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Polycystic Ovary Syndrome in Twins; Role of Leptin.

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Abstract:

Polycystic ovary syndrome (PCOS) is a common endocrinopathy with symptoms such as obesity, insulin resistance and hyperandrogenemia. PCOS could be the result of a genetic disorder. Alteration in the serum level of leptin, as a product of obesity can also be the result of genetic discrepancy. The objective of this study was to find the association between leptin concentration and PCOS, and to ascertain the genetic property of leptin.

One hundred and fifty four female-female twins including 48 pairs of Monozygotic (MZ) and 29 pairs of dizygotic (DZ) twins, aged 15-45, Tehran residents were studied. Clinical, ultrasound and biochemical findings were used to diagnose PCOS.

The incidence of PCOS using biochemical and clinical features was 16.2%. The serum level of leptin was similar between subjects with or without PCOS irrespective of their zygosity. Correlation coefficient between serum leptin levels of MZ twins were more than that of the DZ.

It was concluded that Leptin is likely to be genetically determined. Although the effect of environmental factors cannot be denied. This study did not find any association between the diagnosis of PCOS and leptin level. However, the link between the two may lay with other entities such as eating disorders and/or obesity.

Key Words: Polycystic Ovary Syndrome, Leptin, Twin Study.

Introduction:

Leptin is an important metabolic hormone, influencing processes such as insulin secretion and glucose utilization (1). Leptin plays a role in whole-body energy balance and body weight (2, 3). Animal studies suggest that the absence of leptin results in obesity and infertility (4, 5). Human studies also support the evidence that leptin plays a significant role in reproductive biology. Fluctuation of leptin during menstrual cycle (6, 7), higher leptin level in pre-menopausal women compared with post-menopausal (8), and leptin involvement in gender-related differences in insulin sensitivity (9) all support this role.

Polycystic ovary syndrome (PCOS) as a common endocrine disorder is associated with obesity, hyperinsulinism, insulin resistance and hyperandrogenism (10, 11). PCOS and its association with leptin have been also studied (12, 13, 14). Some studies suggest that elevated leptin levels may cause follicular arrest (15) while others have not found any difference between serum leptin level of PCOS women and those of controls (16, 17). It has been found that below a certain body index (BMI), hyperandrogenic women with PCOS have lower leptin levels than controls. On the other hand, overweight and obese PCOS

subjects produce insufficient leptin for a given fat mass, relative to the degree of hyperinsulinemia, potentially because of the competing effects of adipocyte insulin resistance and androgens on leptin (1). Leptin is a product of the obesity (ob) gene. PCOS endocrinopaphy is also associated with obesity. Moreover, conditions such as hyperinsulinemia and insulin resistance can be found in both subjects with PCOS and or obesity. Whether these two conditions are disorders or symptoms of much broader spectrum of disorders is controversial (18).

Obesity and PCOS can be both the result of genetic discrepancy (1, 19), eating disorders (such as bulimia) (20), or a combination of genetic and environmental factors (21). Twin studies can be used to find any possible genetic link between leptin gene and those of the PCOS. This study aims at measuring leptin level in Monozygotic (MZ) and Dizygotic (DZ) twins with and without PCOS to find any possible link between leptin gene and PCOS.

Materials and Methods:

One hundred and fifty four subjects (77 pairs) including 96 monozygotic (MZ) individuals (48 pairs) and 58 dizygotic (DZ) individuals (29 pairs) were included in the study. They all lived in Tehran, the capital city of Iran.

Since Iran has no twin registry, subjects were recruited through mass media, posters and advertisements in magazines and newspapers. A poster was designed including a short introduction to the PCOS, inviting female-female twins, aged between 15-45 to have a free ultrasound, blood test and an examination. An offer was also made to pay their traveling costs. Five thousand posters were distributed throughout public and private hospitals, major universities in Tehran and also girls' high-schools. Private medical officers and midwives were also notified through Medical Council by leaflets and advertisements in monthly journals distributed to the private practice throughout the whole region within the capital city.

