Comparison of Serum Leptin Level in Women with Unexplained Infertility and Fertile Women in Iran

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About the Author

Dr. Farzaneh Tafvizi has got her Ph.D. in November 2009 in Cellular and Molecular Biology. She has joined Azad University as Assistant Professor from 2010. She has conducted 40 M.Sc. theses and published more than 70 articles in esteemed biological journals and conferences. Her fields of interest are cellular and molecular biology and genetics.

Abstract

Background Various studies have been conducted on the role of leptin in the different types of infertility, including endometriosis, polycystic ovaries and male infertility; however, only a few studies have been conducted on the effect of leptin on unexplained infertility. The purpose of the present study was therefore to compare serum leptin levels in women with unexplained infertility and fertile women as a contributing factor to female infertility in Iran.

Methods The present case–control study was conducted on 40 women with unexplained infertility and 30 fertile women who met the inclusion criteria (FSH < 10, BMI < 30, normal AMH and AFC and age under 35). Serum leptin levels were analyzed on the third day of menstruation by ELISA method.

Results Although the mean serum leptin level was higher in women with unexplained infertility (31.20 ± 2.83 ng/ml) than in fertile women (24.89 ± 2.93 ng/ml), no statistically significant differences were observed between the two groups ($P = 0.13$).

Conclusions No statistically significant relationship was found between serum leptin levels in the two groups. In addition, there were no significant relationships between leptin and other predisposing factors such as hormone profile and antral follicle count.

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Keywords Leptin · Unexplained infertility · ELISA method

Introduction

Reproduction is a complex process relying on the interactions between the anatomical structure and the male and female normal reproductive function; any factor that disturbs it has the potential to cause infertility [1].

Sterility is not achieving pregnancy after a year of unprotected sexual intercourse, which has a prevalence of about 10–15 % in couples in their reproductive ages [2]. Unexplained infertility refers to cases in which the standard diagnostic procedures available do not lead to a specific cause for the infertility. This problem highlights the limitations of our knowledge about the mechanisms controlling human fertility [1]. Various reasons such as mild defects in spermiogram, slight hormonal changes and underlying pathophysiological mechanisms are involved in unexplained infertility [3]. Leptin is a hormonal factor affecting ovulation and the ovary through both paracrine and endocrine actions [4]. It also plays a key role in energy homeostasis and body weight regulation. This substance acts as a neuroendocrine mediator in different systems of the body such as the reproductive system [5]. Neuropeptide leptin is 16 kDa in weight and has 167 amino acids coded by the *ob* gene. The *ob* gene is responsible for obesity in mice, and its equivalent gene in humans is called *LEP*. This hormone reduces glucose oxidation and fat synthesis and increases fat intake [2]. Leptin is mostly secreted from white adipose tissues (adipocytes) and to a lesser degree from other cells such as epithelial gastric cells, placental syncytiotrophoblast cells, hypothalamus, pituitary gland, skeletal muscle, breast epithelium, ovarian follicles and bone marrow [6].

Given the widespread distribution of leptin receptors along the hypothalamic–pituitary–gonadal axis, the regulatory effects of leptin on reproduction depend on a complex set of functions that act through the hypothalamic–pituitary axis and also directly affect target organs such as the placenta and the mammary glands and have major physiological effects on puberty, menstrual cycle, pregnancy, lactation and early embryo development. Other peripheral functions of leptin include the regulation of insulin secretion, reproductive functions, angiogenesis, immune responses, glucocorticoids synthesis and glucose metabolism. The central effects of leptin include the regulation of appetite, temperature, weight and metabolism in the hypothalamus [5].

As for the relationship between leptin and the ovary, high concentrations of leptin were shown to inhibit estradiol and progesterone production and prevent the progression of mature follicles. Leptin is also produced by the ovary, and estradiol has a stimulatory effect on its production. In low concentrations, leptin has stimulatory

effects, and in high concentrations, it has inhibitory effects on gonadotropins [4, 5].

The exact role of leptin in the ovary and in human physiology and pathophysiology requires further investigations. In some studies, high levels of serum leptin concentration were reported to be involved in infertility [7], while in some others, the increase in serum leptin levels was noticed in both fertile and infertile groups and high serum leptin levels in these patients were not a contributing factor of infertility [8]. High serum leptin levels reduce gonadotropin secretion, estradiol production and follicle growth [9]. As the role of leptin has been proven in fertility, several studies have been conducted on the role of leptin in different types of infertility, such as endometriosis and polycystic ovary; however, only a few studies have been conducted to date on the effects of leptin levels on unexplained infertility. The present study was therefore conducted to compare serum leptin levels in women with unexplained infertility and fertile women.

Materials and methods

The present case–control study was conducted after obtaining written informed consent from qualified patients interested in participating in the study. The case group consisted of 40 women with unexplained infertility. Patients with unexplained infertility were chosen from the individuals who had tried but were unable to conceive for a period of at least a year, who had regular menstruation, whose tubes were open and whose uterus cavity was normal in size according to the hysterosalpingography (HSG). In addition, their husbands also had a normal spermiogram (with a concentration of at least 15 million per milliliter, a motility value over 40 % and a morphology of more than 4 % with normal forms).

