Original paper

The Effect of Serum Leptin Level on Subfertility in Patients with Polycystic Ovaries at Different Body Weight Groups in Al-Najaf Province

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Abstract

Background: Subfertility is defined as a disease where couples cannot conceive after 12 months of unprotected intercourse. Adipose tissue is a functional endocrine-like organ that secretes various adipokines (mainly leptin); these adipokines control a wide range of different systematic reactions. Leptin was believed to be released by different cell types (mainly by adipocytes), controlling energy stores and reflecting body fat stores. Elevated serum level of leptin was found to impair oocyte development especially in females with polycystic ovaries **Aim of the study:** Determine the association between serum leptin level and subfertility in women with polycystic ovaries.

Material and methods: A case-control study was done during the period from September 2020 to January 2021. The study includes 88 women, their ages ranging from 18-40, and their BMI ranging between (18.5-40) Kg/m². Forty-four women were subfertile with polycystic ovaries, and the other 44 women are fertile with normal ovarian texture. For both groups, leptin levels were measured.

Result: There is a significant association ($p \leq 0.05$) between serum leptin levels and subfertility, especially in obese females presented with polycystic ovaries compared to the females with normal ovarian texture (control group). This reveals the intimate relationship between impaired reproductive function and increase body fat content that are mainly related to polycystic nature of the ovaries.

Conclusions: The significant association between serum leptin levels and reproductive capacity in females with polycystic ovaries at different bodyweight groups points to the important function of this adipokine in regulating female fertility.

Keywords: Leptin, Polycystic ovaries, Subfertility

Introduction

In the human female, the main reproductive organs consist of the ovaries, two fimbriated oviducts, and a uterus in addition to a vagina [(1)].

Reproduction starts when the oocyte begins to develop and acquire maturity [(2,3)]. *According* to the American Society of Reproductive Medicine Committee, subfertility is defined as a disease that couples cannot conceive after 12 months of unprotected intercourse. The fertility capacity is shown to be decreased with the aging process, especially those who are equal or more than 35 years, so in female patients ≥ (35-40) years old, *subfertility* is defined as the inability to conceive after six months of regular unprotected intercourse; in females aged more than 40 years, fertility evaluation was recommended after three months of regular intercourse. However, in females that had a known defined cause of subfertility, immediate management is mandatory[(4)]. Many factors harmonize to modify female fertility; some are concerned with the dysfunction of ovaries and other reproductive organs, while others are

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related to many environmental and life-style derangements [5]. Subfertility is an expanding problem in the world, influencing about 10-15 percent of the population. The term subfertility is often used instead of infertility because many partners were not infertile but showed a decrement in reproductive capacity and can have a child after fertility treatment [4,6]. The major controller of reproduction and fertility is adipose tissue, which is regarded as an endocrine tissue by releasing various soluble tissue cytokines and adipokines concerned mainly with hypothalamic-pituitary-ovarian axis regulation [5]. Obesity is a chronic inflammatory process associated with an increment in the synthesis and secretion of various adipokines, including leptin. Approximately 30 to 70 % of females that are demonstrating ultrasonographically defined polycystic ovaries were found to be obese [7]. Leptin is one of the earliest adipokines that had been noticed as an endocrine regulator secreted by adipocyte and concerned mainly with energy homeostasis. It is a 167 amino acid peptide, encodes the obesity gene. It has also been found in large amounts in the placenta, hypothalamus, pituitary, ovarian thecal, and granulosa cell in addition to endometrial distribution [6]. These distributing sites reflect their interactive role in controlling appetite and reproduction by multiple paracrine and endocrine factors [8]. Leptin usually exerts its action via leptin receptors. Leptin secretion is correlated with adipose tissue mass mainly (abdominal fat). In a physiological background, leptin is found to inhibit food intake, and at the same time initiate, energy utilization through its action on the hypothalamus. Excess serum leptin level (mainly seen in abdominal obesity) has been implicated in long-term down-regulated leptin receptors in the hypothalamus. In addition to its central action, leptin has been found to modify the steroidogenic signaling pathway in the ovaries via decreasing estrogen and progesterone manufacturing and release by ovarian granulosa cells. An increment in serum level of leptin was implicated in the inhibition of steroidogenesis (i.e., suppressing estradiol formation) and impairing oocyte development and maturation. The unique bimodal effect of leptin depends on its serum concentration, revealing its regulatory effect on the gonadal tissues at a higher serum level (mainly found in obese females) [6]. Females with polycystic ovaries usually have a high body mass index; each of them expresses a deleterious effect on human fertility [9]. Several factors regulate leptin levels in the blood, including excess body fat (obesity), satiety and overfeeding, Glucose and Glucocorticoids, Estrogen, and IL6, which can stimulate leptin secretion. Others act to inhibit leptin secretion, like in a state of decreased body weight and fasting, Catecholamines and adrenergic stimulation, TSH, and Androgens [10]. Polycystic ovarian syndrome (PCOS) is an endocrine disorder characterized by the presence of different forms of menstrual irregularities, anovulatory infertility, and many skin disorders like acne, excessive terminal hair production, which all are attributed to androgen excess; hyperinsulinemia also is common. The Rotterdam Consensus defined PCOS by the presence of ovulatory dysfunction, polycystic ovarian morphology in addition to hyperandrogenism (clinical or biochemical). At least two of these three criteria must be found to establish its full diagnosis. Adipokines (which include leptin mainly) produced by the fatty tissues were implicated in the pathogenesis of this syndrome [9,11]. Polycystic ovary (PCO) is a morphological abnormality defined during female reproductive life. Polycystic ovaries, however, can affect female reproductive capacity, although it is concerned with the ovaries; its involvement in adipose tissue redistribution and obesity has been defined. Unfortunately, the precise pathophysiological processes that have been involved in Polycystic ovary development are obscured. Genetic factors, in addition to other environmental issues that have been implicated. It has been found that polycystic ovaries are equally

