Original Article

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Leptin as a Predictive Marker in Unexplained Infertility in North Indian Population

Obstetrics and Gynaecology Section

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ABSTRACT

Introduction: According to WHO, the primary infertility in India is about 3.9% (age-standardized to 25-49 year) and 16.8% (age-standardized to 15-49 year), using the "age but no birth" definition. Several factors which affect fertility include low sperm production in men, poor egg quality and blocked fallopian tubes in women and also hormonal imbalances. Leptin plays a critical role in women's reproduction and neuroendocrine health. It is used for treating exercise-induced bone loss, eating disorders and infertility.

Aim: To evaluate the serum leptin levels in Indians and to ascertain the relationship between serum leptin levels, Unexplained Infertility (UI) and related variables [height, weight, Waist Hip Ratio (WHR), Body Mass Index (BMI)] between obese infertile, non-obese infertile and healthy subjects.

Materials and Methods: The present case-control study was conducted at the Department of Obstetrics and Gynaecology, King George's Medical University (KGMU), Lucknow, India and funded by Department of Science and Technology, New Delhi, India. The study included 229 female participants in the age group of 18-40 years (120 cases and 109 controls) who were randomly selected. The blood samples were collected from the Infertility Clinic, Queen Mary's Hospital, KGMU, Lucknow, India. All the participants underwent complete physical examination. Initially, the participants were categorized into fertile and infertile groups, they were further divided on the basis of BMI, normal (BMI- 18.5-24.5) and overweight or obese (BMI≥25). Leptin level was measured by Active Human Leptin ELISA kit and BMI of all subjects was calculated in kg/m^2 (weight in kg and height in m).

Results: A highly positive linear correlation (R=0.754, p<0.001) was found between BMI and serum leptin in unexplained infertile women, which indicates a strong relationship between BMI and serum leptin. The variation in serum leptin is explained by the independent variable, BMI. There was a partial positive linear correlation between BMI and serum leptin in the control group. Statistically there was no significant correlation (R=0.109, p=0.258) between BMI and serum leptin in the control group.

Conclusion: The present study clearly demonstrates that level of leptin is higher in unexplained infertile than in the fertile group, and also shows that a strong relationship exists between BMI and serum leptin in the obese group. Serum leptin level was significantly higher in obese than non-obese subjects. Thus, leptin is an important factor for normal reproductive function. Obesity, the main cause of infertility may be controlled by regulating the leptin concentration.

Keywords: Body mass index, Neuroendocrine, Obesity, Reproduction, Serum leptin, Unexplained infertility

INTRODUCTION

Among various health issues, infertility has been always considered as one of the major health problems in different societies [1,2]. Infertility is defined as one year of unprotected intercourse without pregnancy [3]. The term Unexplained Infertility (UI) refers to infertile couples in whom standard investigations, including tests of ovulation, tubal patency and semen analysis are normal. The prevalence of UI has been shown to vary from 22%-28% [4,5]. However, the proportions of couples with UI range from 5% to 37% [6]. The exact aetiology of UI is unknown, but several possibilities have been proposed. Subtle changes in follicle development, ovulation and the luteal phase, as well as sperm concentration and motility at the lower end of the normal range, have been reported in some couples with UI [7-9].

In female endocrinology, relationship between reproduction and metabolism has always been a controversial issue. Low molecular weight IGF-Binding Protein-1 (IGFBP-1), insulin, and amino acids have been introduced as effective signals in changes of body fat and BMI, but these changes have been associated to level of leptin [10]. Leptin, apart from regulating body weight, also plays an important role in maintaining endocrine, reproductive and immune function via food intake suppression and increasing the energy consumption. Leptin deficiency causes obesity as well as leads to reproductive cycle disturbance, hormonal imbalance and disorders of immune system and haematopoietic system and bone metabolism [11]. This signifies the important role of leptin in the physiological process, and the association between abnormal leptin levels and many disorders [12].

