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Association of serum Leptin with Gonadotrophins and Prolactin in Polycystic Ovarian Syndrome Patients attending Tertiary Medical Hospital in Southern Odisha

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ABSTRACT

Introduction: Leptin is an *ob* gene encoded adipocyte derived hormone which serves as a relay link between metabolic signals and brain to regulate the hypothalamic pituitary ovarian axis. Leptin is associated with obesity which is a major cause of PCOS in women of reproductive age group.

Aim: The purpose of study was to evaluate the association of serum leptin concentration with gonadotrophins and prolactin in women with PCOS.

Material and Method: Itwas a case control study conducted for a span of oneyear at one year, at Department of Biochemistry in collaboration with department of Obstetrics and Gynaecology, MKCG Medical College and Hospital from December 2018 to December 2019, where 60 PCOS subjects and 30age matched normal ovulatory controls of 15 – 30 years age group with BMI<25 kg/m^2 were included. BMI was calculated and study population was divided into 2 groups that is Lean PCOS group with BMI < 25kg/m^2 and Obese PCOS group with BMI ≥ 25kg/m^2.Serum leptin, gonadotrophins and prolactin were estimated on 2nd or 3rd day of menstrual cycle. Data was represented as mean and standard deviation and statistical analysis was done in SPSS version 25. Data analysis was done using one-way ANOVA test, correlation was calculated by using the Pearson's correlation method. A 'p' value of <0.05 was taken as significant.

Result: The means and standard deviation of serum Leptin (28.0 ± 17.8, p=0.00), serum LH (13.01 ± 5.6, p=0.001) and serum Prolactin (23.57 ± 5.6, p=0.001) are higher among both the case groups than controls with a higher value observed in obese PCOS which are found to be

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statistically significant. The serum leptin value has significant correlation with serum levels of LH and Prolactin in PCOS cases ($p<0.05$)

Conclusion: Women with PCOS often have high levels of LH secretion. High levels of LH contribute to the high levels of androgens, and along with these low levels of FSH contributes to poor ovum development and in ability to ovulate.

Keywords: Polycystic ovary syndrome **(**PCOS), Prolactin, Leptin, Gonadotrophin, Obesity

INTRODUCTION

Polycystic ovary syndrome(PCOS) is a condition characterized by irregular or absence of menstruation, acne, obesity, and hirsutism. I.F. Stein and M.L. Leventhal were the first researchers to distinguish this reproductive phenomena in1935. Hence itis also called the Stein-Leventhal syndrome.[1] PCOS is a complex metabolic, endocrine and reproductive disorder which affects approximately 5-10%of the female population in developed countries.[1] The manifestation and expression of PCOS symptoms such as polycystic ovaries, high levels of androgen hormones and irregular periods are variable from person to person. Presently, PCOS is diagnosed using Rotterdam criteria (2003) which includesovulatory dysfunction of less than 21 or more than 35 days,hyperandrogenism which may be biochemical or clinicaland polycystic ovaries of more than 10 in USG. Any two out of the three of the above features with exclusion of thyroid disease (TSH), hyperprolactinemia (prolactin) & NCCAH

Leptin, a product of *ob* gene of chromosome 7, is produced in adipose tissues and has a long list of endocrine functions besides being responsible for causing obesity.[2] The role of leptin in reproductive physiology and the pathogenesis of PCOS has been studied in bioactive leptin deficient rats.[3]It was observed that treatment with recombinant leptin helped them to resume fertility but the underlying mechanisms are still not clear.[4-6] Leptin mediates its effects by binding to specific leptin receptors (ObRs) expressed in the brain as well as in peripheral tissues. Alternative splicing generates several isoforms of ObRs. The ObRa isoform (the short leptin receptor isoform) is thought to play an important role in transporting leptin across the blood–brain barrier.[2]The ObRb isoform (the long leptin receptor isoform) mediates signal transduction and is strongly expressed in the hypothalamus, an important site for the regulation of energy homeostasis and neuroendocrine function.[7]