distributed in a non-obese and obese female[12]. The criteria that are used for the diagnosis of polycystic ovaries depends on ultrasonographic identification of polycystic ovarian morphology that met Rotterdam 2003 criteria established by ESHRE/ARSM society which includes the presence of more than 12 follicles, each of them measured 2-9 mm with/without an ovarian volume of more than 10 ml3 that examined during the follicular phase[13].
This study aims to find the association between serum leptin levels and reproductive capacity in females with polycystic ovaries at different bodyweight groups.

Subjects and Methods

A case-control study design was done in the period from September 2020-February 2021. Study sample include 88 females. The cases include 44 patients out of total presented with subfertility and polycystic ovaries. The remaining (44 females) are fertile women who have normal ovaries considered as a control. The age of these women ranges between (18-40) years, and their BMI range between 18.5-40 kg/m² [14] . These females were divided into three subgroups according to their BMI in Kg/m^2. Normal body weight with BMI (18.5-24.9), overweight with BMI (25-29.9), and obese BMI (≥ 30) according to Nuttall FQ (2015) [15]. All females selected were not using hormonal contraceptives for less than or equal to two menstrual cycles, not using any drugs that might affect blood glucose, lipid, and androgens levels at least three months before the study. Out of the total, 44 females with ultrasonographic defined polycystic ovaries met the Rotterdam criteria (presented with subfertility, either primary or secondary) defined as (group A). The other 44 females (control group) involving fertile females with matched age and body weight presented with normal ovarian morphology denoted as (group B); these females attend the clinic for periodic medical examinations. Each patient was subjected to the same clinical, biochemical, and ultrasonographic assessments. All the females included in the study have been randomly selected from the outpatient gynecology clinic at Al-Najaf city/Iraq.

Blood Samples collection

Two milliliters of venous blood were drawn in the early days of menses (menstrual cycle day 2-5) and distributed in a gel tube using a sterile disposable needle and plastic syringes from both groups, the cases, and the control. The blood sample was collected and permitted to clot for about 15 minutes, then the blood centrifuged in about (3000 round /minute) for five minutes using a centrifuge (EBA 20S). After that, the Serum for measuring the hormone leptin was collected and separated, transported into a new disposable tube, frozen, and stored at minus 20 degrees centigrade until complete samples collection before analysis.

Biochemical analysis

Serum level of leptin analyzed using 'Bio Teck USA type ELISA' system. Human-type kits from (Elabscience-USA) were applied for the detection of serum leptin levels in the blood. The precise instruction of the manufacturing source was applied to analyze these two hormones.

Ethical consideration

An informed voluntary consent was obtained from the participant (the cases and control). All the participant fully informed about what will asked, how the collected data will used with the importance of keeping their personal data in private. A full explanation about the procedure of blood drawing and sampling was applied. The participants were informed about their rights and have the right to withdraw at any time they would with a clear explanation on the aim of the research.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for the Social Science) program version 20. Categorical variables were presented as frequencies and percentages, while continuous variables were presented as mean and SD. Shapiro - Wilk test was significant for leptin, which indicated that they were not normally distributed, so the

Mann-Whitney U test was used to compare the cases and controls regarding subfertility and BMI. The data were reported as mean, standard deviation (SD) for every variable. Statistical significance was assumed as P ≤0.05.

