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ORIGINAL ARTICLE

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Serum leptin in polycystic ovarian syndrome with reference to insulin level

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OBJECTIVE(S) : To find out in a case control study whether leptin levels in PCOS patients are altered or not.

METHOD(S) : In 19 obese and 16 non-obese polycystic ovarian syndrome (PCOS) patients and 10 controls we estimated body mass index (BMI), waist-hip ratio, fasting serum glucose, insulin, HOMA score for insulin resistance, leptin, luteinising hormone (LH) follicle stimulating hormone (FSH), total testosterone, and free testosterone levels. Statistical analysis was done with student's t-test and Pearson correlation coefficient.

RESULTS: Serum leptin, fasting serum insulin, HOMA scores for insulin resistance were significantly higher in obese PCOS patients compared to non-obese PCOS patients and controls. LH, total testosterone and free testosterone levels were higher in both obese and non-obese PCOS patients compared to controls.

Conclusion(S): High serum leptin levels observed in obese PCOS patients are related to obesity and not the pathogenesis.

Key words: polycystic ovarian syndrome, leptin, insulin, obesity

Introduction

Polycystic ovarian syndrome (PCOS) is a disorder characterized by chronic anovulation, hyperandrogenism, hyperinsulinemia, and often presence of obesity. Compared to eumenorrheic women in the early follicular phase of the menstrual cycle, PCOS affected women have elevated serum luteinising hormone (LH) and low or low normal follicle stimulating hormone (FSH) levels, with an increased ratio of LH to FSH. The increased LH secretion stimulates thecal cells in the ovary to produce excess androgen and the androgen in turn, stimulates LH secretion, while the vicious cycle goes on. The androgen also inhibits production of sex hormone binding globulin (SHBG) resulting in excess free androgen responsible for hirsutism.

Insulin resistance is an important component of PCOS and altered insulin action precedes the increase in androgens in PCOS. The hyperinsulinemia may cause hyperandrogenism by inhibiting hepatic synthesis of SHBG and by binding insulin like growth factor -1 (IGF -1) receptors in the ovary leading to increased androgen production by thecal cells. In fibroblasts of 50% PCOS patients (PCOS-ser) there is decreased insulin dependent receptor autophosphorylation of tyrosine residue and increased constitutive receptor serine phosphorylation. These defects may cause insulin resistance in women with PCOS-ser [1].

Chronically, insulin exerts stimulatory effect on adipocytes to increase leptin synthesis [2], a protein encoded by "ob gene". Leptin in adipose tissue diminishes glucose oxidation, and lipogenesis while increasing fat utilization (anti-insulin effects) [3]. Because of the intimate association between chronic insulin stiumlation and leptin levels in humans and the prevalence of hyperinsulinemia in PCOS subjects, we tried to examine whether leptin levels in PCOS subects are altered or not.

Methods

The study was carried out on 45 women (35 PCOS subjects - 19 obese and 16 non-obese and 10 age matched healthy women with normal menstrual cycle as controls). Clinical

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diagnosis of PCOS was made from history of chronic oligomenorrhea or amenorrhea plus hirsutism or elevated serum total testosterone or with LH/FSH ratio >2[4]. Valid informed consents were obtained from all women. Serum glucose, insulin, leptin, LH, FSH, total testosterone and free testosterone were measured in all participants from morning blood samples collected after 12 hour fasting. Serum leptin was measured by IRMA, free testosterone and insulin were measured by RIA, total testosterone was measured by chemiluminescence immunoassay, and LH and FSH were measured by ELISA.

In controls and oligomenorrheic PCOS subjects, LH and FSH levels were estimated in the early follicular phase (second or third day of menstruation) from pooled samples. BMI, waist-hip ratio (WHR) and HOMA score for insulin resistance (HOMA-IR)[5] were also calculated in all women.

Statistical significance was estimated using two tailed unpaired t-test. P value <0.05 was considered significant. Correlation analysis between serum leptin, BMI, insulin and free testosterone were done using Pearson Correlation Coefficients.

Results

Table 1 shows age and anthropometric indices of PCOS subjects and controls. Table 2 shows clinical profile of PCOS women. Table 3 shows that compared to controls, obese PCOS subjects (BMI > 25 kg/m²) have significantly higher fasting insulin (P<0.001). LH (P<0.001), LH/FSH ratio (P<0.001), leptin (P<0.001), leptin/BMI ratio (P=0.0002), total testosterone (P<0.001), free testosterone (P<0.001) HOMA-IR score (P<0.001) and significantly lower glucose/insulin ratio (P<0.001). In obese PCOS patients, fasting serum glucose level though within normal range, is still significantly higher (P<0.001) compared to that of controls.