The study was conducted in Avecina Research Center, situated in Shahid-Beheshti University of Medical Sciences, which is one of the major well- known universities in Tehran. Subjects could contact the center during working hours. Full-time research assistance was available to answer the phone calls. A preliminary questionnaire including contact number, addresses and descriptive data were filled out. Appointments were made for sisters during their early follicular phase if they had normal regular menstrual cycles and if amenorrhic, the appropriate arrangements were made. Subjects were instructed to fast overnight and start

drinking 5 glasses of water an hour prior to their appointment for ultrasound examination. Subjects who were on contraceptive pills or any other hormonal medications, those with pregnancy or those who were breastfeeding their babies were excluded from the study. High level of 17-hydroxy progesterone was also considered as exclusion criteria. Twins were agreed that either both or none of the sisters would participate in the study since single sets of data would be useless for the study. In some cases several phone calls were made to make sure that subjects would keep their appointments, are familiar with the study site (the research center), and know the preparatory instructions.

Subjects were briefed upon their arrival. A consent form was signed. A standard questionnaire was filled out containing the result of their examination. Fasting blood was drawn using vacutainer syringes. Blood was then centrifuged at the speed of 2000 RPM for 10-20 minutes, immediately after withdrawal and serums were located in 2cc plastic containers, marked and stored in -80C freezer. Subjects were then asked to drink a syrup containing 75 mg of glucose. Two hours later a blood test was performed. Pelvic ultrasound was performed to visualize the ovaries using Prie-Medial machine and 3.5 MHz trasabdominal and 5 MHz. transvaginal transducer when appropriate. Adam's criteria were adopted to diagnose polycystic ovaries (22) namely the existence of more than 10 peripheral follicles (2-8 diameter) associated with an increase in ovarian stroma. The ovaries were measured in 3 plans and the volume was calculated using the formula: length ´ width ´ thickness ´ 0.5. Vaginal ultrasound was performed in case of obesity when necessary. The sonographer was blind to the clinical and biochemical findings.

Subject's height and weight were measured and Body Mass Index (BMI) was calculated. Hirsutism was evaluated using Ferriman and Gallway Scoring System (23). Marynick score system was adopted to examine the acne severity (24). Oligomenorrhea was defined as less than 8 cycles per year and amenorrhea as 0-2 cycles per year. Regular mensutral cycles were defined as having regular periods every 21 to 35 days.

Clinical symptoms were considered positive if subjects were suffering from hyperandrogenemia (hirsutism, acne), and chronic unovulation (amenorrhea, oligomenorrhea or irregular mensturation).

Biochemical measurements included the followings: Testosterone (T), Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Dehydroepiandrosterone Sulfate (DHEAS), Sex hormone Binding Globulin (SHBG), 17-hydroxy progesterone (17-OHP), Insulin, Fasting blood sugar, 2 hours blood sugar, and leptin.

Serum hormone levels were quantified by well-established RIA methods, using BIO Source Europe S.A., and manufacturing. Leptin was measured by a direct RIA kit (DRG Instruments GmbH, Germany). In all assays intra-assay and inter-assay coefficients of variation didn't exceed 7% and 15%, respectively. Biochemical findings were considered positive if T was more than 1.1 ng/ml and or the ratio of LH/FSH was more than 2.

Student's t-test and Chi-square test were used for comparing quantitative and qualitative variables, respectively. $P<0.05$ was considered statistically significant. Results are expressed as means ± SD. Twin statistical analysis has been explained elsewhere (25).

Results:

More than 33% (33.2%) of the subjects had polycystic ovaries (PCOs) in ultrasound. Abnormal laboratory findings were 21.65% in the whole sample set. When clinical and biochemical findings were both considered as the diagnostic tool. 16.2% of the subjects were diagnosed as PCOS positive (25/154). 18% percent of which were within MZ twins (11/96), while the corresponding value for DZ twins were 8%. (8/58).

Demographic characteristics of MZ and DZ twins with and without PCOS have been shown in Table 1. Overall, no significant difference was found between demographic data of MZ or DZ twins with or without PCOS. Subjects were fairly young with the average age of 21.14(±6.04) for MZ twins and 22.78(±7.60) for DZ twins.

Obesity (those with BMI more than 25 kg/m2) was found in 42.1% of MZ twins in comparison with 30.3% of DZ twins. However, Chi-square test did not show any significant difference between obese and non-obese (BMI less than 25 kg/m2) subjects (p=0.181). Also, a comparison between mean values of BMI for the two groups of MZ and DZ twins were not found to be significant.