Result

Table 1 shows no significant differences between the cases and the control regarding different BMI, P- value > 0.05

Table2 and Figure 1 Show a significant association regarding serum leptin levels in a subfertile patient with PCO compared to the fertile females without PCO.

Table 3 and Figure 2 show a significant association of serum leptin level with a different BMI between subfertile and fertile females. A significant association of serum leptin level was identified in females with normal body weight compared with a matched BMI control. In the second category of overweight patients, a significant association between subfertile females with polycystic ovaries and the control, a significant serum leptin elevation was also identified in obese patients compared to weight-matched fertile females.

Discussion

The ovary is a dynamic-ever-changing organ, which falls under a regulatory system involving the hypothalamic, pituitary, ovarian feedback signals. Gonadotropin-releasing hormone (GnRH) released by the hypothalamus will ultimately stimulate the ovaries to produce the sex hormones (estrogen and progesterone). Leptin can control the reproductive capacity by reflecting the amount of stored energy in the body to gonadotroph neurons. Higher leptin concentration will disrupt the pulsatile gonadotropin secretion, participating in the development of polycystic ovaries via increasing GnRH production [16]. Leptin act as a peripheral signal in regulating many reproductive functions like gametogenesis,

steroidogenesis in the ovary.

Table 1. Anthropometric data for subfertile and fertile females.

		Subfertile females with PCO (n=44)	**Fertile females without PCO** (n=44)	**Total**	**P-value**
BMI(Kg/m²)	**Normal (18.5-24.9)**	9 (20.5%)	9 (20.5%)	18 (20.5%)	0.99
	Over weight (25-29.9)	13 (29.5%)	13 (29.5%)	26 (29.5%)	
	Obese (≥30)	22 (50.0%)	22 (50.0%)	44 (50.0%)	

*Significant P-value is less than or equal to 0.05

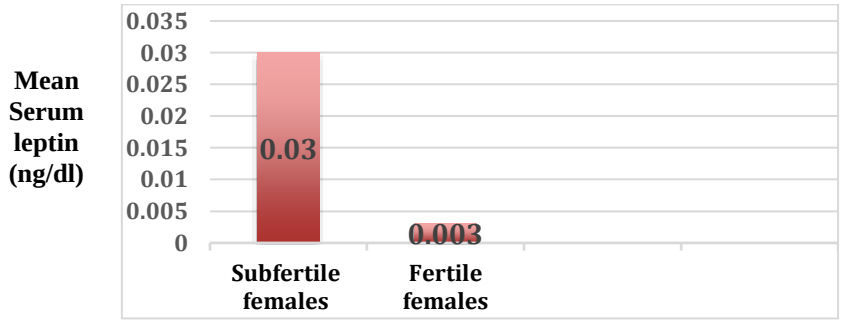

Figure 1. Mean of Serum leptin level in subfertile women with polycystic ovary and fertile females with non- polycystic ovaries.

Table 2. The association between serum leptin level and subfertility in females with PCO compared to fertile females without PCO.

Group	Subfertile females with PCO (n=44) (mean±SD)	Fertile females without PCO (n=44) (mean±SD)	P-value
Leptin(ng/dl)	0.03±0.02	0.003±0.002	< 0.001

*Significant P-value is less than or equal to 0.05

Table 3. Association between serum leptin level and different body weight groups in subfertile females with PCO and fertile females without PCO.

		Subfertile females with PCO Leptin(ng/dl) (n=44) (mean±SD)		**Fertile females without PCO Leptin(ng/dl)** (n=44) (mean±SD)	**P-value**
BMI (Kg/m²)	**Normal (18.5-24.9) (n=9)**	0.04±0.02	**Normal (18.5-24.9) (n=9)**	0.002±0.001	< 0.001
	Over weight (25-29.9) (n=13)	0.02±0.01	**Over weight (25-29.9) (n=13)**	0.002±0.002	< 0.001
	Obese(≥30) (n=22)	0.04±0.02	**Obese(≥30) (n=22)**	0.003±0.002	< 0.001

*Significant P value is less than or equal to 0.05

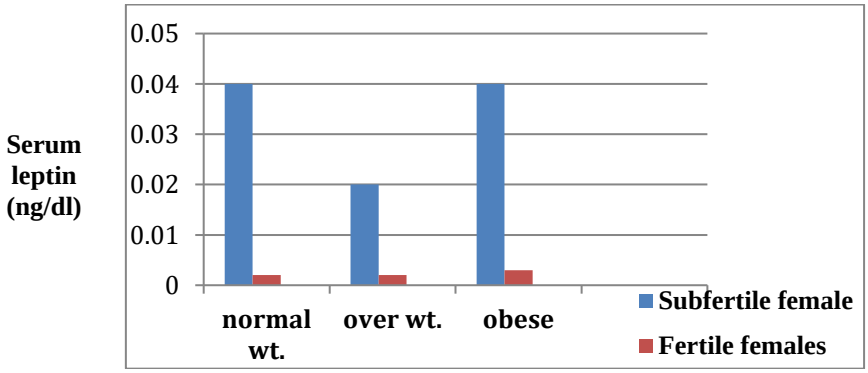

Figure 2. Levels of leptin in subfertile females in comparison to the fertile females. Column no.1 compares subfertile and fertile females with normal body weight, column 2 compares women with overweight, and column 3 compares obese women in both groups**.**