The binding of leptin to the ObRb receptor activates several signal transduction pathways, including Janus Kinase-Signal Transducer and Activator of Transcription-3 (JAK-STAT3), Monophosphate-activated Protein Kinase (AMPK), and the Mammalian Target of Rapamycin (mTOR), have been proposed to be downstream of leptin.[8]Leptin augments secretion of gonadotropin hormones, which are essential for initiation and maintenance of normal reproductive function, by acting centrally at the hypothalamus to regulate gonadotropin-releasing hormone (GnRH) neuronal activity and secretion. The effects of leptin on GnRH are mediated through intraneuronal pathways involving neuropeptide-Y, proopiomelanocortin and kisspeptin. Increased infertility associated with diet induced obesity or central leptin resistance are likely mediated through the kisspeptin-GnRH pathway. Furthermore, Leptin regulates reproductive function by altering the sensitivity of the pituitary gland to GnRH and acting at the ovary to regulate follicular and luteal steroidogenesis. Thus, leptin serves as a putative signal that links metabolic status with the reproductive axis.[9]

Polycystic ovarian syndrome associated with obesity and insulin resistance, both of features are linked to leptin and its receptors. Serum level of leptin is higher in obese women. Taking into consideration the known association between leptin, obesity, and insulin action, it can be assumed that leptin might have a role in PCOS. Thus, PCOS patients may serve as a reliable model to assess the relationship of hyperinsulinemia with leptin concentrations beyond the association of leptin and obesity itself.[10]

Hyperandrogenism is androgen excess, which is the main feature of PCOS. Androgens are part of the steroid hormone family. In the ovary, the first steps of androgen formation are performed in LH-stimulated thecal cells, as these cells express the cytochrome P450c17 gene, with the synthesis of DHEA (dehydroepiandrosterone) and androstenedione. Most of these precursors will be converted to estrogens by granulosa cells, which express the enzyme P450aromatase. But ovaries also directly secrete androgens in the circulation, mainly as androstenedione and testosterone.[11-13]

OBJECTIVES

- To evaluate serum leptin, gonadotrophins and prolactin levels in PCOS cases and controls.
- To find out the correlation between serum leptin, gonadotrophins and prolactin level in PCOS cases.

MATERIAL AND METHODS

Study design

This was a case control study done from December 2018 to December 2019 at Department of Biochemistry in collaboration with department of Obstetrics and Gynaecology. Institutional ethical clearance was obtained before conducting the study. [IEC 673]

Study population

Study population comprised of sixty patients between 15-30 years of age group who were diagnosed as PCOS cases in Gynaecology OPD according to the Rotterdam criteria 2003. They were divided into two groups according to their BMI as Lean PCOS group with BMI < 25kg/m^2 and Obese PCOS group with BMI ≥ 25kg/m^2. Thirty age matched normal ovulatory females with BMI < 25kg/m^2 were randomly chosen from attendants of patients, MBBS students, nursing students and from general populations. All the study participants gave informed consent for participation in the study. According to a pre designed data collection proforma, family and menstrual history were taken and anthropometric measurements were taken. The female patients with known cases of type 2 DM or any chronic disease and using oral contraceptive pills at least 3 months prior to study period were excluded from the study.

Sample Collection

According to the previous year data records of MKCG Medical College and Hospital, average 25 to 30 cases of PCOS had visited the Gynaecology OPD per month. So, we had collected average 15 newly diagnosed PCOS cases according the Rotterdam criteria and also following the exclusion criteria with attrition of 5% and data collection for 4 months dedicated to sample collection as a convenient purposeful sampling. So, the study population comprised of 60 patients

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The blood sample was collected on day 2-3 of menstrual cycle of all controls and blood of PCOS cases were collected independently of menstrual cycle due to irregular menses. Taking all aseptic precautions 5ml blood was drawn from the medial cubital vein. The estimation of hormonal parameters like thyroid hormone, plasma insulin and reproductive hormones were assayed using commercially available kits by Roche Cobas e411 electrochemiluminescence assay maintaining both internal and external quality control in Biochemical diagnostic centre of the hospital.

