Role of kisspeptin in polycystic ovary disease in patients of ALNajaf ALAshrif City, Iraq

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Abstract

Infertility is a typical disease in the reproductive system. This disease is known as the inability to deliver healthy one year after birth effectively in unprotected free endeavors [1]. About 15% of couples on the planet have a problem and are facing failure Initial involvement in pregnancy. Therefore, these husbands can be interpreted as sinners [2]. The infertility in the men may be produced from the reduce the interleukin 17 level and reduced expression of CatSper1 protein in spermatogenesis [3,4]. Polycystic ovary is a disorder in women, where the most prominent features of this disease is the increase of the hormone Androgen, problems in the process of ovulation, and the menstrual cycle irregular, and/or cysts in the ovaries or both, [5] and this disease prevents the development of follicle, a problem in ovulation, microcyte in the ovaries, and changes in the menstrual cycle, PCOS Affects more than 7% of adult women[6]. According to the Office of the National Institutes of Health and Disease Prevention, PCOS regularly affects 5 million women of reproductive age in the United States [7]. Research also suggests that women aged between 18-44 years old by 5% There will be among to 10% of the population [8]. PCOS is often diagnosed in women who they have problems with obesity, acne, menopause or delayed menstruation, excessive hair growth, infertility, and women with polycystic ovaries have higher rates of endometrial cancer, cardiovascular disease, dyslipidemia, and type of diabetes than healthy women [9,10]. Kisspeptin (formerly known as metastatin) is a protein that is encoded by the KISS1 gene in humans. Kisspeptin is a protein that binds G-GRPR54 protein receptors [11]. Kiss1 was originally identified to be of major importance in inhibiting the human genome causing malignant tumors, thus this property has the ability to suppress melanoma and malignant breast cancer. The gene kiss 1 is located on chromosome 1[12]. It is copied into the brain, adrenal gland, and pancreas, and is very expressive during gestation. In the early placenta, Kisspeptin and its receptor were also present at different sites in the kidneys, including in the blood vessels, as well as the smooth vascular muscles and renal tubular cells[13]. Kisspeptin-GPR-54 signals play an important role in the initiation of GnRH in adulthood, puberty can also be affected by a range of environmental factors, and is known to be affected by the metabolic capacity of the person[14]. The secretion hormone is released from the hypothalamus to work on the anterior pituitary gland, stimulating the release of LH and follicle stimulating hormone (FSH)[15].

INTRODUCTION

Infertility is a typical disease in the reproductive system. This disease is known as the inability to deliver healthy one year after birth effectively in unprotected free endeavors [1]. About 15% of couples on the planet have a problem and are facing failure Initial involvement in pregnancy. Therefore, these husbands can be interpreted as sinners [2]. The infertility in the men may be produced from the reduce the interleukin 17 level and reduced expression of CatSper1 protein in spermatogenesis [3,4]. Polycystic ovary is a disorder in women, where the most prominent features of this disease is the increase of the hormone Androgen, problems in the process of ovulation, and the menstrual cycle irregular, and/or cysts in the ovaries or both, [5] and this disease prevents the development of follicle, a problem in ovulation, microcyte in the ovaries, and changes in the menstrual cycle, PCOS Affects more than 7% of adult women[6]. According to the Office of the National Institutes of Health and Disease Prevention, PCOS regularly affects 5 million women of reproductive age in the United States [7]. Research also suggests that women aged between 18-44 years old by 5% There will be among to 10% of the population [8]. PCOS is often diagnosed in women who they have problems with obesity, acne, menopause or delayed menstruation, excessive hair growth, infertility, and women with polycystic ovaries have higher rates of endometrial cancer, cardiovascular disease, dyslipidemia, and type of diabetes than healthy women [9,10]. Kisspeptin (formerly known as metastatin) is a protein that is encoded by the KISS1 gene in humans. Kisspeptin is a protein that binds G-GRPR54 protein receptors [11]. Kiss1 was originally identified to be of major importance in inhibiting the human genome causing malignant tumors, thus this property has the ability to suppress melanoma and malignant breast cancer. The gene kiss 1 is located on chromosome 1[12]. It is copied into the brain, adrenal gland, and pancreas, and is very expressive during gestation. In the early placenta, Kisspeptin and its receptor were also present at different sites in the kidneys, including in the blood vessels, as well as the smooth vascular muscles and renal tubular cells[13]. Kisspeptin-GPR-54 signals play an important role in the initiation of GnRH in adulthood, puberty can also be affected by a range of environmental factors, and is known to be affected by the metabolic capacity of the person[14]. The secretion hormone is released from the hypothalamus to work on the anterior pituitary gland, stimulating the release of LH and follicle stimulating hormone (FSH)[15]. These hormones also lead to decreased sexual maturity and problems of the formation of gametes, and therefore we studied in this research the effect of infertility and infertility problems ovulation. [16] IL-37 is a newly discovered protein belong to Interleukin-1 family member 7. This protein encoded by this gene is a member of the interleukin 1 cytokine family[17]. This cytokine can bind to and maybe a ligand for interleukin 18 receptor (IL1R1/IL-1Rrp), IL-1 cytokine interleukin 37 (IL-37) acts as a natural tonic for fungal infections and acquired immunity, Low IL-37 levels increase the production of cytokines induced by transient-like receptors (TLR) in human monocytes[18]. In addition, human IL-37 recombination into wild-type mice also suppresses inflammatory cytokines and inhibits excessive inflammation, for example in arthritis[19]. Recombinant IL-37 inhibits gene expression of NLRP3 and IL-1β after acute pneumonia, Recombinant IL-37 also increases insulin sensitivity in obesity induced by diet, [20] IL-37 also showed an important effect in curbing cancer cells in renal cell carcinoma and cervical cancer , Some studies have shown that IL-37 can inhibit cancer cell migration, proliferation, and cell proliferation on apoptosis perhaps by blocking the expression STAT3 and phosphorylation , For this reason, we used the IL-37 to find out the effect of the disease on the body's immunity and its relation to infertility and can be considered a diagnostic evidence of the disease [21,22].