The control group consisted of 30 normal subjects who had at least one healthy 2-year-old child (end of lactation). The transvaginal ultrasound was performed for both groups during their follicular phase (the third day of the cycle), and in case at least 5 antral follicles (AFCs) were observed in each ovary, lutein hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), prolactin (PRL) and anti-Müllerian hormone (AMH) measurements were taken on the same day through the ELISA method at Valiasr laboratory, and patients with a normal hormone profile ($FSH < 10$ and $AMH > 10$) finally entered the study.

Those with a history of cardiovascular diseases, cerebrovascular diseases, hypertension, diabetes, thromboembolism, or any specific systemic diseases, gynecologic disorders such as uterine myoma, endometriosis, uterine polyps, ovaries appearing polycystic on ultrasound, acute

or chronic infections, a family history of high blood lipids, obesity, $\text{BMI} \geq 30$, abnormal color image of the uterus, irregular menstrual cycles, receiving medication therapy, recurrent pregnancy loss (RPL), smoking, ovarian tumors, age above 35, husbands' abnormal spermiogram, abnormal hormone profile and having been under hormone therapy for the past 2 months were excluded from the study [3].

The data collection instrument was a questionnaire including information such as age, duration of infertility, menstrual cycle history, history of exercise over the past 3 months (indicating exercise at the gym or at home for at least half an hour, 3 days a week), the color image of the uterus and the husbands' spermiograms and also biochemical parameters of the blood such as FSH, LH, PRL, TSH and AMH. On the third day of the next menstrual cycle, between 8 and 9 a.m., blood samples were taken from the patients' cubital arteries at Valiasr laboratory. After the blood sample collection, samples were centrifuged for 5 min at 3000g; in the next step, their serum was separated and frozen at -70°C . After finishing with the samples, leptin levels were measured by the sandwich ELISA method (DRG Leptin sandwich ELISA, Germany) according to the protocol [3].

Statistical analysis

The results were analyzed in SPSS 19 software. Statistical assessments were conducted using descriptive statistics including the Chi-square test, the t test and the regression analysis. The results obtained were presented as mean $\pm$ SD, and the difference was considered significant at the $P < 0.05$ level.

Results

Demographic Variables and Biochemical Parameters

The results of measuring and comparing the FSH, LH, AMH, PRL, TSH, AFCs count, mean age and BMI between the infertile group and the controls are presented in Table 1. There were no statistically significant differences between the two groups.

In terms of exercise, 7 subjects (17.5 %) in the infertile group and 12 subjects (40 %) in the fertile group were physically active; however, the Chi-square test did not show the difference to be significant between the groups ($P = 0.056$). The subjects were divided into two groups based on BMI, a 18–25 BMI group and a 25.1–30 BMI group; the majority of the women (35 individuals or 87.5 %) from the unexplained infertility group and 25 (83.5 %) from the fertile group were assigned to the 18–25

BMI group and therefore fell in the normal BMI range. The statistical t test did not show the difference to be significant.

Serum Leptin Levels

After ensuring the two groups' homogeneity, the next step was to evaluate serum leptin levels in both the case group and the control group. As shown in Table 1, mean serum leptin levels were 24.89 ± 2.937 (ng/ml) in the normal group and 31.20 ± 2.835 (ng/ml) in the infertility group. Statistical analyses showed no significant differences between the two groups ($P = 0.133$) (Table 1).

Correlation Between Leptin and the Other Variables

Multiple regression analysis is used in examining the relationship between leptin and other underlying factors, including hormonal profile, age and antral follicles, and no significant correlation was found between leptin and these factors in unexplained infertility group. But significant correlation was observed between BMI and leptin levels in the fertile group ($P = 0.003$). Also, serum leptin levels in fertile women were associated with sports activities ($P = 0.05$): Increased physical activity reduced leptin levels (Table 2).

Discussion

Leptin is a neuroendocrine mediator known to act in various systems of the body, including the reproductive system. Leptin affects the reproductive system at different levels of the hypothalamic–pituitary–gonadal axis and has major physiological effects on puberty, maintaining a normal menstrual cycle, lactation and even the newborn's physiology. With poor nutrition or in conditions inducing amenorrhea, serum leptin levels are low, and, on the contrary, in conditions of excessive stored energy such as obesity and also with a polycystic ovary syndrome, serum leptin levels and the follicular fluid are increased and make it possible for leptin deficiency or leptin resistance to contribute to reproductive abnormalities [5].

Several studies have reported the relationship between leptin and infertility; however, few studies have examined the relationship between leptin and unexplained infertility.

Takeuchi and Tsutsumi [10] investigated the role of leptin in ovary dysfunction and showed that leptin levels are associated with the BMI and also do not appear to be directly involved in the regulation of ovarian function.