Analysis methods

The estimation of serum leptin concentration was assayed by using Diagnostic Biochem Canada Inc. Leptin ELISA kit and estimation of serum prolactin, LH and FSH was assayed using commercially available kit of Roche Cobas e411 diagnostics. The LH/FSH ratio was calculated by dividing the value of LH with FSH. The ratio between LH and FSH is < 2 in normal healthy females whereas the ratio in PCOS is >2.

Estimation of Leptin

Leptin was assayed by DBC diagnostics kit which was a sandwich type of enzyme immunoassay. This uses two highly specific monoclonal antibodies i.e., a monoclonal antibody specific for leptin and another monoclonal antibody specific for different epitope for leptin conjugated to biotin.

Statistical Analysis

The statistical analysis was done in SPSS version 25. Data analysis was done using one-way ANOVA test, correlation was calculated by using the Pearson's correlation method and their relationship between variables was shown by scatter plot. A 'p' value of <0.05 was taken as significant.

RESULTS

Sixty (60) PCOS women diagnosed by Rotterdam criteria were taken in the study group as cases. They were divided into two subgroups i.e., Lean PCOS having BMI < 25 kg/m^2 and Obese PCOS having BMI ≥ 25 kg/m^2. Thirty healthy age matched females with BMI < 25kg/m^2 were taken as controls. Table 1 shows mean and standard deviation of BMI of lean and obese PCOS patients were (21.7 ± 2.2) and (31.9 ± 4.7) respectively. Obese PCOS have higher mean and standard deviation for BMI than healthy control (2.7 ±1.8) which is statistically significant. (p = 0.000)Table 2 shows the comparison of mean and standard deviation of hormonal parameters in the three groups. The means and standard deviation of serum LH, serum Prolactin, and serum leptin are higher among both the case groups with a higher value observed in obese PCOS than controls which are found to be statistically significant. (p<0.05)Table 3 shows that reproductive hormones Prolactin and LH correlates significantly with serum Leptin when we take the PCOS cases i.e., both LEAN PCOS and OBESE PCOS. The serum FSH level did not correlate with serum leptin levels in PCOS cases.

DISCUSSION

Polycystic ovarian syndrome (PCOS) is a complex condition that is most often diagnosed by the presence of two of the three following criteria: hyperandrogenism, ovulatory dysfunction, and polycystic ovaries. Because these findings may have multiple causes other than PCOS, a

careful, targeted history and physical examination are required to ensure appropriate diagnosis and treatment.

Leptin is an adipose tissue-derived messenger of the energy stores to brain, which acts as a crucial hormone/cytokine that controls some functions, such as reproduction.[14]It is observed that high leptin levels are likely to exert a negative influence on the normal ovarian function and on fertilization that is required for the development of the embryo and decreased leptin levels disrupt the neuroendocrine regulation of reproduction.[15]We evaluated the association of leptin with BMI which plays a role in regulation of reproductive function. PCOS is important metabolic and endocrine disorder with long term sequel of hypertension, diabetes mellitus and cardiovascular disease.

In the present study we have taken sixty (60) PCOS cases and grouped them into two subgroups of lean and obese according to BMI. We took thirty (30) healthy controls (BMI <25) and compared serum leptin and insulin resistance with cases. All the female participants are between 15-30 years of age and residents of Ganjam district of Southern Odisha.

PCOS affects premenopausal women, and the age of onset is most often peri-menarchal. However, clinical recognition of the syndrome may be delayed by failure of the patient to become concerned by irregular menses, hirsutism, or other symptoms or by the overlap of PCOS findings with normal physiologic maturation during the 2 years after menarche. In our\study the mean age of PCOS women in all the three groups were found to be 22.5 years. The serum leptin level is significantly increased in obese PCOS cases as compared to lean PCOS and healthy controls.

In our study, the mean serum prolactin and LH level are significantly higher in obese PCOS than lean and controls. There is significant positive correlation of serum leptin with serum prolactin and LH level in PCOS cases. The serum prolactin correlates with serum leptin in both lean and obese PCOS groups. When serum LH and FSH are correlated in obese and lean PCOS they don't have significant correlation with serum leptin.

