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Original Article

Assessment of Leptin and Testosterone in women with Polycystic ovarian syndrome.

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is a disorder characterized by chronic anovulation, hyperandrogenism, hyperinsulinemia, and often presence of obesity. Leptin, an adipocyte derived hormone encoded by *'ob'* gene, serves as a link relaying metabolic signals to the neuronal networks in the brain to modulate hypothalamo-pituitary-ovarian axis. Circulating leptin correlates strongly with obesity, which is frequently associated with PCOS. The purpose of study was to evaluate the interrelationship between serum leptin level with body mass index (BMI) and with circulating testosterone in PCOS women. ***Methods:*** A comparative study including 30 women diagnosed as PCOS and 30 age and BMI matched healthy women with normal menstrual cycle as controls was conducted. The age group for the study was 18 to 35 years. Fasting blood samples were drawn to measure serum leptin and serum testosterone. BMI was also calculated. Interpretation of data was done using SPSS version 13. ***Results:*** : Significant positive correlations between leptin levels and BMI in cases and controls ($\rho = 0.683$, $p < 0.001$; $\rho = 0.485$, $p = 0.007$ respectively) were observed. Mean BMI, leptin and testosterone were found elevated in the PCOS women compared to controls but they were not statistically significant. No significant correlation was found between leptin and testosterone. ***Conclusion***: Leptin levels were correlated with BMI both in PCOS women and in the healthy controls. Leptin may not have a direct role in the pathogenesis of PCOS, as the serum levels were not significantly higher in PCOS women and did not correlate with testosterone.

Key words: Polycystic ovarian syndrome, Leptin, testosterone.

Introduction

Polycystic ovary syndrome (PCOS) is one of the common endocrine disorders affecting 5 to 10 % women [(1)]. According to ESHRE/ASRM consensus workshop at Rotterdam in 2003, the diagnosis of PCOS is based on the presence of any two of [(1)] chronic anovulation, [(2)] clinical/ biochemical parameters for hyperandrogenism, and (3) polycystic ovaries on ultrasonography [(2)]. It is also considered as a metabolic disorder, since the components of metabolic syndrome (MetS), namely obesity, glucose intolerance, atherogenic dyslipidemia, and hypertension, are the common features of this syndrome [(3)].

Leptin is a multifunctional adipostatic hormone that is produced by the obese *(ob)* gene. Leptin has been proposed as the peripheral signal indicating the adequacy of nutritional status for reproductive functions. Studies in humans and animals have shown that leptin concentrations in the blood correlate with the amount of adipose tissue in the body, acting as a sensing hormone in a negative feedback control from adipose tissue to the hypothalamus [(4,5)]. PCOS is often associated with obesity and insulin resistance, both of which are features that are linked to leptin and its receptors [(5)]. Hyperandrogenemia is considered to be a key feature in PCOS women. Obesity is associated with alteration in sex steroid balance in both premenopausal and postmenopausal women. These alterations involve both androgens and estrogens and their carrier protein, sex hormone binding globulin (SHBG), which binds testosterone and dihydrotestosterone with high affinity and estrogens with lower affinity. Women with central obesity have lower serum SHBG concentrations in comparison with their age and weight matched counterparts with peripheral obesity. Reduction of circulating SHBG results in an increase in the metabolic clearance rate of circulating SHBG-bound steroids,

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specifically testosterone, dihydrotestosterone and androstenediol [6,7]. The purpose of study was to evaluate the interrelationship between serum leptin level with body mass index and with circulating testosterone in PCOS women.

Material and Methods

The study was carried out on 30 PCOS subjects in the age group of 18 to 35 years and 30 voluntary age and BMI matched healthy women with normal menstrual cycle as controls. The study was conducted at Kempegowda Institute of Medical Sciences & Hospital. The diagnosis of PCOS was fulfilled as per Rotterdam criteria. Presence of at least two criteria from clinical, hormonal and abdominal USG category was considered diagnostic of PCOS. Patients with diabetes mellitus, hypertension, renal and liver failure and other endocrine disorders and patients receiving hormonal / non-hormonal treatment for PCOS were excluded from the study. The institutional ethical committee approved the study protocol. Informed consent was obtained from all the participants.

