Iraqi Journal of Biotechnology, 2024, Vol. 23, No. 3, 83-89

Leptin Gene Polymorphism (rs7799039), Some Serum Hormones and Lipid Profile Parameters in Iraqi Women with PCOS

[1]Fadaa A. Mahmoud, [2] Ismail A. Abdulhassan

[1]College of Health and Medical Techniques, AL-Bayan University
[2]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

Received: October 5, 2023 / Accepted: November 7, 2023 / Published: December 30, 2024

Abstract: Polycystic ovary syndrome (PCOS) is a complicated, heterogeneous disorder, which affects 6–20% of women of reproductive age, causes a range of problems in the reproductive, metabolic, and endocrine systems. Polycystic ovaries, hyperandrogenism, and prolonged anovulation are its defining features. It appears differently as a result of a number of interconnected factors, including heredity, dietary habits, and environmental exposure. This study aimed to study of leptin gene polymorphism rs7799039 and some serum parameters in Iraqi women with polycystic ovary syndrome. By using ELISA kits, the levels of leptin and the soluble leptin receptor (sOB-R) were measured. Except for anti-mullerian hormone (AMH), which was assessed by Elisa, and the lipid profile parameters, which were measured by spin 200 full auto, other hormones were measured by cobas E411. HRM-PCR was used to determine the genotypes and allele frequency of rs7799039 SNP at Leptin gene. PCOS patients had significantly ($p<0.05$) higher serum leptin and sOB-R levels than controls (4303.28±71.55 *versus* 3854.84 ±213.96 pg /ml and 144.09 ±5.51 *versus* 122.16 ±7.64 ng/ml, respectively). The frequency of GG genotype was in PCOS patients significantly lower than in apparently healthy subjects (18.3 % *versus* 60%, respectively),while the frequencies of GA and AA genotypes were significantly greater in PCOS patients compared to controls that appear to be healthy (56.7% *versus* 36.6% and 25.0% *versus* 3.4%, respectively).The G allele frequency was higher in apparently healthy subjects than in PCOS women (78.3% *versus* 46.6%, respectively) while A allele frequency was significantly greater in PCOS patients compared to people who appear to be healthy (53.4% vas 21.7%, respectively). It was concluded an A allele-related risk factor for PCOS in iraqi patients. Sgnificant difference between GA and AA genotypes in estrogen hormone and significant difference between GG,GA and AA genotypes in AMH hormone were noted.

Keywords: leptin gene, hormones and lipid profile.

Corresponding author: (Email: fathaa.m@albayan.edu.iq).

Introduction

Polycystic ovary syndrome (PCOS) is a frequent endocrinological condition in women of reproductive age which characterized by prolonged anovulation, increased androgen production, and polycystic ovarian morphology (1). Numerous degrees of obesity, dyslipidemia, oxidative stress, insulin resistance (IR), and other metabolic disorders have been identified through studies (2) .dyslipidemia is one of the most frequently seen symptoms in PCOS women (3).

Insulin resistance and a high prevalence of obesity are linked to polycystic ovarian syndrome (PCOS). The *ob* gene's product, leptin, circulates in both its free and bound forms and is

implicated in the control of energy balance and obesity. The most significant leptin-binding protein is the soluble leptin receptor (sOB-R), which affects the level of physiologically active (4).

PCOS may result from a combination of genetic, epigenetic, and lifestyle factors (5). Despite being an inherited disorder, obesity makes polycystic ovarian syndrome worse.Obesity and PCOS are intimately associated, as shown by epidemiological evidence and more recently verified by genetic research. It indicates that adipokines production by visceral and subcutaneous fat affects metabolic process (6).In addition, Comparing the PCOS group to the control group, the PCOS group was significantly overweight (7).

Also, PCOS has a substantial heritable component, as shown by familial clustering and twin studies, however the exact cause is still unknown. Numerous novel risk loci and potential PCOS genes have been discovered by genome-wide association studies (GWAS). Despite these results, fewer than 10% of heritability has been explained by association studies (8).

Several polymorphisms studied in PCOS patients and in different populations.one of the Iraqi studies for example was on variation in the GSTO2 gene for glutathione-S-transferase omega 2 (GSTO2) and the prevalence of polycystic ovary syndrome in Iraqi women (9). Also, Tabark and Abdul Hussein(10) study on Large Deletion of Sex Hormones Binding Globulin SHBG Gene in Iraqi Women with Polycystic Ovary Syndrome and found that , PCOS patients could be due to the novel deletion of this gene. The studies about the leptin gene and its association with PCOS are not enough .So, the aim of the present study is to study the association of one of the polymorphisms at the leptin gene (G2548A,rs7799039) and association with polycystic ovary syndrome in a sample of Iraqi women.