Overall, 28.5% of subjects had hirsutism,(14.3% severe, 7.1% moderate and 7.1% mild). 48.7% had acne (38.3% mild, 10.4% moderate and none were severe), 2.6% were amenorrehic, 7.7% had oligomenorrhea and 29.9% had irregular menstruation.

Table 2 shows the clinical features of subjects with and without PCOS within the two groups of MZ and DZ twins. As it has been demonstrated in the table, hirsutism was found to be more common among MZ twins with PCOS (P= 0.0006) in comparison with those without PCOS. Fisher test was used for comparing

variables such as amenorrhea and infertility and no significant difference was found between those with or without PCOS.

Laboratory findings suggest that SHBG has been significantly lower in MZ twins with PCOS (p = 0.016). Comparison of other laboratory measurement did not show any significant difference between the 2 groups. Moreover, the mean level of leptin in serum was not significantly differ between the two groups of subjects with or without PCOS for MZ twins (P = 0.111), or for DZ twins (P = 0.289) after matching for BMI.

When the serum level of leptin was compared between the two groups of PCOS+ and non PCOS- irrespective of their zygosity, the mean level was 11.62(±7.67) and 13.84(±9.92) and comparing the mean values, no significant difference was found (p = 0.073).

Correlation between the serum level of leptin for MZ twins (Twin1-Twin2) resulted in rMZ = 0.757. This means that there is a similarity between the serum leptin levels of MZ twin sets (Figure 1). The corresponding value for DZ twins however was rDZ = 0.384 (Figure 2), suggesting that (rMZ > rDZ). A comparison between these two correlations suggests a significant difference (z= -3.44, P = 0.0003).

The data were also analyzed by model-fitting approach to test genetic effect. In this analysis, for each quantitative variable trait (Y), the data was fitted in three models as follows:

Model 1: Y=m+G+C+E

Model 2: Y=m+G+ E

Model 3: Y=m+C+E

m is the overall mean for each trait. The second model (Y=m+G+ E), when compared to the first model (Y=m+G+C+E), results in a p value. The lower the value, the greater is the environmental effect. The p value for leptin comparing GE vs GCE was 0.775. Genetic effect was also tested by comparison of model 3 (Y=m+C+E) and model 1. The p value which resulted as the comparison between CE and GCE was 0.012 suggesting that leptin level is determined by genetic factors.

Table 1. Descriptive data of MZ (n=96 individuals, 48 pairs) and DZ (n=58 individuals, 29 pairs) twins with and without PCOS.

	MZ N =96		P Value	DZ N=58		P Value
	PCO-(n=79)	PCO+(n=17)		PCO-(n=50)	PCO+(n=8)	
Age (year)	23.3(±5.05)	24.84(±3.12)	0.231	22.11(±4.79)	23.42(±3.87)	0.466
Menarche(year)	12.53(±1.27)	12.59(±0.94)	0.863	12.79(±1.32)	12.5(±1.5)	0.573
Weight(Kg)	60.27(±14.9)	62.24(±7.09)	0.598	56.62(±10.93)	61.38(±11.1)	0.260
Height(m)	1.61(±6.62)	1.59(±6.50)	0.230	1.60(±5.69)	1.62(±7.64)	0.546
BMI(kg/m2)	23.3(±5.05)	24.84(±3.13)	0.231	22.11(±4.79)	23.42(±3.87)	0.466

Table 2. Clinical symptoms of MZ (n=96 individuals, 48 pairs) and DZ (n=58 individuals, 29 pairs) twins with and without PCOS.

	MZ N =96		P Value	DZ N=58		P Value
	PCO-(n=79)	PCO+(n=17)		PCO-(n=50)	PCO+(n=8)	
Hirsutism	42.9%	57.1%	0.006*	56.3%	43.8%	0.165
Acne	60.8%	39.2%	0.407	75%	25%	0.404
Amenorrhea	50% (n=1)	50%(n=1)	0.585∞	100%(n=2)	-	0.472∞
Infertility	66.7%(n=2)	33.3%(n=1)	0.715∞	100%(n=2)	-	0.472∞
Irregular menstruation	50%(n=14)	50%(n=14)	0.15	77.8%(n=12)	22.2%(n=6)	0.33

*P<0.005 when PCOS- and PCOS+ in MZ group were compared.