Table 1. Age and anthropometric indices of study subjects

Parameter	Obese PCOS (n=19)	Non-obese PCOS (n=16)	Control (n=10)
Age	22.53 ± 6.83	23 ± 5.27	21.9 ± 3.07
BMI	31.49 ± 4.18[a,b]	22.76 ± 1.48[b]	21.97 ± 1.02
WHR	0.87 ± 0.02 [a]	0.85 ± 0.03	0.83 ± 0.02

Data = mean ± SD. [a] P <0.001 between obese PCOS subjects and controls. [b] P <0.001 between obese and non-obese PCOS subjects.

Table 4 depicts that compared to controls, non-obese PCOS subjects (BMI <25 kg/m²) have also significantly higher LH (P<0.001) LH/FSH ratio (P<0.001), total testosterone (P<0.001), free testosterone (P<0.001) free testosterone/total testosterone ratio P<0.001). However in contrast to the findings in obese PCOS subjects, leptin, leptin/BMI ratio, insulin, glucose/insulin ratio, HOMA-IR score do not significantly differ between non-obese PCOS subjects and controls. Fasting serum glucose in non-obese PCOS subjects, though within normal range, is still higher (P=0.016) compared to that of controls.

Table 2. Clinical features of PCOS (N=35)

Feature	No. of cases	Percent
Amenorrhea	2	5.7
Oligomenorrhea	33	94.3
Hirsutism (perimenarcheal)	9	25.7
Hirsutism (nonperimenarcheal)	23	65.7
Acanthosis nigricans	2	5.7
Obesity (BMI >25 kg/m^2)	19	54.3
Clitoromegaly (Clitoral length > 10 mm)	2	5.7

Table 3 Biochemical parameters of obese PCOS and controls

Parameter	Obese PCOS (n=19)	Control (n=10)	P value
Glucose (mg/dl)	88.53 ± 5.14	82.6 ± 2.50	<0.001
Insulin (µU/ml)	28.07 ± 9.57	12.47 ± 1.63	<0.001
Glucose/Insulin ratio	3.59 ± 1.44	6.73 ± 0.96	<0.001
HOMA-IR score	6.12 ± 2.06	2.66 ± 0.31	<0.001
LH (µIU/ml)	11.13 ± 1.63	5.04 ± 0.62	<0.001
FSH (µIU/ml)	4.43 ± 0.96	4.75 ± 1.24	<0.48
LH/FSH ratio	2.64 ± 0.72	1.12 ± 0.29	<0.001
Leptin (ng/ml)	24.64 ± 4.5	14.92 ± 1.43	<0.001
Leptin/BMI ratio	0.77 ± 0.08	0.68 ± 0.04	<0.001
TT (ng/ml)	1.55 ± 0.29	0.64 ± 0.10	<0.001
FT (pg/ml)	3.06 ± 2.19	0.89 ± 0.35	<0.001
FT/TT ratio	2.13 ± 1.71	1.45 ± 0.67	<0.14

Values are mean ± SD TT = total testosterone, FT = free testosterone. HOMA-IR = HOMA for Insulin Resistance

Table 4. Biochemical parameters of Non-obese PCOS and Controls

Parameter	Non-obese PCOS (n=19)	Control (n=10)	P Value
Glucose (mg/dl)	85.25 ± 2.44 (P=0.016)	82.6 ± 2.50	0.016
Insulin (mU/ml)	12.59 ± 1.40 (P=0.853)	12.47 ± 1.63	0.853
Glucose/Insulin ratio	6.87 ± 0.97 (P=0.727)	6.73 ± 0.96	0.727
HOMA-IR score	2.64 ± 0.26 (P=0.878)	2.66 ± 0.31	0.878
LH (mIU/ml)	10.31 ± 1.8 (P=0.000)	5.04 ± 0.62	<0.001
FSH (mIU/ml)	4.09 ± 1.22 (P=0.17)	4.75 ± 1.24	0.17
LH/FSH ratio	2.71 ± 1.05 (P=0.19x10^{-3})	1.12 ± 0.29	<0.001
Leptin (ng/ml)	16.32 ±2.35 (P=0.07)	14.92 ± 1.43	0.07
Leptin/BMI ratio	0.72 ± 0.08 (P=0.07)	0.68 ± 0.04	0.07
TT (ng/ml)	1.6 ± 0.32 (P=0.06x10^{-8})	0.64 ± 0.10	<0.001
FT (pg/ml)	3.65 ± 1.99 (P=0.05x10^{-3})	0.89 ± 0.35	<0.001
FT/TT ratio	2.33 ± 1.37 (P=0.0413)	1.45 ± 0.67	0.041

Values are mean ± SD TT =total testosterone, FT = free testosterone HOMA-IR = HOMA for Insulin Resistance

Table 5. Correlation coefficients of leptin with BMI, insulin and free testosterone for participants

Participant	BMI	Insulin	Free Testosterone
Obese PCOS	r = + 0.888	r = + 0.923	r = + 0.803
Non-obese PCOS	r = + 0.831	r = + 0.837	r = + 0.828
Control	r = + 0.894	r = + 0.811	r = + 0.772

r = Pearson's correlation coefficient.