It is an important adipokine and regarded as a link between the degree of nutrition and reproduction [17]. Leptin was found to be implicated in growth and development by regulating energy storage and sexual hormonal production [18]. Adipokine dysregulation has been commonly seen in obese and overweight females, in addition to females with polycystic ovaries [19]. Abnormalities in adipokine production and release are commonly found in females with polycystic ovaries. Our results show a significant increment in serum leptin level in females with PCO compared to the non-PCO females independent of BMI, as shown in table 2. Jalilian and Nomair found the same result [20,21]. Olszanecka-Glinianowicz, *et al*. also recognized a significantly elevated leptin concentration in females with PCO concerning BMI-matched females with normal ovaries [22]. On the other hand, the clinically significant difference in serum leptin level between females with and without polycystic ovaries is consistent with many studies. In contrast Baig, *et al*. (2014) have demonstrated a non-significant difference in leptin between these females and control[16]. The current study revealed a non-significant difference regarding serum leptin concentration between lean, overweight, obese females who have PCO; this disagrees with Olszanecka-Glinianowicz,

et al. and Rizk et al. findings [22, 23]. A significant elevation in serum leptin levels in females with PCO, and among these females, a significant increment in serum leptin concentration was defined in obese and overweight females compared to females with normal BMI. The difference between previous studies and current data may be related to the sample size and type of sample collection. Regarding serum leptin level and the fertility capacity, we found a significant elevation in serum leptin in subfertile females with polycystic ovaries compared to the control females of normal non-polycystic ovaries at different body weight groups. These findings reveal that the dysregulated serum leptin in these subfertile females resulted from the increment in their body weight and several factors intrinsic to the ovaries. Baldani DP *et al.* (2019) found the same significant change in leptin level in females with polycystic ovaries compared to females with normal ovaries irrespective of their body weight [7]. Leptin was extensively distributed in reproductive tissues, with its important direct regulatory effect on ovarian folliculogenesis. At higher concentrations, it acts to disrupt LH-induced estradiol production by the ovary, thereby affecting steroidogenesis [20]. Leptin is also found to play an important role in the process of implantation and early embryonic development. A high serum level of this adipokine was found in females who suffer from subfertility for a different duration than a normal fertile female. Leptin was also found to be implicated in the initiation of ovulation at its normal level [24]. Thus, elevated serum leptin is seen to be implicated in anovulatory infertility.

In the blood, leptin is circulated into two forms, the free form, the active form, and a protein-bound form. Leptin displays its action via binding to the soluble leptin receptor that is circulating in plasma. In females with normal body weight, the bound form of leptin dominates. While in obese females, the leptin circulates mainly in free form, this is attributed to the small concentration of soluble leptin receptor. Interestingly, many previous studies reveal conflicting results regarding circulating leptin levels among women with PCO at different BMI [23]. The current study reveals a significant elevation of serum adipokines level (leptin) in lean females with PCO (BMI 18.5-24-9) compared to the females with normal ovarian texture, as shown in table 3. This finding Reveals that a disrupted adipokines secretion does not only relate to obesity that is presented in most of them but may be related to factors intrinsic to the ovaries and their development. The same result was found by Baldani DP *et al.* (2019). Such findings can highlight the important role of the leptin hormone in PCO development. While in overweight and obese females, it has been shown that an elevation of serum leptin occurred significantly in overweight and obese females who were presented with PCO in contrast to a matched females with normal ovaries, as shown in table 3. The same significant result was found by Olszanecka-Glinianowicz, *et al*. (2013). This finding is theoretically anticipated because leptin is synthesized mainly by adipocytes, and an elevated BMI will result in an increment in leptin production. Palomba S. (2018) found that the increment in serum leptin level was implicated in the pathogenesis of multiple diseases with a significant impairment of reproductive function [25].

Conclusion

The significant association that has been found between serum leptin levels at different body weight groups, especially in females with polycystic ovaries, points to the important function of this adipokine in reproductive impairment, especially in overweight and obese females in comparison to the control group.

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