A pre-structured and pre-tested proforma was used to collect the data. Baseline data including age, BMI, detailed medical history, clinical examinations and relevant investigations were included as part of the methodology. Serum leptin and serum testosterone were measured in all participants from morning blood samples collected after 12 hours of fasting. Serum leptin was measured by Sandwich ELISA method (Diagnostic Biochem Canada Inc. Cat.No. CAN-L-4260;Version:8.1;August 2009). Serum testosterone was measured by electrochemiluminescence immunoassay (Elecsys 2010 analyzer, Roche Diagnostics). Body mass index (BMI) was calculated as the ratio of weight (Kg) to height squared (m^2). SPSS software version 13.0 was used for statistical analysis. Comparisons between groups were performed using the Mann-Whitney test. Correlation analysis between BMI, serum leptin and serum testosterone were done using Spearman's rank order correlation coefficients. A P value < 0.05 was considered statistically significant.

Results

Results on continuous measurements are presented as Mean ± SD. The basic characteristics and mean distribution of biochemical parameters in the cases and controls are depicted in Table 1. There was no significant difference in age between the two groups. Slightly higher mean was recorded in BMI, leptin and testosterone in cases than in controls but difference in mean between the two groups was not statistically significant (P>0.05). Correlation of leptin with testosterone and BMI is depicted in Table 2. Significant positive correlation between leptin levels and BMI in cases and controls (ρ= 0.683, p < 0.001; ρ= 0.485, p = 0.007 respectively) was found in our study, which is shown in figure 1. No significant correlation could be found between leptin and testosterone in cases (ρ= - 0.025, p = 0.896) or controls (ρ= - 0.214, p = 0.256). Though correlation between BMI and testosterone showed change in control groups (ρ= 0.455, p = 0.012), there was no significant correlation could be found between BMI and testosterone in cases (ρ= - 0.086, p = 0.652).

Parameters	Cases with PCOS (n=30)	Controls (n = 30)	P value
Age (years)	23.37 ± 4.09	23.73 ± 3.81	0.744
BMI ((kg/m2)	24.00 ± 4.41	22.51 ± 2.31	0.126
Serum Leptin	10.61 ± 12.52	9.01 ± 4.87	0.813
Serum Testosterone	51.02 ± 24.34	46.44 ± 21	0.564

Table 1: Mean distribution of biochemical parameters in PCOS cases and controls. Values are expressed as means ±SD.

Parameters	Cases		Controls	
	ρ value	P value	ρ value	P value
Leptin and BMI	0.683	< 0.001*	0.485	0.007*
Leptin and Testosterone	- 0.025	0.896	-0.214	0.256
BMI and Testosterone	- 0.086	0.652	0.455	0.012*

*denotes significant correlation

Table 2: Correlation between various parameters.

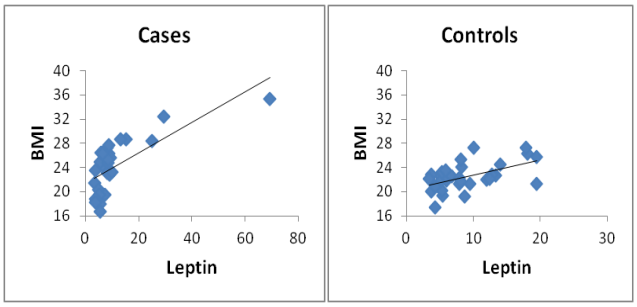

Figure 1: Correlation scatter plot of leptin vs. BMI in cases and controls

Discussion

The heterogenous polycystic ovary syndrome (PCOS) is defined as a syndromal complex consisting of hirsutism/ hyperandrogenism, chronic anovulation, menstrual disturbances, and features of the metabolic syndrome such as obesity, insulin resistance, dyslipidemia, and endothelial dysfunction. It results from an abnormal secretory pattern of GnRH and leads to a high LH/FSH ratio and a hyperandrogenic state [8].

C.Karlsson et al concluded that both the short and long isoforms of the leptin receptor are expressed in human ovarian cells and that immunoreactive leptin is present in human follicular fluid. In addition, leptin significantly suppressed LH-induced estradiol production. These findings are consistent with an endocrine action of leptin on the human ovary, with possible implications for female reproduction in health and disease [9]. Leptin acts as permissive signal to activate the reproductive axis and to maintain normal reproductive function [10,11]. Ovarian follicular cells and testicular Leydig cells express a functional leptin receptor, and leptin mRNA is synthesized in granulosa and cumulus cells of preovulatory human follicles [9,12]. Expression of leptin receptors in granulosa cells, which synergies with glucocorticoids to promote steroidogenesis, indicates that leptin exerts direct regulatory action in ovarian folliculogenesis [5]. With the exception of rare cases of gene mutations, human obesity is normally associated with hyperleptinemia (leptin resistance) rather than leptin deficiency. Increasing obesity is positively correlated to the numbers of anovulatory cycles, and high leptin levels directly inhibit ovarian steroidogenesis, leading to ineffective follicular maturation [8]. Endocrine and/or direct paracrine effects of leptin on the gonads include antagonism of the stimulating effects of several growth factors and hormones [insulin-like growth factor I (IGF-I), insulin, glucocorticoids] on gonadotropin-stimulated steroidogenesis in ovarian cells as well as inhibition of testosterone production in Leydig cells [12]. In contrast, leptin deficiency results in down-regulation of the HPG axis, resulting in low levels of circulating sex steroids [13].