Many studies have document elevated levels of prolactin in PCOS patients.[16-19]Hernandez et al suggested hypothalamic deficiency of dopamine could be a cause of mild elevation in prolactin level in PCOS.[20] Shibli-Rahhal et al (2009) found the association between prolactin, leptin and obesity and suggested that prolactin may play a role in modulation of body weight and composition, and it remains unclear whether weight gain is associated with hyperprolactinemia due to stimulation of lipogenesis or disruption of CNS dopaminergic system[21] .Study done by RoshniSadaria et al (2019) showed that there is mild increase in prolactin level, but no significant correlation was present between leptin and prolactin. In our study there is a significant correlation of Prolactin in PCOS cases.[22]

Various studies have documented that on administration of leptin in leptin deficient *ob* mice there is restoration of lactation, concluding that leptin is one of the various factors modulating prolactin secretion.

In our study, there is a significant positive relationship between leptin and LHwhich confirms the findings of Mohiti-Ardekaniand Taarof et al in 2009.[23]During amenorrhea, leptin plays a possible role as a primary signal in receiving LH secretion pulses. LH pulses in mid-luteal and early follicular phases has led to speculation that leptin fluctuations regulate LH plasma intensity.[24]On the other hand, it has been reported that leptin has a direct regulatory action in ovarian folliculogenesis. Furthermore, leptin has been shown to modulate LH-stimulated

oestradiol production in the ovary.[25] Although, leptin is extensively present in reproductive tissues, its correlation with reproductive hormones is still obscure.

A study by Farooq et al (2013) reported that serum leptin has a strong negative correlation with LH, FSH, and testosterone in the fertile obese as well as in normal males and females, which were statistically significant.[26]

The mean level of serum FSH doesn't correlate with serum leptin concentration in our study which is similar with M Baig et al (2019). They did not find any correlation of leptin with FSH, LH and estradiol.[27]

The LH/FSH ratio were high in PCOS cases which shows that serum LH is raised and FSH is decreased in PCOS. Previously the LH/FSH ratio > 2 were taken as diagnostic criteria in women with infertility. Since one of the main causes of infertility in reproductive age group female is PCOS so we did not take LH/FSH ratio as criteria for diagnosis of PCOS women with irregular menses as it is of less importance now.

In present study lack of correlation with FSH and LH/FSH ratio with serum leptin concentration does not support the role of leptin on gonadotropin secretion and ovarian steroidogenesis in PCOS

Limitation of our study

We did not evaluate the other reproductive parameters like testosterone and estradiol which could have association with leptin in PCOS due to unavailability of test parameters.We did not take overweight category females without PCOS as controls for better analysis of level of leptin with overweight PCOS females.The study requires a very large sample size for better correlation values between the parameters.

Recommendations

We could evaluate the molecular mechanism such as polymorphism studies for further validation of our observations as leptin is extensively present in reproductive tissues, its correlation with reproductive hormones is still obscure in various studies.

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Table 1: Anthropometric parameters of Research subjects

PARAMETERS	CONTROL (n=30) (BMI<25)	LEAN PCOS (n=30) (BMI<25)	OBESE PCOS (n=30) (BMI>25)	TOTAL (n=90)	'p'VALUE (ANOVA)
AGE (yrs.)	22.5 ± 2.8	22.0±2.8	22.8 ± 3.7	22.4± 3.13	0.602
HEIGHT (cm)	152 ± 5.4	148.7 ± 4.5	157.8 ± 6.6	152.9±6.7	0.000**
WEIGHT (kg)	48.9 ± 5.0	48.7 ± 4.8	79.8 ± 13.5	59.2±17.09	0.000**
BMI (kg/m^2)	20.7 ± 1.8	21.4 ± 2.2	31.9 ± 4.7	24.8±6.0	0.000**

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 2: Comparison of Leptin, Prolactin, LH ,FSH and LH/FSH values of the research subjects