Wertel et al. [8] and Gogacz et al. [11] investigated serum leptin levels in infertile patients belonging to three

Table 1 Comparison of the examined variables (demographic variables and biochemical parameters of the blood) in the case and control groups

Variables	Unexplained infertility group	Fertile group	<i>P</i> value
Age (year)	29.30 ± 4.20	31.03 ± 3.76	0.07
BMI (m/kg ²)	23.60 ± 0.27	23.66 ± 0.33	0.87
FSH (MIU/I)	4.57 ± 0.255	4.88 ± 0.305	0.43
LH (MIU/I)	3.88 ± 0.25	3.49 ± 0.30	0.19
AMH (ng/ml)	2.98 ± 0.17	3.03 ± 0.15	0.82
PRL (MIU/I)	9.71 ± 0.81	9.58 ± 0.990	0.92
TSH (MIU/I)	2.15 ± 0.15	2.26 ± 0.15	0.60
Leptin (ng/ml)	24.89 ± 2.937	31.20 ± 2.835	0.13

Table 2 Correlation between leptin and the others variables in both the case and control groups

Factors	<i>P</i> value in unexplained infertility group	<i>P</i> value in fertile group
BMI (m/kg ²)	0.09	0.00
Age (years)	0.57	0.21
LH (MIU/I)	0.80	0.99
FSH (MIU/I)	0.47	0.14
TSH (MIU/I)	0.37	0.76
PRL (MIU/I)	0.22	0.20
AMH (ng/ml)	0.35	0.56
R AFC	0.53	0.04
L AFC	0.97	0.70
Sport	0.69	0.05

different groups, the unexplained infertility, the endometriosis and the polycystic ovary groups. Serum leptin levels were reported to be similar in the three groups. BMI similarity in the three groups was noted as the main reason for their lack of difference in leptin levels. Demir et al. [3] compared leptin levels in two groups, the group of women with unexplained infertility and the group of fertile women. Despite the two groups' similar BMIs, this study found serum leptin levels to be significantly higher in the group of women with unexplained infertility than in the group of fertile women.

In the present study, mean leptin levels were higher in women with unexplained infertility than in fertile women, though in terms of BMI, the majority of them belonged to the normal group. Studies conducted by Shafi and Afzal [12] showed that obesity, which is accompanied by increased leptin, leads to infertility, since high leptin levels have an inhibitory effect on the growth of immature follicles and prevent oocyte maturation.

In the present study, no significant correlation was found between leptin and BMI in infertile group.

Hardie et al. [13] studied the leptin cycle during the different phases of the menstrual cycle and pregnancy and showed that serum leptin levels have a direct correlation with

body fat percentage and BMI and that serum leptin concentration are higher in the obese than in the underweight although leptin concentrations are not very different in their cerebrospinal fluid. Given the relationship between leptin and BMI, the present study is consistent with the views argued by Shafi and Hardie. In women, obesity and consequently increased leptin are associated with menstrual dysfunction, decreased fertility, increased risk of miscarriage and obstetric and neonatal complications; in men, obesity is associated with abnormal semen parameters and can have adverse effects on fertility; therefore, couples trying to conceive are recommended to get to a BMI of 20–25 [2].

According to the results of the present study, a significant indirect correlation was observed between leptin and exercise in control group, and patients with a history of regular exercise had significantly lower serum leptin levels.

Studies show that 8 weeks of aerobic exercise during the follicular phase of menstruation significantly decreases plasma leptin in obese women [14].

Ozcelik et al. [15] investigated the effect of aerobic exercise on leptin concentrations in obese women and found that leptin concentrations significantly decrease after exercise and body fat mass also decreases.

It therefore appears that leptin levels are lower in individuals adhering to regular exercise compared to other individuals given the reduced body fat and despite having a normal weight, because leptin is secreted from the adipose tissue and decreased adipose tissue decreases leptin levels.

It is therefore essential to combine the effects of exercise and hormones in obese women with unexplained infertility. Exercise also affects certain hormone concentrations such as insulin, cortisol and testosterone, which can influence leptin concentrations [16]. Understanding the role of leptin in the physiology and pathophysiology of the human reproductive process requires further studies in this area. To obtain more accurate results, another study should be conducted on patients with unexplained infertility who have a BMI > 30 and its relationship with other predisposing factors should then be assessed [17, 18].

The mean serum leptin levels between the group of women with unexplained infertility and the group of fertile women showed no statistically significant differences; however, the results showed that the serum leptin level is associated with BMI and physical activity in normal group. The disparity of results reported by different studies examining serum leptin might be due to the variations between sample sizes studied, the use of different kits and differences in the dietary customs and lifestyles of different nations.

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Authors' contribution Farzaneh Tafvizi contributed to project development, data collection and manuscript writing. Masomeh Masomi collected and analyzed data.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval The present case–control study was conducted after obtaining written informed consent from qualified patients interested in participating in the study.

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