ORIGINAL ARTICLE
SERUM LEPTIN LEVEL IN ADOLESCENT GIRLS WITH POLYCYSTIC OVARY SYNDROME: CORRELATION WITH ANTHROPOMETRIC AND ENDOCRINE PARAMETERS

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Background: Polycystic Ovary Syndrome (PCOS) is linked with hyperinsulinemia and insulin resistance, characteristics that are linked to the level of leptin and leptin receptors. This study was planned to estimate serum leptin levels in adolescent girls with PCOS and its relation with endocrine and anthropometric parameters. **Methods:** Fifty (50) girls with PCOS, aged 16–20 years, and 60 age-matched normal subjects were included in the study. Study duration was from Jun 2014 to Dec 2016. Patients were taken from Sir Ganga Ram Hospital, Lahore. Normal subjects (controls) were students of Fatima Jinnah Medical University, Lahore. Serum levels of Fasting blood sugar, leptin, testosterone, and serum insulin were estimated. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was used to calculate insulin resistance. Body mass index (BMI) was calculated using the standard formula. **Results:** Mean age of adolescent girls with PCOS was 19 ± 2.8 years. BMI of PCOS patients was increased non-significantly compared to normal subjects. Fasting blood sugar and serum testosterone level was non-significantly decreased in PCOS patients compared to normal subjects. Level of serum insulin was significantly increased in patients compared to normal subjects. On the other hand, serum leptin level was increased in patients as compared to normal subjects. A weak correlation between leptin hormone, serum insulin and insulin resistance was observed. Significant positive correlation was observed between BMI of PCOS women and their leptin hormone. **Conclusion:** In adolescent girls, increased leptin level showed a significant correlation with BMI, whereas its correlation with endocrine parameter was weak.
Keywords: PCOS, adolescent, Leptin, BMI, Insulin, HOMA-IR

Pak J Physiol 2017;13(1):3–6

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a wide spread metabolic and hormonal disorder among reproductive women. The PCOS incidence among adolescence is between 11–26%.[1] This disease is characterized by hirsutism, menstrual disorder, ovarian dysfunction, infertility, obesity, hyperandrogenism and insulin resistance. The reason of PCOS is multifactorial, including both environmental and genetic issues.[2]

Hyperinsulinemia and the reduced insulin sensitivity are associated with lipid disorders which are known risk factors for coronary heart disease.[3] Hyperinsulinemia in association with insulin resistance may raise the level of androgen formation and its serum level by the adrenal gland and ovary by decreasing the level of sex hormone binding globulin.[4]

Insulin resistance may be the major cause of POCS. In 50–70% of PCOS patients there is Insulin resistance which may results hyperinsulinemia.[3] It also increases the risk of long-term metabolic diseases, like cardiovascular disease and diabetes.[5] But it is controversial that increase risk of these diseases are related with abnormalities of hormone due to PCOS or it may be linked to metabolic changes leading to obesity in PCOS women.[6]

Obesity is a common clinical feature in 50–60% PCOS women.[7] Adiposity is observed in PCOS patients and have an important role in metabolism through the synthesis of adipokines.[8] BMI may be the main factor associated with diabetes mellitus and coronary heart disease in PCOS women.[6]

Leptin protein with molecular weight 16 Kda synthesized in adipose tissue. It has a function to suppress intake of food and triggers pathways involve in production of energy. In PCOS women there is a direction relation of serum leptin was observed with BMI. This further supports the importance of abdominal fat mass in the secretion of leptin.[9]

Leptin improves the sensitivity of insulin in peripheral tissues and also effect the function of β–cell of pancrease.[3] It also has an important role in reproductive dysfunction.[10] insulin excess may be associated with higher circulating levels of leptin. Higher level of leptin maybe one of the cause of hyperandrogenism or anovulation.[11] Studies on the leptin in PCOS have controversy, some study indicate high levels of leptin[12], while others show no relationship with PCOS.[13,14]

Association of testosterone with BMI and waist circumference showed that as the BMI increased, the serum testosterone levels showed a significant

elevation. This showed that increase fat tissue and BMI is related with imbalance of sex steroid hormons.[15]

Although PCOS is a known disorder, the diagnosis of PCOS may be missed in adolescence, as menstrual irregularity, overweight and acne are common in girls. Study was planned to estimate the level of serum leptin in adolescent girls with PCOS and its correlation with endocrine and anthropometric parameters

