Serum Leptin levels in ovarian polycystic disease and its correlation to body weight

Maad M. Shalal   FICOG

Najmah M. Miran   FICOG, CABGO, DOG

Zina I. Mahddi   MBChB

Summary:

Background: Poly cystic ovary syndrome is a common disorder in women of reproductive age, it is associated with disturbance of reproductive, endocrine and metabolic functions. The pathophysiology of PCOS appears to be multifactorial and polygenic. Leptin seems to play an important role in pathophysiology of PCOS especially in women with BMI ≥25kg/m2.

Objectives: To assess leptin level in both PCOS and healthy women and explore the relation to their body weight and body mass index.

Patient and Methods: A total of 120 women were enrolled in this study, 60 women (50%) had PCOS (study group) and the reminder 60 women (50%) were healthy women and considered as control group. BMI was calculated first. Both groups were further subdivided into two groups; BMI of ≥25kg/m2 and BMI of <25kg/m2. Pelvic U/S scan was done to confirm the diagnosis.

Hormonal profile (FSH, LH, LH/FSH, testosterone, TSH, T4 and prolactin) were measured. RBS was measured. Serum leptin level was measured in patient with PCOS and the control by Enzyme linked immunesorbant assay (ELISA) test. Correlations between leptin levels and age, Wt, Ht, BMI, and serum hormone levels (FSH, LH, testosterone and prolactin) were studied.

Results: Mean serum leptin levels were not significantly higher in patients with PCOS compared to the control group (P =0.052). Leptin levels were found to be higher significantly in the overweight subgroups both in patients with PCOS and in the control women. Leptin levels were found to be higher in over weight patients with PCOS compared to over weight control P value =0.0001. Serum leptin levels were significantly correlated with BMI in both patient with PCOS and control group (r=0.918 and r=0.846) respectively. Serum leptin concentration had a positive correlation with serum LH concentration in PCOS group (r=0.327). Leptin levels had strong positive correlation with serum LH, FSH, and testosterone in over weight PCOS subgroup women and (r=0.592, 0.609 and 0.604) respectively.

Conclusion: Leptin levels are higher in patient with PCOS compared to control groups. Leptin levels were correlated with the amount of fatty tissue not only in PCOS group but also in healthy women. Leptin play an important role in pathophysiology of PCOS of women with BMI ≥25 kg/m2.

Key word: polycystic ovary syndrome, serum leptin, body mass index.

Introduction:

Polycystic disease of the ovary is an extremely common disorder in women of reproductive age. It has a heterogeneous collection of signs and symptoms that form a spectrum of disorder with mild presentation in some, while in others a severe disturbance of reproductive, endocrine, and metabolic function.[1, 2] Despite being heterogeneous in nature, the hallmarks of the disease are hyperandrogenism and chronic anovulation.[1] The prevalence of PCOS in general population is about 20-33% of women in reproductive age.[2, 3] The major features of polycystic ovarian syndrome (PCOS) include menstrual dysfunction, anovulation, and signs of hyperandrogenism [4]. Leptin may plays role in the pathophysiology of PCOS. [5].

stores. Secretion of leptin is affected by various Leptin is a hormone secreted mainly from the adipose tissue, and its serum

level reflects the amount of fat hormones like insulin, androgens and estrogens. Besides regulating the energy metabolism of the body, leptin has important actions on the reproductive system, which makes it an important link between the adipose tissue and hypothalamus – pituitary – gonadal axis. It seems that, in women with PCOS insulin resistance in adipocyte limits insulin stimulated leptin secretion. Additionally, higher abdominal (visceral) fat accumulated in these patients also secrete less leptin than subcutaneous fat which send inappropriate signals to the brain resulting in progressive insulin resistance and increase body weight, this will cause increase serum leptin level with eventual decompensation of reproductive function. [6] At ovarian level, high leptin concentration may impair ovarian function by reducing the response to Gonadotrophin stimulation. Beginning from 10 ng /ml of serum level (high physiological dose) leptin antagonize the synergistic effect of insulin like G.F-1 on FSH and LH stimulated steroid genesis in

Corresponding author: Najmah M. Miran
*Dept .Obgyn college of medicine/ Baghdad teaching hospital.
Email:najmah_74@yahoo.com

both granulosa and theca cell. Thus high leptin concentration in the ovary may suppress estrogen production and interfere with the development of ovarian follicle and oocyte maturation. [6] The combined effect of obesity, insulin resistance, androgen excess and high basal LH level in PCOS have lead to suggestion that leptin may play a part in pathogenesis of PCOS. [6]

Aim the of study
To assess leptin level in both PCOS and healthy women and explore the relation to their body weight and body mass index.

