, with no identifiable etiology, diverse etiologies have been associated with POF: genetic aberrations, autoimmune ovarian damage, iatrogenic factors, infectious agents, toxins and environmental factors (18). POF seriously interferes with fertility and family planning.
Three diagnostic criteria comprise POF:

- Amenorrhea lasting more than 4 months.
- Age less than 40 years.
- Serum follicle stimulating hormone > 40 mIU/ml on two occasions at least one month apart (19).

The **aims** of the study are to:

1. Compare serum cortisol level of patients with polycystic ovary syndrome and patients with premature ovarian failure with controls.

2. Compare serum levels of FSH, LH, PRL, testosterone, T3, T4, and TSH in patients with PCOS and POF with controls.

3. Find the number & the % of PCOS and POF women having low serum thyroid hormones.

4. Compare serum cortisol, FSH, LH, PRL and testosterone in PCOS women having low thyroid hormones with those in control women.

**Subjects and Methods**

One hundred forty five women were included in the study. Fifty seven women who were diagnosed as polycystic ovary syndrome (PCOS) following the criteria formulated by the National Institutes of Health in 1990 (8), their mean age was 34.2 years (range 24-47 yr) and thirty women who were diagnosed as premature ovarian failure (POF) according to the three criteria (19), their mean age 32.7 yr (range 24-39 yr), were studied as patients.

The PCOS and POF women were recruited from the IVF and fertility center at Al-Batoul Teaching Hospital in Mosul for the period from May- July 2013.

Fifty eight age matched women, mean age was 32 yr (range 20-43 yr), with regular menstrual cycle comprised the control group, they were volunteers from relatives & from the outpatient clinic.

All patients were in the reproductive age group, had no history of previous ovarian surgery, & had not received any medication that could affect adrenal hormone metabolism.

All the subjects were examined physically; medical history was taken after obtaining an informed consent. Blood samples were taken between 8:30-9 am, for the measurement of serum cortisol, FSH, LH, PRL, testosterone, T3, T4 and TSH. Another blood sample was taken at 5 pm for measurement of post afternoon serum cortisol because of diurnal rhythm. The measurement of hormones was done using Minividas & ELFA technique (Enzyme Linked Fluorescent Assay).
Patients with PCOS and POF were studied on a random day. Control women were sampled in the early follicular phase (day 2-5) of the cycle.

Statistical analysis

Data categorization & coding performed via Microsoft 2007. Descriptive & analytic statistics was carried out by using Minitab Version 16.2 software statistical program. Mean ± SD was done for measurable values.

Chi square test of independence was used for two categoral variables.

Independent t- test for two means, paired t-test and one way analysis of variance ANOVA were used where required.

p- Values ≤ 0.05 were considered statistically significant throughout data analysis.

**Result**

A total of 145 women were included in the study. Fifty seven women were infertile with polycystic ovary syndrome (PCOS), thirty were infertile with premature ovarian syndrome (POF), and fifty eight women were fertile used as controls. The mean serum morning cortisol concentration in patients with PCOS and POF were within normal range although they were significantly lower than in control subjects (Table 1).

Table 2 shows that serum LH was significantly higher in patients with PCOS as compared to its level in controls. Serum FSH was significantly higher in patients with POF as compared to patients with PCOS & controls. Patients with PCOS & POF have higher level of prolactin as compared to controls.

Concerning serum testosterone, its concentration is higher in patients with PCOS as compared to patients with POF & controls. There was no statistical difference between the three groups with regard to thyroid hormones.

There was a highly significant difference between morning & afternoon serum cortisol concentration in patient groups & controls (Table 3).

Out of 87 infertile women (PCOS& POF), 23 women (26.44%) had low serum T3 level. out of 57 patients with PCOS, 13 women (56.52%) had low T3level, & out of thirty women with POF only 10 women (43.48) had low T3( table4)

Table 5 depicts that PCOS women with low thyroid hormones had significant increase in serum prolactin, testosterone, LH, as compared with control women.

While serum FSH was lower than in controls although it was within normal values.

Concerning serum cortisol, its concentration was lower (121.9±61.3) in PCOS women.
when compared to controls (147.3±59.3) although it did not reach significance.

**Discussion**

The morning serum cortisol concentration in women with PCOS were within normal range while serum testosterone level was increased as compared to normal, this agrees with the finding of Ferdinand *et al* 2010(20), Rento&Alessandra 2012(21) although serum cortisol was significantly lower than in controls, that serum cortisol metabolism & regeneration from inactive glucocorticoids can be disrupted in PCOS, thereby contributing to determining adrenal hyperandrogenic state & the increased adrenal androgen secretion in PCOS is usually explained by amplified CRH-ACTH secretion, activated by diminished cortisol feedback signaling via increased cortisol metabolism(10).