PATIENTS AND METHODS

It was a case control study; 50 girls with PCOS, age range 16–20 year and 60 age-matched normal subjects with no history of any disease were included in the study. The study was conducted from Jun 2014 to Dec 2016. Patients were consecutively taken from Gynaecology and Endocrinology Units of Sir Ganga Ram Hospital, Lahore. Normal controls were students of Fatima Jinnah Medical University, Lahore. Serum levels of fasting blood sugar, leptin, testosterone, and serum insulin were estimated. Insulin resistance was calculated with Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), and BMI was calculated as:

$$[Weight (Kg)]/[Height (m)]^2$$

The study protocol was approved by the Local Ethics Committee. Informed written consent was obtained from all participants of the study. Rotterdam criteria was used for the diagnosis of PCOS and it included clinical signs of oligomenorrhoea (six or less menses/year) or amenorrhoea (no menses in the last six months), hyperandrogenism, and polycystic ovaries (by ultrasonographic examination).[16]

Exclusion criteria were hypothyroidism, pregnancy, use of oral contraceptives, hyper-prolactinemia, anti-diabetic and anti-obesity drugs, or any hormone containing drug. Patients with any metabolic, neoplastic or other medical illness were also excluded from the study.

Blood samples were taken in the early follicular phase in normal participants, and in any phase of menstrual cycle in PCOS patients. Serum level of testosterone, insulin, fasting blood glucose levels, and leptin were estimated.

Serum leptin, testosterone, and insulin were estimated by Eliza technique using standard kits. Fasting blood glucose level was estimated by standard kit of Merck. HOMA-IR for insulin resistance was calculated with the formula[17]:

$$Fasting\ plasma\ glucose\ (mg/dl) \times serum\ insulin\ (mU/l)/405$$

Data was analysed using SPSS-18 and presented as Mean±SD. The two groups were compared using Student's t-test. Pearson correlation was used for correlation between two variables and $p<0.05$ was considered statistically significant.

RESULTS

Mean age of adolescent with PCOS and of normal control subject was 19 and 18 years respectively. BMI of PCOS patients was increased as compared to the BMI of controls but the differences were not significant. Fasting blood sugar was non-significantly less in PCOS patients as compared to controls. Level of serum insulin was significantly raised ($p<0.001$) in patients compared to normal subjects. Serum testosterone level was raised in patients compared to normal subjects. Raw volume of serum leptin level was raised in patients compared to normal subjects.

When Pearson's correlation was studied between parameters in the patients, there was a significant positive correlation between insulin resistance and fasting insulin ($r=0.99$). A direct correlation was also observed between fasting insulin and testosterone ($r=0.426$).

A weak correlation between leptin, serum insulin and insulin resistance was observed. Significant positive correlation was observed between BMI of PCOS women and leptin hormone ($r=0.532$). (Table-1).
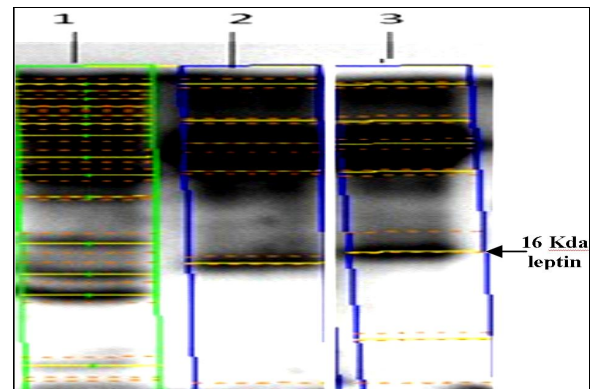

Figure-1: 18% SDS-PAGE Electrophoresis
Track 1: Standard Ladder, Track 2: Patient's serum,
Track 3: Normal serum

Table-1: Study parameters in girls aged 16–20 year

Variables	PCOS (n=50)	Controls (n=60)
Age (year)	19.0±2.8	18.4±3.2
BMI	26.86±1.684	23.60±1.949
Fasting blood Glucose (mg/dl)	108.3±5.4	95.0±8.5
Fasting serum Insulin (ìU/ml)	9.5±12.4*	3.3±2.6
Serum testosterone (ηg/ml)	1.1±0.78	0.67±0.38
HOMA-IR	1.9±2.7	0.7±0.6
Raw volume of serum leptin	1226398.25	180214.53