Patients & methods:
A prospective Case-Control study was conducted at obstetric and gynecology department at Baghdad Teaching Hospital over a period of one year from January 2010 to January 2011. 120 patients were enrolled in this study aged 20-35 years, 60 women with PCOS and 60 women healthy control group. From all women, a detailed history was taken about menstrual cycle, abnormal hair growth, weight gain, acne, endocrine abnormality and any medication taken. Also clinical examination was done and abdominal ultrasound study was done for all women. The diagnosis of PCOS requires the presence of two of the following three criteria
Oligoovulation or anovulation.
Clinical or/and biochemical sign of hyperandrogenism.
Polycystic ovaries.
Their clinical signs were taken as one of the parameter in this study. Clinical features of hyperandrogenism; hirsutism & acne were included. Ferriman and Gallwey score were used to evaluate the degree of hirsutism. . Menstrual irregularity ranging from oligo- menorrhea to secondary amenorrhea was included as well.
The control group consisted of 60 healthy women with regular cycle, no clinical evidence of hyperandrogenism, and no ultrasonographic evidence of PCO.
Exclusion criteria:
Women with Thyroid dysfunction for both groups.
Women with Prolactin dysfunction for both groups.
Women with Diabetes Mellitus for both groups.
Women who take medication or hormonal treatment 3 month before the study for both groups.
Women already diagnosed with congenital adrenal hyperplasia.
In all studied groups, we measure the serum level of LH, FSH, prolactin, TSH, testosterone, T4 and random blood sugar. Other samples were collected for leptin, these samples were left to stand at room temperature for at least 30 minutes to allow the blood to clot and then centrifuged for 10 minutes then freezing at -20 C^0 and kept there without thawing till the day of testing then serum leptin was measured using ELISA sandwich kits with the range of the assay from zero to 100 ng/

ml.Body mass index (BMI) of all participants was calculated [BMI = body weight (kg)/height^2 (m^2)]. According to BMI, Patients and control groups were subgrouped as over weight (BMI $\geq$25 kg/m^2) and normal weight (BMI < 25 kg/m^2). Out of both 60 patients with PCOS and 60 control women, 30 were normal weight and 30 were overweight.
Statistical Analysis: Analysis of data was carried out using the available statistical package of SPSS-18 (Statistical Packages for Social Sciences- version 18 "PASW" Statistics).
Data were presented in simple measures of frequency, percentage, mean, standard deviation, standard error of the mean, and range (minimum-maximum values).

The significance of different means (quantitative data from different PCOS groups and from control group) was tested using independent student-t-test for the difference between two means, while different percentages (qualitative data from both groups) were tested using Pearson Chi-square test (χ^2-test). Pearson correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation. Statistical significance was considered whenever the P value was less than 0.05.

Results:
The mean of age, Wt, Ht, BMI, mean Leptin concentration and hormonal parameter (prolactin, FSH, LH, testosterone) of both group are shown in Table1 with their P value.The mean age, weight, height, and BMI did not differ among the groups and the P value is not significant(P=0.1).Serum level of LH is significant elevated in PCOS group and the mean LH of 13.69±2.21 in PCOS group and of 5.10±0.50 in control group and the difference between groups was statistically significant (P=0.0001).Mean FSH of 4.62±0.57 in PCOS group and of 6.29±0.63 in control group is different and was statistically significant (P=0.0001). Mean LH /FSH ratio of 2.91±0.45 in PCOS group and of 0.78±0.11 in control group and the difference between groups was statistically significant (P=0.0001). Mean testosterone level was 1.31±0.40 in PCOS group and of 0.47±0.18 in control group and the difference between groups was statistically significant (P=0.0001).The mean prolactin did not differ among the groups and was not statistically significant (P=0.718). The mean leptin levels were 33.61±23.82 and 26.62±13.87 ng/ml in the study group and in the control group respectively. Though leptin levels were higher in patients with PCOS, this difference was not statistically significant (P=0.52) (table 1).