It has been found that compared to non-obese PCOS subjects, obese PCOS subjects have significantly higher serum insulin (P=0.03), glucose/insulin ratio (P<0.001), HOMA score for insulin resistance (P<0.001). Though serum fasting glucose level of both obese and non-obese PCOS subjects were within normal limit, obese PCOS subjects have more fasting serum glucose than those of non-obese PCOS subjects (P=0.02). Leptin/BMI ratio is of borderline significance (P=0.051).

As seen from Table 5, it is evident that serum leptin is positively correlated with BMI, fasting insulin and free testosterone in both obese and non-obese PCOS women and in normal controls.

Discussion

In our study, in PCOS subjects, inappropriate gonadotropin secretion was evidenced by increased LH/FSH ratio compared to that in the controls. Similar results were published by Arroyo et al [6].

Increased insulin levels and HOMA-IR scores were seen in obese PCOS subjects compared to controls and non-obese PCOS subjects. HOMA-IR scores were much higher in obese PCOS subjects than the normal range values (2.1-2.7). Fasting glucose to fasting insulin ratio of less than 4.5 is a sensitive and specific index for insulin resistance in a group of obese PCOS subjects [9] as seen in our study. Furthermore, acanthosis nigricans, a peripheral indicator of insulin resistance was present in two of the 19 (10.5%) obese PCOS women.

Both total testosterone and free testosterone levels were elevated in obese and non-obese PCOS women compared to those in the controls in our study. However, there was a disproportionate increase in free testosterone. These findings were similar to those reported by Nestler et al [8] who found serum free testosterone concentration in obese PCOS subjects disproportionately higher than serum total testosterone concentrations due to reduction in serum SHBG concentration.

Leptin levels were significantly higher in obese PCOS subjects compared to those in normal controls. However, non-obese PCOS women did not show higher leptin levels compared to those of the controls. Absence of increased leptin concentration in non-obese PCOS women diminishes the likelihood of any effect of leptin in PCOS pathophysiology. Rather the correlated factor appeared to be the obesity as judged by BMI. Relation of leptin with obesity in humans was previously established[9]. Furthermore, our finding of absence of relation between leptin and PCOS was corroborated by three previous studies[10-12], wherein age and weight matched PCOS subjects and controls did not differ in leptin concentration.

Conclusion

PCOS subjects have elevated LH/FSH ratio, free testosterone/ total testosterone ratio, hyperinsulinemia and insulin resistance compared to control. However, increased serum leptin is not a constant finding in all patients of PCOS. In fact, it is associated with obesity and hence increased leptin is found in obese PCOS patients only.

References

1. Dunaif A. Insulin action in the polycystic ovarian syndrome. *Endocrinol Metab Clin North Am 1999; 28: 341-59.*
2. Caro JF, Sinha MK, Kolaczynski JW eta l. Leptin: The tale of an obesity gene. *Diabetes 1996; 45: 1455-62.*
3. Bryson JM, Phuyal JL, Swan V et al. Leptin has acute effects on glucose and lipid metabolism in both lean and gold thioglucose-obese mice. *Am J Physiol Endocrinol Metab 1999; 277: E417-E422.*
4. Talbott EO, Guzick DS, Sutton-Tyrrell K et al. Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. *Arterioscler Thromb Vasc Biol 2000; 20: 2414-21.*
5. Kanauchi M, Yamano S, Kanauchi K et al. Homeostasis model assessment of insulin resistance, quantitative insulin sensitivity check index, and oral glucose insulin sensitivity index in nonobese, nondiabetic subjects with high-normal blood pressure. *J Clin Endocrinol Metab 2003; 88: 3444-6.*
6. Arroyo A, Laughlin GA, Morales AJ et al. Inappropriate gonadotropin secretion in polycystic ovary syndrome: influence of adiposity. *J Clin Endocrinol Metab 1997;82:3728-33.*
7. Legro RS, Finegood D, Dunaif A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. *J Clin Endocrinol Metab 1998; 83: 2694-8.*
8. Nestler JE, Powers LP, Matt DW et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with polycystic ovary syndrome. *J Clin Endocrinol Metab 1991; 72: 83-9.*
9. Considine RV, Sinha MK, Heiman ML et al. Serum immunoreactive leptin concentrations in normal weight and obese human. *N Engl J Med 1996; 334: 292-5.*
10. Mantzoros CS, Dunaif A, Flier JS. Leptin concentrations in the polycystic ovary syndrome. *J Clin Endocrinol Metab 1997;82:1687-91.*
11. Laughlin GA, Morales AJ, Yen SS. Serum leptin levels in women with polycystic ovary syndrome: the role of insulin resistance/ hyperinsulinemia. *J Clin Endocrinol Metab 1997;82:1692-6.*
12. Rouru J, Anttila L, Koskinen P et al. Serum leptin concentrations in women with polycystic ovary syndrome. *J Clin Endocrinol Metab 1997; 82: 1697-1700.*