Women with PCOS were found to have high serum LH concentration as compared to controls & this in agreement with Dunait *et al* 1992(16) and Yens 1980(17) that the hyperandrogenism & hyperinsulinemia in PCOS affect the secretion of gonadotropins in favour of increased LH which contributes to the mechanism of anovulation and the elevated concentration of LH is due to abnormal feedback by estrogen.

There was no significant differences between groups in regard to afternoon cortisol level & this disagrees with the finding of Ferdinand *et al* 2010(20) who found that the evening cortisol levels were somewhat higher in PCOS than in controls. Presently, we have no explanation for this but it may be because we measured post afternoon cortisol and not evening and night cortisol level as we cannot obtain the evening samples from the subjects and this is a limitation in our study.

The afternoon serum cortisol concentration was significantly lower than the morning serum cortisol concentration level in the three groups & this can be explained by the diurnal variation in serum cortisol level.

Deficiency of thyroid hormones has many profound end-organ effects, which also include those in the reproductive system of the human female(22).

56.52% of the PCOS women have hypothyroidism & this result agrees with the finding of Raber *et al* 2003(6).hypothyroidism may cause insulin resistance, which may lead to PCOS(23).

PCOS patients with hypothyroidism have a significant increase in serum prolactin levels as compared to controls, this finding agrees with what was found by Roberts&Ladenson 2004(22,24) who found that longstanding hypothyroidism can interfere with gonadotropin secretion by increasing serum prolactin levels.

In the present study, patients with PCOS and hypothyroidism exhibited high serum testosterone levels as compared to controls as what was found by Ghosh *et al* 1993(25) and Iptisam *et al* (24) as low sex hormone-
binding globulin (SHBG) is associated with increase in testosterone concentration & it has been claimed that changes in SHBG levels play a central role in the underlying pathophysiological mechanisms of hormonal as well as ovarian morphological changes in hypothyroidism \(^{(24)}\).

One drawback of our study is that serum SHBG levels were not evaluated; previous studies have found that hypothyroidism is associated with low serum SHBG.

Furthermore, Ghosh \(et\ al\) \(^{(25)}\), Raber \(et\ al\) \(^{(6)}\) and Janssen \(et\ al\) \(^{(26)}\) who found an increase of LH-FSH in hypothyroidism as what was found by our study.

In PCOS patients with low thyroid function, cortisol level did not differ from the control subjects & it was within normal values, this agrees with the finding of Janssen \(et\ al\) 2004 \(^{(26)}\) who found that cortisol may be normal.

Premature ovarian failure (POF) seriously interferes with fertility and family planning, POF may be either spontaneous or induced, women with POF can have autoimmune dysfunction of other endocrine organs, and the organ associations are: thyroid disease (more common), adrenals \(^{(18)}\).

women with POF demonstrated low morning cortisol levels and significantly low serum testosterone levels as compared to controls and this what was found by Norbert \(et\ al\) 2013 \(^{(27)}\) this raise the question whether this hypogonadism originates in adrenals or ovaries, while results support lower androgen levels in women with POF, the limitation is the small number of women in whom morning cortisol levels is available.

Serum FSH levels were significantly higher in POF women than their levels in controls that is explained by the ovarian follicle depletion in those patients \(^{(19)}\).

Serum LH levels were also significantly higher in patients with POF as compared to controls and this can be due to low serum testosterone level.

In conclusion, patients with PCOS have altered hormonal function and hypothyroidism and consequently, further studies might be needed to confirm the relationship between ovary & thyroid functions, there might be an interaction between them.

Recommendation: further studies for hormonal functions in PCOS women after replacement therapy with thyroid hormones, and another study in POF women with larger sample size to demonstrate the relation between serum cortisol and androgen levels.

References

Serum Cortisol in:

Serum Cortisol in:


Serum Cortisol in:

Table (1): The differences in mean morning cortisol levels between patient women and control women.

<table>
<thead>
<tr>
<th></th>
<th>Control group [n= 58]</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCOS [n = 57], 125.2 ± 65.1</td>
<td>22.1</td>
<td>0.060</td>
</tr>
<tr>
<td>POF [n = 30], 116.1 ± 68.4</td>
<td>31.1</td>
<td>0.029</td>
</tr>
</tbody>
</table>

* No significant differences between groups in regard to evening cortisol level.

** Independent t-test for two means was used.

Table (2): Comparison between mean parameters of the three groups (n= 145).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCOS [n=57] Mean±SD</th>
<th>POF [n=30] Mean±SD</th>
<th>Control [n=58] Mean±SD</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (mIU/ml)</td>
<td>8.41 ± 12.76</td>
<td>6.14 ± 3.59</td>
<td>3.86 ± 0.72</td>
<td>0.005</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>6.13 ± 3.45</td>
<td>20.46 ± 32.71 ab</td>
<td>7.18 ± 0.85 bc</td>
<td>0.001</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>28.95 ± 21.22</td>
<td>25.81 ± 17.36</td>
<td>11.45 ± 3.75 bc</td>
<td>0.001</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>1.21 ± 3.19</td>
<td>0.60 ± 0.35</td>
<td>0.26 ± 0.19 abc</td>
<td>0.017</td>
</tr>
<tr>
<td>T3 (nmol/l)</td>
<td>1.78 ± 2.77</td>
<td>1.69 ± 2.76</td>
<td>1.77 ± 0.59</td>
<td>0.984</td>
</tr>
<tr>
<td>T4 (nmol/l)</td>
<td>86.53 ± 12.95</td>
<td>85.63 ± 12.63</td>
<td>89.14 ± 14.71</td>
<td>0.433</td>
</tr>
<tr>
<td>TSH (mIU/ml)</td>
<td>2.80 ± 1.20</td>
<td>2.71 ± 1.21</td>
<td>3.04 ± 1.21 bc</td>
<td>0.395</td>
</tr>
</tbody>
</table>