Table 1: The Mean of different variables (age, Wt, Ht, BMI, LH, FSH, LH/FSH ratio, testosterone, prolactin and Leptin) in both groups with their P values.

	PCOS(n=60)	Control(n=60)	P value
	Mean±SD (Range)	Mean±SD (Range)	
Age (years)	26.40±6.09 (29-35.0)	28.0±4.36 (20.0-35.0)	0.100
Weight (Kg)	78.49±8.61 (65.0-92.0)	77.51±12.25 (70.3-91.0)	0.611
Height (cm)	157.65±18.93 (167-171)	159.30±6.95 (155-168)	0.526
BMI (Kg/m^2)	24.43±2.54 (20.5-28.3)	24.46±2.42 (20.4-28.1)	0.944
LH (IU/L)	13.69±2.21 (9.0-18.1)	5.10±0.50 (3.5-6.3)	0.0001*
FSH (IU/L)	4.62±0.57 (3.8-5.7)	6.29±0.63 (5.0-7.3)	0.0001*
LH/FSH.	2.91±0.45 (2.0-4.0)	0.78±0.11 (0.5-1.1)	0.0001*
Testost. (ng/ml)	1.31±0.40 (0.8-2.5)	0.47±0.17 (0.2-0.9)	0.0001*
Prolactin (ng/ml)	12.29±4.12 (5.0-22.0)	12.07±2.70 (6.9-17.9)	0.718
Leptin (ng/ml)	33.61±23.82 (7.7-81.4)	26.62±13.87 (6.5-51.0)	0.052

*Significant difference between two means using Student-t-test for two independent means at 0.05 level of significance.

Table 2; Shows the mean ± SD of leptin level in each sub group for PCOS and control. Mean leptin measurements (± SD) in the study group was 51.52 ± 19.43 in over weight PCOS were significantly higher than that of over weight control with mean of 38.72 ± 8.43 and P value was 0.002 which is highly significant. Mean leptin measurements ± SD of 51.52 ± 19.43 in over weight PCOS were significantly higher than that of normal weight PCOS with mean of 15.71 ± 10.64 and P value was 0.0001 which is highly significant. Mean leptin measurements ± SD of 38.72±8.43 in over weight control group were significantly higher than that of normal weight control group with mean of 14.51±4.16 and P value was 0.0001 which is highly significant. Mean leptin measurements ± SD of 15.71 ± 10.64 in normal weight PCOS group did not differ from that of normal weight control group which is 14.51 ± 4.16 with P value was 0.568 which is not significant. These all shown in table 2.

Table 2: The mean Leptin concentration in over weight and normal weight controls and women who had PCOS.

	Over weight (≥25)	Normal weight (<25)	P value
	Mean±SD (Range)	Mean±SD (Range)	
PCOS(n=60)	51.52±19.43(15.2-81.4)	15.71±10.64(7.7-49.4)	0.0001*
Control(n=60)	38.72±8.43(20.9-51.0)	14.51±4.16(6.5-25.8)	0.0001*
P value	0.002*	0.568	

*Significant difference between two means using Student-t-test for two independent means at 0.05 level of significance.

In table 3 Show the correlation analyses that were performed between leptin level and BMI both in PCOS group and control group. Leptin levels correlated strongly positive with BMI both in patients with PCOS and in control (r = 0.918, P= 0.001 and r = 0.846, P= 0.0001, respectively).

Table 3: The correlation of Leptin with BMI in both PCOS and control groups

BMI kg/m^2	Leptin level	
	PCOS	Control
R	0.918**	0.846**
P value	0.0001	0.0001

**Correlation is significant at the 0.01 level using t-tests.

Table 4, Shows the correlation analyses that was performed between leptin level and the other parameter in PCOS and control groups. We found a non significant correlation between leptin level and age in PCOS groups. Also it shows positive significant correlation between serum leptin with serum LH in PCOS group at 0.01 levels.