MATERIALS AND METHODS

Blood and serum samples were collected from Infertility women with primary and secondary infertility disease in addition To control group (healthy women) who attended Fertility Center. The average age of infertility patients was 26.97 ± 0.69 years. All samples 215 were collected (Samples with interference to other diseases were excluded) and The samples that were tested 88. Control group (fertile and healthy women ) were obtained from 18 samples and 70 samples were patients. After the hormones (LH ,FSH, Prolactin and Estradiol) and biomarker(Kisspeptin 1) were measured by ELISA method (Huma Germany origin), while lipid profile and total protein were measured by using a spectrophotometer. Tests were conducted in laboratories of Department / Faculty of Science / University of Kufa. ELISA kits used in this study were LH (LH231F), FSH (FS232F) , Prolactin (PR234F) and Estradiol (ES180S) (CALIBIOTECH company USA in Origin) and human Kisspeptin-1 ( E-EL- H2129). While lipid profile and total protein kits was BIOLABO company French manufacturer of Reagents for Medical Biology.
Statistical analysis:
Statistical analyses of all result were carried out by the help of Graphpad prism version 5)software statistical package using t-test (with p value at level of significant less than 0.05) to compare values of result between groups. Result values were expressed as mean ± SE, number of patient, or percentage.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Patient</th>
<th>Control</th>
<th>P –value (p&lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>191.4 ± 3.970</td>
<td>140.6 ± 3.532</td>
<td>Significant</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>145.7 ± 3.985</td>
<td>98.50 ± 5.766</td>
<td>Significant</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>123.2 ± 3.928</td>
<td>71.58 ± 3.735</td>
<td>Significant</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>29.09 ± 0.7955</td>
<td>19.70 ± 1.153</td>
<td>Significant</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>40.90 ± 0.9307</td>
<td>49.33 ± 1.605</td>
<td>Significant</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.13 ± 0.1429</td>
<td>11.48 ± 0.1443</td>
<td>No significant</td>
</tr>
<tr>
<td>RBC (X 10^12/L)</td>
<td>4.336 ± 0.0510</td>
<td>3.987 ± 0.0762</td>
<td>Significant</td>
</tr>
<tr>
<td>LH (mlu/ml)</td>
<td>4.598 ± 0.5028</td>
<td>3.776 ± 0.3437</td>
<td>No significant</td>
</tr>
<tr>
<td>FSH (mlu/ml)</td>
<td>5.507 ± 0.7796</td>
<td>3.188 ± 0.2313</td>
<td>No significant</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>38.44 ± 3.360</td>
<td>34.14 ± 7.147</td>
<td>No significant</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>25.41 ± 3.599</td>
<td>10.97 ± 0.7644</td>
<td>significant</td>
</tr>
</tbody>
</table>