*$p<0.001$

DISCUSSION

PCOS is linked with hyperinsulinemia and insulin resistance which in turn is linked to leptin hormone. In women with PCOS a direct correlation exists between BMI, serum leptin, and serum insulin indicating hyperleptinemia.[18,19]

In our subjects, the mean age of adolescent PCOS was 19 years. A study also found the age range

for adolescent with PCOS 15–18 years.[20] In our study the BMI of PCOS patients was non-significantly increased as compared to their controls. It is reported that obesity and increased BMI are clinical features of PCOS that may interfere with successful fertility treatment. In this regard, it was found that irrespective of PCOS status elevated BMI was inversely associated with the total number of oocytes retrieved from the ovary.[21] In PCOS infrequent ovulation leads to increased LH and reduced FSH. The latter prevents the granulosa cells of the ovary from producing oesterogen, while LH is stimulating the ovary to produce androgens. The end results is hyperandrogenism and reduced oestrogen due to failed follicular development. At this point the leptin-insulin ratio potentiates the vicious cycle of this axis.[22]

Present study observed that the level of fasting blood sugar was increased in PCOS patients as compared to normal subjects. In PCOS specially in adolescence, insulin resistance may be the first identifiable risk factor.[23]

According to our study the serum level of insulin was considerably increased in patients as compared to normal subjects. It is proved that insulin stimulates ovarian steroidogenesis via increasing the ovary LH receptors and the sensitivity of pituitary gonadotropes to GnRH action.[24] Leptin is primarily synthesised and secreted into the blood by the white adipose tissue, hence, its blood levels are positively correlated to body fat.[25]

We found that the level of testosterone was increased in patients as compared to controls. Findings from a research show that PCOS patients had increased levels of testosterone, insulin and insulin resistance. Data of this study indicates that increased level of circulating leptin is associated with hyper-androgenaemia, increased LH concentrations, and insulin resistance.[26]

Present study observed an increased raw volume of serum leptin level in patients as compared to their controls. There is evidence that leptin acts as a paracrine mediator and shows a relation between reproduction and energy homeostasis.[7] Double effect of leptin was reported on reproduction and energy homeostasis. The complex interplay between metabolism and reproductive function is essential for species survival. High leptin level directly inhibits follicular maturation. Also, hyperleptinemia seen in obesity and underlying leptin resistance interact with the hypothalamic-pituitary-gonadal axis at several levels leading to reproductive dysfunction.[27]

Hyperinsulinemia acts together with LH to enhance androgen production in the ovary. Insulin also inhibits the production of sex hormone binding globulin in the liver which enables more free testosterone into the circulation.[28,29] According to another study it is possible

that leptin affects androgen levels via its impact on insulin secretion instead of a direct alteration of serum testosterone.[15]

The Pearson's correlation was studied between parameters in the patients and it was observed that there was a considerable correlation between insulin resistance and fasting insulin. We also found a direct correlation between fasting insulin and testosterone. Findings of a study showed that PCOS women had higher levels of testosterone, insulin, and insulin resistance.[15]

Present study found a weak correlation between leptin, serum insulin, and insulin resistance. Insulin increases leptin *m*RNA in fat cells confirming its role in leptin secretion.[15] According to a study, in PCOS women there is an increased level of insulin but no increase in the level of leptin.[30] On the other hand, another study reported a strong positive correlation between leptin and insulin resistance.[15]

According to our study a significant correlation was observed between BMI and leptin hormone in PCOS women. Our study is inline with studies which found that increased leptin levels directly correlate with the BMI.[19,31] Disorders of serum leptin and SHBG are common pathological manifestations in different syndrome types of PCOS.[32] A direct correlation exists between BMI and leptin in PCOS patients which may suggest that the interaction between neuropeptide Y neurons and leptin is altered in PCOS.[33]

Our study is inline with a study which reported that lack of relationship of leptin with insulin, insulin resistance, and testosterone is probably due to heterogeneity of PCOS group.[1]

CONCLUSION

In adolescent girls, increased leptin level showed a significant correlation with BMI, whereas its correlation with endocrine parameter is weak. There is need of further study on large group of adolescent girls.

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