Leptin has a molecular mass of 16 kDa and has 167-amino acids coded by the ob gene and an adipocyte derived hormone that acts as a major regulator for food intake and energy homeostasis. Leptin deficiency or resistance can result in profound obesity, diabetes and infertility in humans [13]. Earlier it was believed that leptin is produced exclusively by adipose tissue [14]. Studies have shown that leptin is also produced by human ovarian follicles - both in granulosa and cumulus cells. It was confirmed at mRNA and protein levels that human pre-ovulatory follicles express the leptin gene [15]. Males with unexplained infertility experienced little change in sperm concentration and lower motility, while in females there were few changes in follicle development, ovulation and the luteal phase [7-9]. In an earlier study, it has been shown that the concentration of serum leptin was equal in idiopathic infertile and fertile endometriosis patients [16]. However, leptin concentration was shown to be increased in serum and peritoneal fluid of endometriosis patients compared to control women [17]. Moreover, the relationship of serum leptin and unexplained infertility has not been well described.

Leptin functions primarily as an antiobesity hormone. In healthy individuals serum leptin concentration positively correlated with fat composition [18], while it negatively correlated when energy intake was less and fat energy stored was declining [19]. Measurement of BMI and waist circumference helps in studying the association between leptin and indirect measurement of adiposity [20-23]. Only a few studies have been done to show the relationship of leptin concentrations with BMI in a local population [24-26]. In most individuals, obesity levels were high and this shows the relationship between BMI and body fat percentage [18].

No data are available in the literature for levels of serum leptin in UI among the Indian population. Studying leptin levels in Indians may be relevant in the light of reported association of serum leptin with obesity and UI.

The aim of this study was to evaluate the serum leptin levels in Indians and to ascertain the relationship between serum leptin levels, UI and related variables (height, weight, WHR, BMI) between obese infertile, non-obese infertile and healthy subjects.

MATERIALS AND METHODS

This case-control study was conducted in the Department of Obstetrics and Gynaecology, King George's Medical University (KGMU), Lucknow, India. The study was approved by the Ethical Committee of KGMU (ref no -53ECMIIB/P1). Consents were obtained from all subjects included in this study irrespective of the cause of infertility.

The study included 229 female participants (120 cases and 109 controls) in the age group of 18 to 40 years who were randomly selected. The blood samples were collected from the Infertility Clinic, Queen Mary's Hospital, KGMU, and Lucknow. All the participants underwent complete physical examination. At the time of enrolment and blood collection, information about the participants, including height, weight, Waist Circumference (WC), and Hip Circumference (HC) was obtained.

All the participants were asked to fill a questionnaire about family history of obesity and genetic diseases. Pregnant women and those who were on medication during the course were excluded from the study. In the present study, the infertile female cases were compared with the fertile females of the control group of the same age to find out the role of serum leptin in causation of unexplained fertility. Initially women were categorized into fertile and infertile groups which were further divided into obese and nonobese groups on the basis of BMI, normal (BMI-18.5-24.5) and overweight or obese (BMI≥25) and BMI of all subjects was calculated in kg/m^2. Serum leptin level was measured by active human leptin ELISA kit using an ELISA reader.

STATISTICAL ANALYSIS

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean±SD. Quantitative variables were compared using unpaired t-test between two groups. Qualitative variables were compared using Fisher's-exact-test. Pearson correlation coefficients were used to determine the relationship between BMI and leptin concentrations in each group, while correlation was defined as a measure of the strength of a linear relationship between two variables. A p-value of <0.05 was considered statistically significant. Data were entered in MS EXCEL spreadsheet and analysed by using SPSS version 21.0.

RESULTS

The demographic (age, BMI and WHR) and clinical {Thyriod Stimulating Hormone (TSH), Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Prolactin (PRL) and leptin} characteristics of two groups (fertile and unexplained infertile) are summarized in [Table/Fig-1]. Comparing the mean demographic and clinical characteristics of the two groups, t-test showed significantly (p<0.01or p<0.001) different and higher WHR, FSH and leptin, while it showed significantly (p<0.001) different and lower TSH and LH in unexplained infertile group as compared to fertile group. However, mean age, BMI and PRL did not differ (p>0.05) between the two groups i.e., they were found to be statistically the same.