∞ Fisher Test was used

Table 3. Laboratory findings of MZ (n=96 individuals, 48 pairs) and DZ (n=58 individuals, 29 pairs) twins with and without PCOS.

	MZ N =96		P Value	DZ N=58		P Value
	PCO-(n=79)	PCO+(n=17)		PCO-(n=50)	PCO+(n=8)	
LH(mIU/ml)	6.25(±4.52)	5.29(±3.58)	0.318	5.99(±3.68)	4.06(±1.38)	0.11
FSH(mIU/ml)	6.05(±2.20)	5.29(±2.0)	0.116	5.68(±1.8)	5.5(±2.19)	0.787
LH/FSH	1.09(±0.72)	1.19(±1.17)	0.609	1.19(±1.0)	0.99(±1.14)	0.591
T(ng/ml)	10.41(±77.43)	99.52(±53.32)	0.378	3.6(±14.73)	210.43(±663.93)	0.35
DHEA(ng/ml)	1983.32(±1399.41)	2163.22(±1545.23)	0.795	2448.48(±1306.26)	2336.71(±1096.87)	0.804
SHBG(nmol/L)	55.05(±25.99)	41.21(±23.83)	0.016*	57.25(±24.29)	48.95(±19.92)	0.317
17-OHP(ng/ml)	1.53(±1.36)	1.65(±2.34)	0.751	11.61(±1.09)	13.9(±7.2)	0.135
Insulin(mIU/ml)	14.73(±7.46)	15.02(±11.06)	0.882	12.31(±4.88)	13.11(±9.38)	0.695
FBS(umg/dl)	77.48(±17.61)	76.42(±27.86)	0.822	72.92(±13.81)	74.5(±13.19)	0.741
2HBS(umg/dl)	81.37(±26.25)	92.77(±75.24)	0.286	82.74(±34.31)	64.72(±33.21)	0.154
Leptin	12.53(±8.22)	15.77(±10.41)	0.111	10.36(±6.73)	7.83(±4.85)	0.289

*P<0.016

Discussion

Using clinical and laboratory measures for diagnosis, 16.2% of subjects were found to have PCOS. The incidence of PCOS has been found to vary between 16-25% (11, 25, 26, 27, 28) in normal populations, those with symptoms and family studies. Most of those studies used ultrasound and or biochemical findings as the golden standard. In this study a combination of clinical and biochemical findings were used for diagnosis of PCOS consistent with the recommendation of the 1990 National Institute of Child Health and Human Development (NICHD) Conference (29). The overall incidence of PCOS in our data set is similar to most family studies. It was interesting to find that the incidence of PCOS for MZ twins were more than

twice of that of DZ twins. Another twin study which was done on 34 female-female twin pairs of Australian women using ultrasound (as model 1) and/or biochemistry (as model 2) as the golden standard showed the incidence of 50% and 38% respectively (21) which is more than that of normal population. The result of current study also shows the incidence of PCOS is higher among twin sets.

This study considered more precise measures in terms of diagnosis and yet the incidence of PCOS in MZ twins was also found to be higher than that of the DZ twins. Both of these studies and many other familial studies suggest that a genetic background does in fact exist for PCOS. At the same time having discordant MZ twins suggests that the mode of transition is not autosomal dominant, rather a more complex model should be sought. In our study 13 MZ twin pairs (27.08% of the subjects) had discordant results meaning that one twin had PCOS and the other sister was normal in terms of both clinical and biochemical symptoms.

Another interesting fact is that the incidence of PCOS using ultrasound diagnosis was higher (33.2%) than that of the biochemical (21.65%) alone or a combination of clinical and biochemical diagnosis (16.2%). This finding is consistent with U.S. publications where practitioners prefer not to use ultrasound as the only measure for diagnosis of PCOS. The presence of polycystic ovaries may be the result of excessive levels of circulating androgens from any source which will lead to the disruption of follicular development and the accumulation of many small, atretic follicles in the ovaries cortex, producing a polycystic appearing picture. Moreover, during early follicular phase, antral follicles appear on the surface of the ovary which may lead to the wider transversal measurements and may also be mistaken as the PCO appearance, obscuring the diagnosis of PCO from normal ovary.