In our study, slightly higher mean BMI was recorded in cases than in controls but the difference in mean BMI between the two groups was not statistically significant (P>0.05). Higher mean serum leptin was recorded in PCOS subjects compared to controls but the difference between them was not statistically significant (P>0.05). This finding was consistent with study done by Mantzoros CS et al who concluded that circulating leptin levels in women with PCOS, although increased compared to those in lean individuals, do not differ from those in age and weight-matched controls [14].

The results of the present study showed that a significant positive correlation exists between serum leptin and BMI both in PCOS subjects ($p < 0.001$) and controls ($p = 0.007$) suggesting that leptin is secreted from adipocytes into circulation and by acting as a sensing hormone to hypothalamus informing the brain about abundance of body fat. This finding was consistent with study done by Chakrabarti J[5], Tayfun Alper et al[15] and Javed Mohiti-Ardekani et al[16]. Study done by Chakrabarti J showed that irrespective of BMI, PCOS population had higher leptin levels. This observation is because leptin is predominantly synthesized by adipocytes, and higher BMI is observed in the PCOS group than in control women [5]. Tayfun Alper et al observed in their study that serum leptin levels and BMI were significantly high in PCOS women. Although leptin production mainly occurs in adipose tissue, when the difference in body fat mass between PCOS and controls was corrected for, the difference in the leptin levels remained significant. This finding suggested that there might be other reasons for the increase in the serum leptin concentration in PCOS cases [15]. Study done by Javed Mohiti-Ardekani et al showed a significant high total and free leptin in PCOS women as compared to controls and total leptin levels correlated significantly with BMI in both PCOS women and controls [16]. In contrast, G.A.Laughlin et al found that leptin levels in PCOS did not differ from those of normal cycling women with similar BMI or adiposity [17]. Rouru J et al also found in their study that serum leptin concentrations were not significantly different in PCOS and control subjects [18].

In present study, higher mean testosterone was recorded in cases compared to controls but difference between cases and controls were not statistically significant (P≥0.05). No significant correlation could be found between leptin and testosterone in cases ($\rho = -0.025$, $p = 0.896$) or controls ($\rho = -0.214$, $p = 0.256$). Chakrabarti J et al found significantly higher mean baseline serum testosterone level in PCOS women compared to control. But, when serum testosterone was analyzed as a function of serum leptin, no significant correlation could be found between testosterone and leptin in either of the groups [5]. Ramanand SJ et al concluded that correlation of leptin with FSH and testosterone is influenced strongly by obesity and PCOS. There was no significant correlation between leptin and testosterone in normal weight PCOS women and in normal weight controls. But highly significant inverse correlation between leptin

and testosterone was found in obese PCOS women and obese controls [1]. In our study, though changes were observed with respect to correlation between BMI and testosterone in controls (ρ= 0.455, p = 0.012), no significant correlation could be found between BMI and testosterone in cases (ρ= - 0.086, p = 0.652). One of the established metabolic effects of obesity on circulating endogenous hormones is the progressive reduction in SHBG with increasing BMI. In premenopausal women, increased BMI, waist-hip ratio or abdominal obesity have been associated with either no change or an increase in total testosterone concentrations. Most of the studies in premenopausal women have shown an increase in free testosterone with increasing body weight. Ephia Yasmin et al also could not found any correlation between BMI and testosterone. The dynamics between increased clearance due to reduction of circulating SHBG and compensatory increased production of androgens may explain the lack of a positive correlation between plasma testosterone and BMI [6].

Conclusion

Our findings showed that leptin levels were correlated with BMI both in women with PCOS and in the healthy controls. Leptin levels were found to be correlated with the amount of fat tissue not only in women with PCOS but also in healthy women. Leptin may not have a direct role in the pathogenesis of PCOS, as the serum levels were not significantly higher in women with PCOS and did not correlate with testosterone. Because our study consisted of a limited number of PCOS subjects and controls from a single population, further studies with larger number of PCOS subjects will be beneficial in elucidating the relationship between leptin, testosterone and BMI in polycystic ovarian syndrome subjects.

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