Table 4: The correlation of serum Leptin with different parameters in both PCOS and control groups.

Variable		Leptin level	
		PCOS (n=60)	Control (n=60)
Age (years)	r	0.203	0.235
	P value	0.120	0.070
Weight (Kg)	r	0.880**	0.637**
	P value	0.0001	0.0001
Height (cm)	r	0.107	-0.030
	P value	0.414	0.819
LH (IU/L)	r	0.327**	-0.391**
	P value	0.011	0.002
FSH (IU/L)	r	0.198	-0.344**
	P value	0.129	0.007
LH/FSH.	r	0.211	-0.046
	P value	0.105	0.725
Testost. (ng/ml)	r	0.021	0.067
	P value	0.871	0.613
Prolactin(ng/ml)	r	-0.103	-0.161
	P value	0.433	0.218

*Correlation is significant at the 0.05 level using t-tests.
**Correlation is significant at the 0.01 level using t-tests.
*Correlation is significant at the 0.05 level using t-test.
**Correlation is significant at the 0.01 level using t-test.

Table 5, Shows the correlation analyses that were performed between serum leptin levels and serum LH, FSH, LH/FSH ratio and testosterone in both over weight PCOS and normal weight PCOS groups. Our results found significant positive correlation between serum leptin level and serum LH, FSH, and testosterone in only over weight PCOS group at 0.01 levels. There is a non significant correlation between Leptin levels and serum LH, FSH and testosterone in normal weight PCOS women.

Table 5: The correlation between serum leptin level and LH, FSH, LH/FSH, and testosterone in over weight and normal weight PCOS women.

Variables		Leptin level	
		Over weight PCOS (n=30)	Normal weight PCOS (n=30)
LH IU/ml	r	0.592**	0.289
	P value	0.0001	0.044
FSH IU/ml	r	0.609**	0.179
	P value	0.0001	0.344
LH/FSH	r	0.269	0.203
	P value	0.150	0.283
Testost. ng/ml	r	0.604**	-0.088
	P value	0.0001	0.643

Discussion:

In this study, all patients were carefully selected regarding weight, height and BMI, the result of our study suggest that serum leptin levels were somewhat higher in patients with PCOS compared with controls, but the difference was not statistically significant (P value 0.52), this is because we calculate the whole numbers of cases in both groups. Our result is in agreement with previous study done by Telli et al 2002, who found that there were no significant difference in leptin level in both PCOS and control groups. [7] Study done by Mendonca H.C. et al 2004 on PCOS and healthy groups, they found that Estradiol correlated with leptin in PCOS patients regardless their weight also show that mean leptin levels were not significantly higher in patients with PCOS compared to the control group and it is also higher in obese subgroup in both groups.[8] De, Sujit Kumar et al 2007, found that age and weight matched PCOS subjects and controls did not differ in leptin concentration and high leptin level observed in obese PCOS are related to obesity and the pathogenesis of PCOS. Also high level insulin found in PCOS.[9] Also our result is in agreement with previous study done by Mantzoros et al 1997, Chapman et al 1997, Gennarelli et al 1998, and Laughlin et al 1997.[10, 11,12,13] A study by Sepilian et al 2006, who tried to find positive relationship between leptin and insulin resistance, found that leptin to be no different in women with PCOS than