Skin problems namely hirsutism and acne were found to be high in our sample set. Higher rate of obesity among MZ twins may lead to more clinical symptoms as it was the case in this study. The rate of hirsutism is found to be higher among MZ twins with PCOS+ (57.1%) as compared with the corresponding value for DZ twins (43.8%). As Table 2 shows the difference of hirsutism rate between PCOS+ and PCOS- subjects in MZ twins were found to be significant.

This finding is further supported by the fact that SHBG was lower in MZ twin sets with PCOS+. It is well known that obesity leads to higher metabolism rate of androgens which in the absence of SHBG leads to hyperandrogenemia (30).

Although some studies have found a lower level of leptin for PCOS+ subjects (15), this finding has not been supported by others (12, 31). Our data shows a lower level of leptin for PCOS+ subjects although the difference between PCOS+ and PCOS- subjects is not significant. When the subjects are further classified using the zygosity, no significant difference can be found either. Moreover, when correlation between twin pairs was analyzed, a significant correlation was found between MZ twins suggesting a similarity between the serum levels of leptin for each pair. This finding suggests that serum level of leptin may be regulated by genetic factors.

Model analysis was adopted to find whether leptin level is determined by genetic factors. Within twin differences and between twins differences showed that serum levels of leptin are determined by genetic factors. However, the role of environment cannot be ruled out since the fat mass and obesity can also decrease the leptin produced in the body.

In conclusion, findings suggest that the level of leptin does not differ in subjects with or without PCOS. The serum level of leptin is genetically determined but environmental factors such as obesity may alter its secretion in PCOS subjects. Obesity, itself may be under genetic control or may be the result of some other problems such as eating disorders.

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References:

1. Remsberg, K.E., Talbott E.O., Zborowski J.V., Evans R.W., McHugh-Pemu K. Evidence for competing effects of body mass, hyperinsulinemia, insulin resistance, and androgens on leptin levels among lean, overweight, and obese women with polycystic ovary syndrome. Fertil. Steril., 2002:78:479-86.

2. Pelleymounter M.A., Cullen M.J., Baker M.B., Hecht R., Winters D., Boone T. Effects of the obese gene product on body weight regulation in ob/ob mice. Science., 1995; 269:540-3.

3. Halaas J.L., Gajiwala K.S., Maffei M., Cohen S.L., Chait B.T., Rabinowitz D. Weight reducing effects of the plasma protein encoded by the obese gene. Science. 1995; 269:543-6.

4. Barash I.A., Cheung C.C., Weigle D.S., Ren H.P., Kabigting E.B., Kuijper J.L. Leptin is a metabolic signal to the reproductive system. Endocrinol., 1996; 137:3144-7.

5. Chehab FF, Lim M.E., Lu R.Correction of the sterility defect in homozygous. obese female mice by treatment with the human recombinant leptin. Nature. 1996;12:318-20.

6. Teirmaa T., Luukkaa V., Rouru J., Koulu M., Huupponen R .Correlation between circulating leptin and luteinizing hormone during the menstrual cycle in normal-weight women. Eur J Endocrinol., 1998:139:190-4.

7. Messinis I.E., Milingos S., Zikopoulos K., Kollios G., Seferiadis K., Lolis D. Leptin concentrations in the follicular phase of spontaneous cycles and cycles superovulated with follicle stimulating hormone. Hum Reprod., 1998;13:1152-6.

8. Shimizu H., Shimonura Y., Nakanishi Y., Futawatari T., Ohtani K., Sata N. Estrogen increase in vivo leptin production in rats and human subjects. J Endocrinol., 1997; 154:285-92.

9. Fernandez-Real J.M., Casamitjana R., Ricart-Engel W. Leptin is involved in gender-related differences in insulin sensitivity. Clin Endocrinol.,1998; 49:505-511.

10. Ovalle F., Azziz R. Insulin resistance, polycystic ovary syndrome, and type 2 diabetes mellitus. Fertil. Steril. 2002; 77:1095-1105.