Figure 1: level of Kisspeptin in women with PCOs:
(A) Showed compare between patient and healthy : * significant difference (p < 0.05).
(B) Showed compare between type of PCOs disease (primary and secondary)

Figure 2: level of IL-37 in women with PCOs:
(A) Showed compare between patient and healthy : * significant difference (p < 0.05).
(B) Showed compare between type of PCOs disease (primary and secondary)
RESULTS
The result showed a significant increase (p<0.05) of Kisspeptin in patients infertile women with polycystic ovary disease (1390 pg/ml ± 109.1) compared with control group (415.3 pg/ml ± 56.06) showed in figure 1 A. The result showed a significant decrease (p<0.05) of IL-37 in patients infertile women with polycystic ovary disease (36.99 pg/ml ± 3.891) compared with control group (169.3 pg/ml ± 26.65) showed in figure 2A. The result in kisspeptin and IL-37 showed slightly increased in secondary infertility when compare with primary infertility but no significant (p>0.05) between them showed in figures (1B)(2B). The result showed a significant increase (p<0.05) of Prolactin hormone in patients infertile women with polycystic ovary disease (25.41 ng/ml ± 3.599 ) compared with control group (10.97 ng/ml ± 0.7644) ,but no significant (p>0.05) between primary and secondary infertility . While no significant in LH ,FSH, E2 between patient and control , the result showed a significant increase (p<0.05) of cholesterol, TG, LDL, VLDL and total protein in primary and secondary infertile women with polycystic ovary disease compared with control group. Also the result showed a significant decrease (p<0.05) of HDL in patients infertile women with polycystic ovary disease compared with control group, showed in table (1) .The result in lipid profile(TG ,cholesterol, LDL,VLDL) showed slightly increased in secondary infertility when compare with primary infertility while in HDL slightly decrease in secondary infertility when compare with primary infertility but no significant between them. Also the result showed significant increase (p<0.05) in Hb (12.13 ± 0.1429) and RBC (4.336 ± 0.0510) in women with polycystic ovary disease when compare with controls (11.48 ± 0.1443,3.987 ± 0.0762respectively ), while no significant (p> 0.05) in WBC between patient and control. The result showed negative correlation between kisspeptin and IL-39 (figure 4) ,while positive correlation between kisspeptin and periods infertility (figure 5).

DISCUSSION
In the current study, the level of Kisspeptin-1 has a significant increase (p<0.05) in the serum of women with polycystic ovary disease compared with fertile women. The potential effect of kisspeptin in direct control of ovarian function may indicate that deregulation of the ovarian KISS1 system may participate in the ovarian phenotype, including ovulatory dysfunction and cystic ovaries (23). Gonadotropins control the ovarian expression of KISS1 as positively (24). On the other hand, local mediators also play important role in the control of ovarian KISS1 expression. All those observing suggest a possible role of locally produced kisspeptin on ovulation control. further, kisspeptin derived from ovaries have been newly suggested to play role in the adjustment of gonadotropin secretion (25,26 ).The study appeared that serum kisspeptin levels were significantly higher in women with PCO , the correlation between kisspeptin concentrations and serum FSH level were negatively correlated, this study agrees with Umit Gorkem (27). Other study showed The levels of kisspeptin were high in PCO disease and these levels had a positive relationship with LH levels. There are some data related to changes in kisspeptin levels and its relationship to metabolic and hormonal disorders in PCO disease (28). Some other studies confirmed higher kisspeptin levels in women with PCOS, in agreement with this study (29,30). Present study also understand that women with PCOS have higher levels of LH than ovulation without PCOS, this agree with Umit Gorkem (27).however, there is a need for other studies to obvious whether this phenomenon might play a role in the deregulation of gonadotropin secretion in PCOS. It appears possible that the human female kisspeptin system could

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y = -0.0027x + 40.786
R^2 = 0.0059
\]

Figure 4: The correlation between kisspeptin-1 and IL-37 in women with polycystic ovary.