in controls and that leptin level reflect the degree of adiposity and not that of hyperinsulinaemia.[14] Maria J.et al 2007, found that there were no significant differences in mean serum leptin concentrations or in secretary burst characteristics between PCOS and normal women. Although the 24-hour serum leptin concentration correlated with BMI in both normal and PCOS women, leptin secretary burst mass correlated with BMI only in normal women. [15] Some studies reported increased leptin levels in PCOS and proposed that leptin play a strong role in the pathogenesis of the syndrome, this is done by Brzechffa et al 1996, Vicennati et al 1998, and El Orabi et al 1999 who found that leptin levels were higher in patients with PCOS compared to control. [16, 17, 18] This result doesn't coincide with our results. Hahn S, et al 2006 thinks that abnormal secretion of leptin, leptin resistance and decreased soluble leptin receptor levels is probably a specific feature of PCOS. [19] A study by Blagovest et al 2009 on 45 women with PCOS and 20 control women and found that serum leptin levels in PCOS patients were higher than in control group independently BMI, and probably the hyperleptinaemia is due to leptin resistance and may be characteristic of the syndrome. This study doesnot agree with our study. [20] When leptin level were compared between overweight and normal weight women in PCOS group, the mean leptin measurement found to be significantly higher in obese group; this result coincides with result of Mantzoros CS et al 1997, Telli et al 2002, Mendonca H et al 2004,Sujit Kumar et al 2007, Gennarelli et al 1998 and Pirwany et al 2001.[10,7,8,9,12,21] This result reflect that there is a positive strong correlation between serum leptin level and BMI in women with PCOS. Also we compared between overweight and normal weight women in control group, the mean leptin measurement found to be significantly higher in over weight group; this indicate that high leptin levels is not present only in over weight PCOS group but also in over weight control group, and this will reflect the degree of adiposity in both groups. Our result is coincide with result done by Telli et al 2002, Mendonca H, et al 2004, ,Gennarelli et al 1998 and Pirwanyet al 2001.[7,8,12,21] The normal weight patients with PCOS is compared with normal weight control in our study and show that leptin level is elevated but the difference statically not significant between them, this was similar to the results reported by by Mantzoros et al 1997, Telli et al 2002, Mendonca et al 2004, Gennarelli et al 1998 and Laughlin et al 1997. [7, 8, 10, 12,13] Remsberg et al 2002[22] who did not find any correlation between leptin and testosterone. Regarding to the age, we found that the mean patient's age was 26.40 ± 6.09 years. We found that leptin levels don't alter significantly with different age. our result disagree with result by EL-Gharib MN et al 2010[23], who found a significant positive correlation between leptin, BMI, Age in Clomifine C responder women and an insignificant positive correlation between Leptin and Insulin. In Clomifine Citrate resistant women we found a

significant positive correlation between leptin, BMI, Age and a significant positive correlation between age and Insulin. [23]

Conclusion:
Leptin was found to be correlated with the amount of fatty tissue and high BMI not only in patients with PCOS but also in healthy women. Leptin was noted to play a direct role in the pathogenesis of PCOS especially in those with BMI ≥25kg/m², as the serum level correlate with LH levels and androgen, and were higher in over weight control compared to normal weight control.

Author contribution:
Study conception: Dr. Maad M. Shalal
Study design: Dr. Maad M. Shalal & Dr. Najmah M. Miran
Acquisition of data analysis: Dr. Zina I. Mahddi
 Interpretation of data: Dr. Maad M. Shalal & Dr. Najmah M. Miran
Drafting of manuscript: Dr. Najmah M. Miran