* One-way ANOVA-test for more than two means with Tukey`s pair-wise comparison was used.

Table (3): The comparison between mean morning and post afternoon cortisol levels in the three groups.

<table>
<thead>
<tr>
<th>Women`s group</th>
<th>Number</th>
<th>Cortisol level (ng/ml)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Morning Mean ± SD</td>
<td>Post afternoon Mean ± SD</td>
</tr>
<tr>
<td>PCOS</td>
<td>57</td>
<td>125.2 ± 65.1</td>
<td>105.5 ± 52.9</td>
</tr>
<tr>
<td>POF</td>
<td>30</td>
<td>116.1 ± 68.4</td>
<td>106 ± 69.1</td>
</tr>
<tr>
<td>Control group</td>
<td>58</td>
<td>147.3 ± 59.3</td>
<td>103.6 ± 47.1</td>
</tr>
</tbody>
</table>

* Paired t-test for two means was used.
Table (4): Relationship between thyroid hormones and type of ovarian problem.

<table>
<thead>
<tr>
<th>Women’s group</th>
<th>No.</th>
<th>Low</th>
<th>Normal &amp; high</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>PCOS</td>
<td>57</td>
<td>13</td>
<td>56.52</td>
<td>44</td>
</tr>
<tr>
<td>Premature ovarian failure</td>
<td>30</td>
<td>10</td>
<td>43.48</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>23</td>
<td>100.00</td>
<td>64</td>
</tr>
</tbody>
</table>

* Chi-square test was used.

Table (5): The comparison in hormones between low thyroid hormones PCOS (n = 13) and control groups (n = 58).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Low thyroid function PCOS [n=13]</th>
<th>Mean ± SD</th>
<th>Control women [n=58]</th>
<th>Mean ± SD</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin (ng/ml)</td>
<td>30.5 ± 22.5</td>
<td></td>
<td>11.45 ± 3.75</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.62 ± 0.25</td>
<td></td>
<td>0.26 ± 0.19</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>8.18 ± 5.93</td>
<td></td>
<td>3.86 ± 0.72</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>FSH (Miu/ml)</td>
<td>5.90 ± 1.87</td>
<td></td>
<td>7.18 ± 0.85</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Cortisol level (ng/ml)</td>
<td>121.9 ± 61.3</td>
<td></td>
<td>147.3 ± 59.3</td>
<td></td>
<td>0.171</td>
</tr>
</tbody>
</table>

* Independent t-test for two means was used.

الملخص

الخلفية: متلازمة تكيس المبيض الأكثر شيوعا كعنصرً مرضي للعمر الجنسي للنساء في عمر التكاثر. هناك تقارير تشير إلى التضارب في تركيز الكورتيزول الأساسي والمحفز في مصل الدم، مما كثر أو قليل أو لا يتغير. يُعرف فشل المبيض المبكر بالعلاقة بين غياب الطمث، نقص المنشط الجنسي وارتفاع مستوى الأوتروستيول للجيب والهرمون اللويتي في مصل الدم قبل عمر 40 سنة.
الهدف من الدراسة: 1. مقارنة تركيز الكورتيزول في مصل الدم للنساء المصابة بمتلازمة تكيس المبايض وفشل المبايض المبكر مع نساء السيطرة.

2. مقارنة تركيز البروتينات المحفز للجيب و البروتينات المحفزة للجيب مع نساء السيطرة.

3. لمعرفة النسبة المئوية للنساء المصابات بتراكيز تركيز الكورتيزول وفق تراكيز المبتقيات للفحوصات.

المشاركون والطرق المتبعة: شملت الدراسة سبعة وخمسون امرأة مصابة بمتلازمة تكيس المبايض بعد إتمام فحوصات المتابعة لمدة عامين. وتمت تجميع البيانات من خلال البيانات الإحصائية المتوفرة.

النتائج: تختلف تركيزات البروتينات المحفزة للجيب بحسب الفحوصات. تأثرت نتائج الدراسة بالعوامل الحيوية والبيئية.

الاستنتاج: تأثرت تركيزات البروتينات المحفزة للجيب بحسب الفحوصات. تأثرت نتائج الدراسة بالعوامل الحيوية والبيئية.