[Table/Fig-2] shows the values of BMI for obese and non-obese groups. For each group, comparing the mean demographic and clinical characteristics within the groups (i.e., non- obese vs. obese), Tukey's test showed significant (p<0.001) different and higher leptin levels in unexplained infertile obese subjects as compared to non-obese subjects. Comparing the mean demographic and clinical characteristics between the groups (i.e., fertile vs. unexplained infertile), Tukey's test showed significantly (p<0.001) different and lower TSH and LH and higher leptin in unexplained infertile non-obese as compared to fertile non-obese subjects. In contrast, in obese, the mean LH was lowered significantly (p<0.05), while FSH and leptin were significantly (p<0.01 or p<0.001) higher in unexplained infertile as compared to fertile subjects. However, mean age, WHR and PRL did not differ (p>0.05) within and between the groups i.e., they were found to be statistically the same.

The distribution of leptin in the two groups according to age (lower age: ≤30 years and higher age: >30 years) and BMI (lower BMI: <25 kg/m^2 and higher BMI: ≥25 kg/m^2) is shown in [Table/Fig-2]. Comparing the mean leptin levels within the groups, Tukey's test showed significantly (p<0.001) different and higher leptin levels in higher BMI subgroup as compared to lower BMI subgroup in unexplained in fertile group. However, it did not differ (p>0.05) between the two age subgroups in both the groups and BMI infertile group. Similarly, on comparing the mean leptin levels between the groups, Tukey's test showed significantly (p<0.001) different and higher leptin levels in unexplained infertile group as compared to fertile group in subgroups of age and BMI.

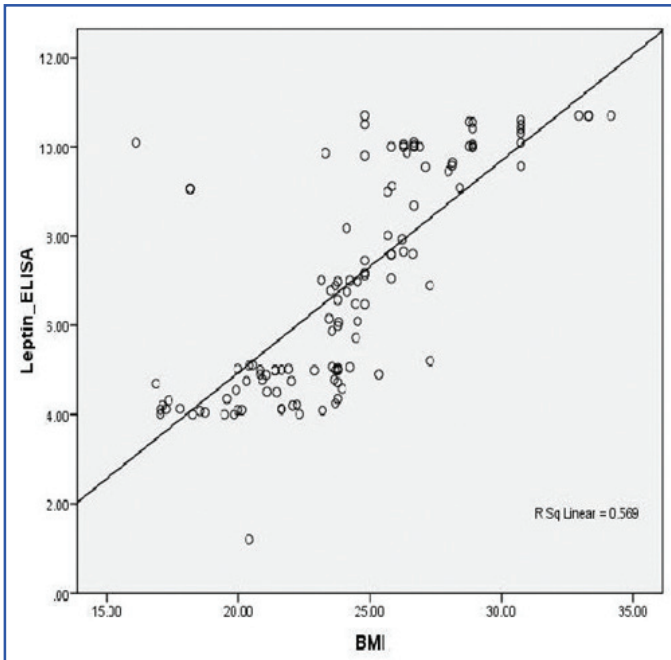
There was a highly positive linear correlation between BMI and serum leptin in the cases. A strong significant correlation (R=0.754, p<0.001) was found in BMI and serum leptin in cases. Its large value indicates a strong relationship between BMI and serum leptin