Parameters	CONTROL (BMI<25)	LEAN PCOS (BMI<25)	OBESE PCOS (BMI>25)	TOTAL (n=90)	pVALUE (ANOVA)
LH (IU/L)	8.2 ± 4.3	9.0 ±5.1	13.01 ± 5.6	10.10 ±5.4	0.001**
FSH (IU/L)	4.4 ± 1.5	4.6 ± 1.9	5.2 ± 1.91	4.7 ±1.8	0.232
PRL (ng/ml)	18.1 ± 4.3	22.2 ± 6.2	23.57 ± 5.6	21.2 ± 5.9	0.001**
LEPTIN (ng/ml)	5.2 ± 2.1	5.8 ± 2.2	28.0 ± 17.8	13.3 ± 14.6	0.000**
LH/FSH RATIO	1.9 ± 1.0	2.2 ± 1.2	2.7 ± 1.4	2.3 ±1.2	0.047

*. Correlation is significant at the 0.05 level (2-tailed).

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**. Correlation is significant at the 0.01 level (2-tailed).

Table 3: Correlation of Leptin with Prolactin and LH values in PCOS cases

	PCOS CASES (n=60)	
PARAMETERS	**'r' VALUE**	**'p' VALUE**
1.PROLACTIN (ng/ml)	0.279	0.031*
2.LH (IU/L)	0.338	0.008**
3.FSH (IU/L)	0.093	0.478

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Figure 1: Scatter diagram showing positive correlation of serum leptin with LH hormone in PCOS cases

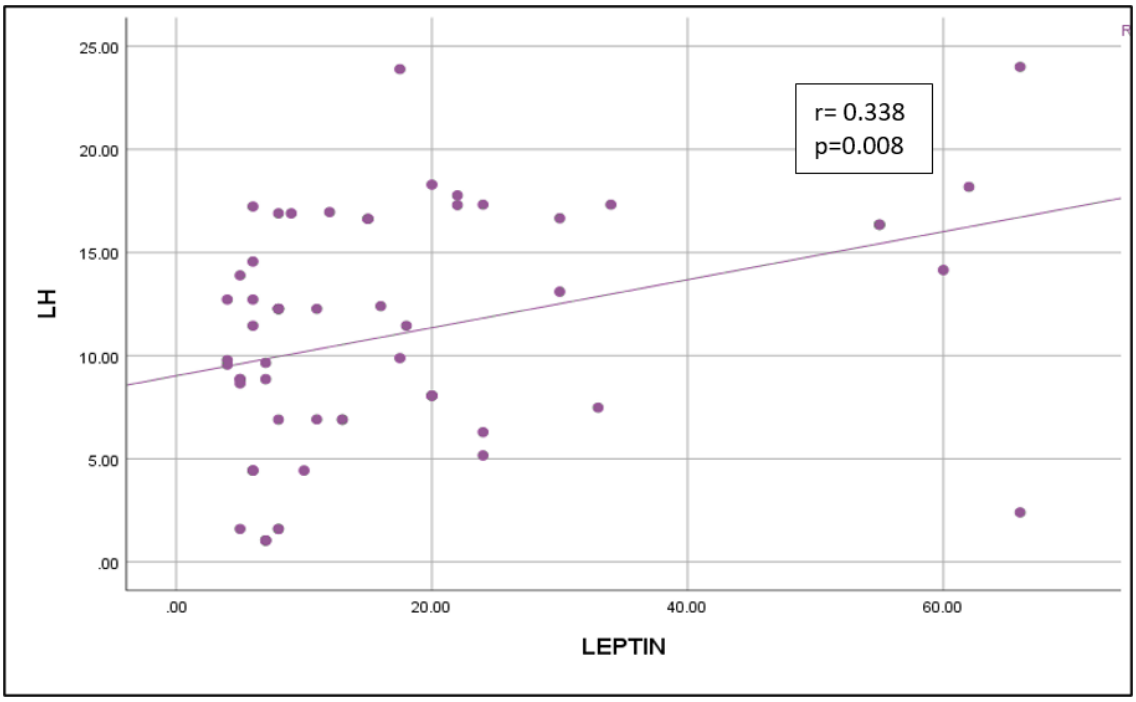

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Figure 2: Scatter diagram showing positive correlation of serum leptin with Prolactin hormone in PCOS cases