11.Acien P. Quereda F., Matalin P., Villarroya E., Lopez-Fernnandez J.A., Acien M., Mauri M., Alfayate R. Insulin, androgens, and obesity in women with and without polycustic ovary syndrome: a heterogeneous group of disorders. Fertil. Steril. 1999 ; 72:32-40.

12. Lindheim S.R. Sauer M.V., Carmina E., Chang P.L., Zimmerman R., Lobo R.A. Circulating leptin levels during ovulation induction: relation to adiposity and ovarian morphology. Fertil. Steril. 2000;73: 493-8.

13. Carmina E., Ferin M., Gozalez F., Lobo R.Evidence that insulin and androgens may participate in the regulation of serum leptin levels in women. Fertil. Steril.,1999;72:926-31.

14. Rouru J., Anttila L., Koskinen K., Penttila T.A., Irjala K., Huupponen R. Serum leptin concentration in women with polycystic ovary syndrome. J Clin Endocrinol.1997; Metab. 82:1697-1700.

15. Agarwal S.K., Vogel K., Magoffin D.A. Leptin antagonizes IGF-I augmentation of FSH-stimulated Oestradiol production in human granulose cells. Hum Reprod.,1997: 12:68-71.

16. Mantoros C.S. Dunaif A., Flier J.S. Leptin concentrations in the polycystic ovary syndrome. J Clin Endocrinol Metab.,1997; 82:1687-91.

17. Laughlin G.A., Morales A.J., Yen S.C.C. Serum leptin concentrations in polycystic ovary syndrome :relation to anthropometric and metabolic parameters. Clin Metab.,1997;82:1692-6.

18. Legro R.S., Strauss J.F., Molecular progress in infertility :PCO. Fertil. Steril.2002; 78:569-76.

19. Sanders E. PCOS development influences by multiple genes. Fertil. Steril 2002; 78:473-8.

20. Jahanfar S., Eden J.A. Bulimia nervosa and polycystic ovary syndrome. Gynecol Endocrinol.,1995; 9:113-7.

21. Jahanfar S. Eden J.A., Ngyent T.V., Warren P., Seppala M. A twin study of polycystic ovary syndrome. Fertil. Steril., 1995;63:478-86.

22. Adams J., Polson D.W., Franks S. Prevalence of polycystic ovaries in women with unovulation and idiopathic hirsutism. Br. Med. J.1986; 293:355-59.

23. Ferriman D., Gallwey J.D. Clinical assessment of body hair growth in women. J Clin Endocrinl Metab. 1961;21:1440-1447.

24. Marynick S.P., Chakmakjian Z.H., McCaffree D.L., Herndon J.H. Androgen excess in cystic acne. N Eng J Med.,1983; 308: 981-985.

25. Jahanfar S., Garret D.K., Eden J.A. How reliable are biochemical tests in the diagnosis of PCOS? Aut J Med Scien., 1994;15:1-4.

26. Govind A, Obhari M.S., Clayton R.N. Polycystic ovaries are inherited as an autosomal dominant trait: Analysis of 29 PCO and 10 control families. J Clin Endocrinol Metab1999;84:38-43.

27. Farquhar C.M., Birdsall M., Manning P., Mitchell J.M., France J.T.The prevalence of polycystic ovaries on ultrasound scanning in an population of randomly selected women. Aust NZ J Obstet Gynecol.,1995; 34:67-72.

28. Adams J., Polson D.W., Franks S. Prevalence of polycystic ovaries in women with unovulation and idiopathic hirsutism. Br Med J.1986: 293:355-359.

29.Zawadzki J.K., Dunaif A. Diagnostic criteria for polycystic ovary syndrome:towards a rational approach. In: Dunaif A., Givens J.R., Haseltine F., Merriam G.R., eds. Polycystic ovary syndrome. Boston, MA: Blackwell Scientific, 1992:377-84.

30. Acien P., Quereda F., Matallin P., Villarroya E., Lopez-Fernandez J.A., Acien M., Mauri M., Alfayate R. Insulin, androgens, and obesity in women with and without polycystic ovary syndrome: a heterogeneous group of disorders. Fertil Steril1999;72:32-40.

31. Rouru J., Anttila L., Koskinen P., Penttila T.A., Irtala K., Huuppana R. Serum leptin concentrations in women with polycystic ovary syndrome. J Clin Endocrinol Metab., 1997;82:1697-700.