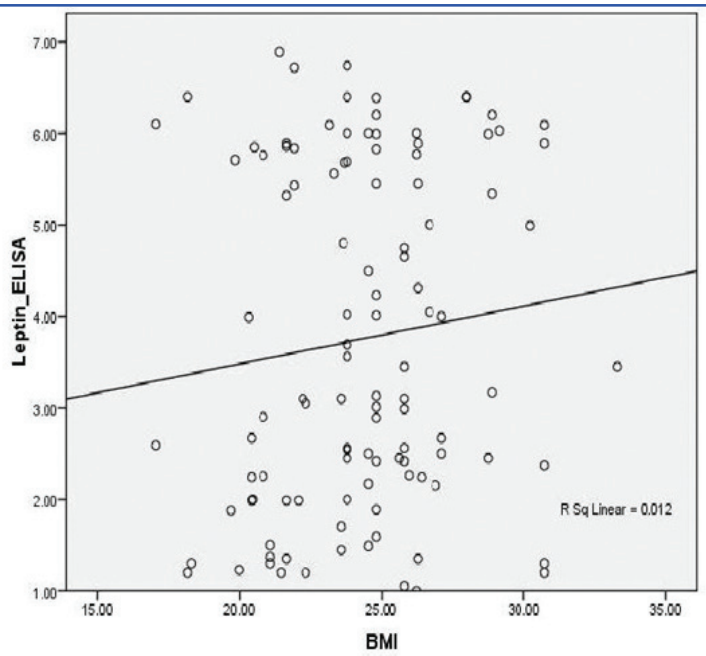
Parameters	Cases	Controls	p-value
Age (years)	29.52 ± 4.43	29.58±4.01	0.925
Height (cm)	147.39 ± 6.69	145.78±6.58	0.067
Weight (kg)	52.29 ± 8.92	51.54±6.9	0.48
BMI (kg/m^2)	24.1 ±3.9	24.31±3.19	0.671
WC (cm)	95.65± 10.99	82.95± 9.64	<0.001*
HC (cm)	99.89±10.11	91.49±7.36	<0.001*
WHR	0.96± 0.12	0.91± 0.14	<0.005*
TSH (ug/ml)	2.88±9.261	2.97±0.819	0.469
LH (ug/ml)	5.95±2.437	6.23±2.317	0.374
FSH (ug/ml)	7.79 ± 1.885	7.33±2.023	0.070
PRL (ug/ml)	13.162 ± 3.536	14.07±3.886	0.063
Leptin ELISA (ng/ml)	6.89±2.46	3.75±1.84	<0.001*

[Table/Fig-1]: Distribution of demographic and clinical characteristics (Mean±SD) of two groups at presentation.
Applied unpaired t-test for significance. *Significant

Variable	Fertile		Unexplained infertile		p-value
	N	Mean ± SD	N	Mean ± SD	
Age(yrs): ≤30 >30 p-value	77 32	3.66±1.79 3.98±1.97 0.902	83 37	6.80±2.41 7.09±2.59 0.912	<0.001* <0.001*
BMI(kg/m^2): <25 ≥25 p-value	70 39	3.71±1.90 3.83±1.76 0.987	77 43	5.54±1.78 9.31±1.44 <0.001*	<0.001* <0.001*

[Table/Fig-2]: Distribution of leptin in two groups according to age and BMI.
Applied unpaired t-test for significance. *Significant





[Table/Fig-3]: Correlation between serum leptin vs BMI in cases. **[Table/Fig-4]:** Leptin in relation to BMI in the control group.

(R=0.754). For R sq 0.569, above one half (56.9%) the variation in serum leptin levels was explained by the independent variable BMI [Table/Fig-3]. There was a partial positive linear correlation between BMI and serum leptin levels in control group. Statistically there was no significant correlation (R=0.109, p=0.258) between BMI and serum leptin levels in control group [Table/Fig-4].

DISCUSSION

Fertility is the natural capability of producing offsprings. As a measure, "Fertility rate" is the measure of kids conceived per couple [3]. Globally, according to the Centers for Disease Control and Prevention, USA, out of the total infertility cases, 1/3 each pertain to male and female infertility. The remaining instances of infertility are because of a mix of variables from both accomplices. For roughly 20% of couples, the cause could not be diagnosed [27].