References:
1. Michael T. Sheehan. Polycystic Ovarian Syndrome: Diagnosis and Management. Clinical Medicine & Research 2004 Volume 2, Number 1: 13-27.
2. Edmonds D. Keith. Polycystic Ovarian Syndrome and secondary amenorrhoea. Dewhurst>s textbook of Obstetrics and Gynaecology, 7ᵗʰ edition, Blackwell publishing, 2007 Chapter 39 :377-396.
 3. Bonnar John ,William Dunlop. Recent Advances in Obstetrics and Gynaecology 23 . Royal Society of Medicine Press Ltd 2005;147-158.
 4. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril. Feb 2009;91(2):456-88.
5. http://www.rcsb.org/pdb/cgi/explor.cgi?pid=252711358456 and Page =oxpdbld=1AX8 (2005-2007) Karl Harrison.
6. Erbil Dogan, Bulent Guleki . ROLE of SERUM LEPTIN ELEVATION in OBESE WOMEN with POLY CYSTIC OVARY SYNDROM. Dokuz Eylul University Department of Obestetrics and Gynnecology, Division of Reproductive Medicine and IVF Unit May 3, 2011: 164.
7. Muhittin H. Telli, M.D., Mulazim Yildrim, M.D., and Volkan Noyan, M.D Serum leptin levels in patients with polycystic ovary syndrome. Fertil Steril (2002); 77:932-935.
8. Mendonca H.C., Montenegro R.M., Foss M.C., Silva de Sa M.F., and Ferriani R.A. Positive correlation of serum leptin with estradiol levels in patients with polycystic ovary syndrome. Braz J Med Bio Res (2004); 37 (5): 729-736. [www.IVSL.org]
9. De Sujit Kumar, Sarkar Chandan , Chaudhuri Subhaschandra, Chowdhury Subhankar and Lahiri Tapan Kumar. Serum leptin in polycystic ovarian syndrome with reference to insulin level. J Obstet Gynecol India (2007); Vol. 57, No. 4: Pg 339-341.
10. Mantzoros CS, Dunaif A, Flier JS. Leptin concentration in the polycystic ovary syndrome. J. Clin. Endocrinol. Metab. (1997); 82: 1687-91.
11. Chapman IM, Wittert GA, Normman RJ. Circulating leptin concentrations in polycystic ovary syndrome: relation to anthropometric and metabolic parameter. Clin Endocrinol (1997); 46:71-75.
12. G.Gennarelli1, J.Holte1, L.Wide, C.Berne and H.Lithell. Is there a role for leptin in the endocrine and metabolic aberrations of polycystic ovary syndrome? Hum Reprod (1998):vol.13 no.3 pp.535–541. [www.IVSL.org]
13. Laughlin G.A., Morales A.J., and Yen S.S.C. Serum Leptin Levels in Women with Polycystic Ovary Syndrome: The Role of Insulin Resistance/ Hyperinsulinemia. J Clin Endocrinol Metab (1997) ;82: 1692-6.
14. Sepilian VP, Crochet JR, Nagmani M. Serum soluble leptin receptor levels and free leptin index in women with polycystic ovary syndrome: relationship to insulin resistance and androgens. Fertil Steril (2006); 85: 1441-7.
15. Maria J. Iuornoa, Leila Z. Islama, Paula P. Veldhuisc, David G. Boydb, Leon S. Farhyb, Michael L. Johnsonb,c, John E. Nestlera, William S.and Evansb,d. Leptin secretory burst mass correlates with body mass index and insulin in normal women but not in women with polycystic ovary syndrome. Metab Clinic and Exper (2007); 56: 1561–1565
16. Brzechffa PR, Jakimiuk AJ, Agarwal SK, et al. Serum immunoreactive leptin concentration in women with polycystic ovary syndrome. J Clin Endocrinol Metab (1996); 81: 4166-9
17. Vicennati V, Gambineri A and Calzoni F. Serum leptin in obese women with polycystic ovary syndrome is correlated with body weight and fat levels. Metab. (1998); 47:988-92.
18. El Orabi H, Ghalia AA, Khalifa A, Mahfouz H, El Shalkani A and Shoieb N. Serum leptin as an additional possible pathogenic factor in polycys-tic ovary syndrome. Clin Biochem. (1999); 32:71–5.
19. Hahn S, Haselhorst U, Quadbeck B et al. Decreased soluble leptin receptor levels in women with polycystic ovary syndrome. Eur J Endocrinol. (2006) ; 154: 287-294.
20. Blagovest Pehlivanov and Mitko Mitkov. Serum Leptin Levels Correlate With clinical and biochemical indices of insulin resistance in women with poly cystic ovary syndrome. The European Journal of Contraception and

Reprod. Health Care, April (2009). 14 (2): 153-159.

21. Pirwany IR, Fleming R, Sattar N, Greer A, & Wallase AM. Circulating leptin concentration and ovarian function in Polycystic ovary syndrome. Eur J Endocinol (2001);145: 289- 294.

22. Remsberg KE, Talbott EO, Zborowaski JV, et al. Evidence for competing effects of body mass. Hyperinsuinemia, insulin resistance, and androgen on leptin levels among lean, overweight, and obese women with polycystic ovary syndrome. Fertil Steril (2002); 78: 479- 86.

23. EL-Gharib MN and Badawy T. Role of Insulin and Leptin In The Pathogenesis of PCOS. Departments Of Obstet & Gynaecol And Clinical Pathology, Tanta Faculty Of Medicine, Tanta University, Tanta, Egypt. (2010); pg 1-17.