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y = 0.0009x + 3.6255
R^2 = 0.0559
\]

Figure 5: The correlation between kisspeptin-1 and fertility period in women with polycystic ovary.
be a helpful therapeutic target in PCOS patients, Experimental studies have proposed that the expression hypothalamic KISS1 was sensitive to variation in the steroid environment and metabolism (23). On a functional level, sexual dimorphism is fully characterized by the menstrual cycle. LH surge is the more significant characteristic of this. Normally, the positive feedback of estrogen leading to create LH-peak. Kisspeptin relays this influence to the GnRH neurons, and thus to the LH cells (31), in our data showed higher kisspeptin in women with secondary polycystic ovary disease when compare with primary polycystic ovary disease. In the current study, the level of interleukin 37 has a significant increase (p < 0.05) in the serum of women with polycystic ovary disease compared with fertile women. IL-37 protein is present fundamentally in the cytoplasm of peripheral blood mononuclear cells (PBMC) and constitutively at low levels in normal people and can be upregulated by cytokines and inflammatory stimuli (32). The data in the present study confirm that PCOS is a proinflammatory state, IL-37 are recently discovered anti-inflammatory cytokines (33,34). Levels of cytokines (IL-37) were significantly decreased in obese and non-obese PCOS patients compared with obese and non-obese controls. These findings seem to be in line with the case that PCOS is a proinflammatory state because there shows to be an imbalance between pro and anti-inflammatory mediators in patients with PCOS. However, the increase in pro-inflammatory cytokines and referred to obesity, because there appears to be a strong association between inflammation and PCOS. Hence, inflammation seems to be affected both by obesity and PCOS. Although IL-37 is newly discovered from IL-1 family and according to results and studies it has been shown to decrease in women with PCOS, there is an inverse association with IL-1, according to studies, indicating that IL-1 is elevated in women with PCOS (35). IL-1 levels in the serum of LH (p < 0.0001) and significant decline in the serum level of FSH (p < 0.05) in infertile women with PCO in comparison with control group. Study the changes in the levels of LH (elevation) and FSH (decline) in PCO were recorded by many studies and considered as a characteristic feature of PCO (37,38). Low levels of gonadotropin can be refer to the increased production of androgen in PCO. Increasing the level of LH may directly promote the synthesis of androgen. However, it has been suggested that the height of LH levels result from weak negative reactions to LH secretion, due to excessive androgen action on the pituitary axis (39). Our results showed a significant elevation in the serum level of LH (p < 0.0001) and significant decline in the serum level of FSH (p < 0.05) in infertile women with PCO in comparison with control group. In this study the frequency of hyperprolactinemia in PCO has increased in comparison with control. When a single blood sample was taken from the patient, 40% of PCO patients had hyperprolactinemia blood (40). Further, this study data indicate that hyperprolactinemia in PCO patients reflects just increased estrogen uptake since they have demonstrated a gradual increase in circulating estrogen levels. Prolactin Basically, this phenomenon is related to the increase in estrogen alone, since it was not possible to show statistically correct variations of estradiol. Taking into account that estrogen stimulates the Prolactin secretion (41), it can be assumed that hyperprolactinemia was found in patients who have greater secretion of estrogens, especially esters. Further, our data don’t allow us to determine the development of estrogen in such patients because estrogen is largely the product of peripheral aromatase conversion. Which can be produced from the ovary and adrenal glands (42). In our data no highly significant between primary and secondary polycystic disease. In many study to note the effects of polycystic ovaries on the lipid profile, total cholesterol, TG, and LDL levels were statistically higher, in our data higher in secondary polycystic disease compare with primary polycystic disease, and HDL was lower in PCOS patients than healthy females with close proximity to age groups, in our data lower in secondary polycystic disease compare with primary polycystic disease. In another study similar to those observed in PCOS patients. Furthermore, similar results were found in some studies that PCOS patients were had high level in lipid profile such as high total cholesterol, LDL, TGs concentrations and low levels of HDL control (43,44,45). These studies are consistent with our results. Our results showed a significant elevation in the serum level of viscosity (p < 0.05) in infertile women with PCO in comparison with the control group. Increased viscosity of blood observed in young women with PCOS is clearly an early detection marker that contributes to cardiovascular risk (46). The study concluded that the length of the period of infertility, the significant increase in the level of kisspeptin and some of the biochemical and physiological parameters measured in this study may provide an indication of increased infertility in women with ovarian disease.

**References**

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