The present study was carried out on 229 female participants (120 cases and 109 controls), aged between 18 to 40 years and who were randomly selected. Female age is an important factor because the rate of fertility diminishes with the advancement in age and women who conceive at higher age are at a greater risk of pregnancy complications.

The present study has demonstrated that the mean serum leptin level is significantly higher in women with unexplained infertility than fertile control females. BMI was also found to be increased in women with unexplained infertility than fertile control females. Our study is consistent with the studies of Dermir B et al., and Tafvizi F et al., who have shown that the mean serum leptin level was higher in women with unexplained infertility than in fertile women. Infertile couples, who after evaluation do not reveal the cause of infertility, are diagnosed as case of unexplained infertility [26,27]. However, many cases of unexplained infertility may be due to other factors also [7-9].

High BMI may lead to low pregnancy rates even after utilizing artificial methods like stimulation of ovulation and assisted conception [28]. In the present study we found a strong relationship between BMI and serum leptin in the obese group. Serum leptin level was significantly higher in obese than non-obese subjects. This was in agreement with previous studies [29]. In one study, the leptin levels in unexplained infertility group were more than that in explained infertility and normal fertile groups [30]. Previously Afzal R et al., demonstrated that over-weight accompanied by hyperleptinemia is generally associated with infertility in females. Increased leptin level as a result of overweight may deregulate the hypothalamic pituitary gonadal system, leading to reproductive dysfunction, including infertility [31].

There may a different underlying pathophysiologic mechanism in unexplained infertility that may not be fully described by current knowledge. Previously Dermir B et al., showed a significant increase in serum leptin levels in the unexplained infertile group compared to the fertile group [26]. Their results might help in understanding the pathophysiology of unexplained infertility. Smith GD et al., suggested that leptin is a cytokine which affects pathophysiology of infertility [32]. Caprio M et al., suggested that it is possible that leptin plays a dual role in regulating reproduction. Low leptin levels may negatively influence the neuroendocrine regulation of reproduction, with a threshold level being permissive to normal reproduction [33].

Conversely, elevated leptin levels may negatively influence normal ovarian function and/or embryo development and viability [32]. There was a positive linear correlation between BMI and serum leptin in infertile women. A strong significant correlation (R=0.754, p<0.001) was found between BMI and serum leptin cases. This finding is consistent with the earlier studies carried out in predominantly overweight and obese population [33-35].

Obese people have excessive amounts of leptin and their brains do not receive the signal which tells the individual to stop eating (leptin resistance). Fat cells produce leptin, the fat cells pass the signals to the brain to tell the individual to stop eating, but the brain does not get the signal. As a result, the individual's satiety is not fulfilled with the result that the individual becomes obese, which in turn may cause infertility [36].

Leptin levels change during the menstrual cycle. In a recent study, researchers have shown higher serum leptin levels in certain phases of menstrual cycle in women, and significant correlations were found in the follicular phase between leptin and prolactin as well as between leptin and free testosterone in males. It was concluded that between the first to fifth days of the cycle leptin levels were more stable compared to those produced later in the cycle [37]. Based on a previous finding [26], in the present study, leptin levels on the third day of the menstruation cycle were used for precise cycle-independent measurements.

LIMITATION

Financial and time constraints led us to conduct a random study. Further studies are needed to show the relationship between leptin, UI and obesity by taking a larger sample size of the population.

CONCLUSION

The present study clearly demonstrates that leptin is higher in cases of UI than in the control group, and also shows that a strong relationship exists between BMI and serum leptin in the obese group. Serum leptin level was significantly higher in obese than non-obese subjects.

Thus leptin is important for normal reproductive function. Obesity, the main cause of infertility may be controlled by regulating the leptin concentration. Therefore, weight management should be the first line of treatment in cases of unexplained infertility. The role of leptin in reproduction may be a useful tool for treatment of reproductive pathologies. Further studies are needed to elaborate the relationship between leptin, UI and obesity.

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