Materials and methods

This case-control study was carried out during the period from July 2022 to October 2022, at Infertility Care and IVF center of Kamal AL-Samaraay Hospital/ Baghdad. The study includes 120 Iraqi women as a sample size divided into two groups 60 PCOS patients and 60 apparently healthy controls.

The investigator interviewed and examined the PCOS patients(n= 60)in order to determine and record the necessary data after the adopted questionnaire, which included patient's history, clinical examination, laboratory investigation, and abdominal ultrasound. The diagnosis was made in accordance with Rotterdam ESHRE/ASRM criteria. Following the extraction of genomic DNA according to the Transgenbiotech Company's technique, agarose gel electrophoresis was utilized to verify the presence and integrity of the isolated DNA. Nanodrop equipment was used to assess DNA sample concentration..A ratio of (1.8) is generally accepted as “pure” for DNA. High Resolution Melting (HRM) PCR used for identifying polymorphism rs7799039 at leptin gene. By using an ELISA assay, the levels of leptin and leptin receptor hormones were determined. AMH is measured by Elisa, while the other hormones are all measured using Cobas E411 tests: in addition to lipid profile by spin 200 complete auto. To analyze various study parameters, the Statistical Analysis System- SAS (2018) program(11) was utilized. In this study, the least significant difference (LSD)

test and the chi-square test were both utilized to make comparisons between means and percentages. The odd ratio was also used to identify risk factors.

Results and discussion

Table (1) showed a significant increase in the serum leptin levels in PCOS patients compared with apparently healthy controls (4303.28 ±71.55 *versus* 3854.84 ±213.96 ng/ml, respectively).these results are in agreement with Yuanyuan Peng *et al* (12) who found that serum leptin levels were significantly higher in PCOS patients than in apparently healthy controls. This result is clearly expected because leptin is predominantly synthesized by adipocytes, which increase in obesity and higher BMI which considered as main features of PCOS(13). In addition, the levels of leptin receptor in serum were also in PCOS patients higher than in apparently healthy controls as (144.09 ±5.51 *versus* 122.16 ±7.64 pg/ml, respectively). These results disagree with the results of Vicken *et al.* (14) who found no significant difference between PCOS patients and apparently healthy controls as related with sOB-R levels in serum, whereas, the results of the present study are in agreement with the results of Xiaoyu *et al.*(15) who found an increase in the plasma sOB-R in PCOS patients compared with controls. Also , the present results disagree with Susanne Hahn *et al.* (4) who found that sOB-R levels were low in PCOS patients compared with controls and supposedly compensate diminished leptin action that PCOS might cause leptin resistance.

Table (1): The concentrations of serum leptin and Sob –R in PCOS patients *versus* apparently healthy subjects. (mean± SE)

Groups	Serum leptin (ng / ml)	Serum leptin receptor (Sob-R) (pg/ml)
Apparently healthy subjects (Control)	3854.8 ±214.0	122.2 ±7.6
PCOS[1] patients	4303.3 ±71.6	144.1 ±5.5
***t* test**	360.31 *	18.858 *
***p* value**	0.0153	0.0232

[1] PCOS means polycystic ovary syndrome.

The frequencies of genotypes and alleles for Leptin gene at rs7799039 SNP in PCOS patients versus apparently healthy subjects are presented in table (2). The GG genotype(wild-type) percentage was in PCOS patients lower than in apparently healthy controls (18.3% *versus* 60%,respectively).this result means that the mutations in PCOS patients were equal to two fold those in controls.Kargasheh *et al.* (16) found there is a significant difference in the rs7799039 polymorphism's GG genotype frequency between PCOS patients and healthy controls, and these findings concur with those of the current study.

In comparison to controls, PCOS patients had considerably greater percentages of the GA and AA genotypes (56.7% and 25% versus 36.6% and 3.4%, respectively). The frequency of the G allele was 78.3% in the control group and 46.6% in the PCOS patient group, whereas the frequency of the A allele was 21.7% in the control group and 53.4% in the PCOS patient group. These findings concur with those of Shilpa Shetty *et al.*(17), who found that in the seemingly healthy control subjects, the G allele frequency was considerably greater than

the A allele frequency. According to the findings, the rs7799039 SNP of the leptin gene is associated with an A allele-related risk factor for PCOS incidence in Iraqi women.

Table (2): Genotypes and allele frequencies for leptin gene at rs7799039 SNP in PCOS patients *versus* apparently healthy subjects.

Genotypes Rs7799039	Control [1] n (%)	PCOS[2] Patients n (%)	*Chi square* (X^2)	Odd Ratio (95% CI)	*p*- value
GG	36 (60.00%)	11 (18.33%)	--	Ref.	--
GA	22 (36.67%)	34 (56.67)	14.4	5.05 (2.13-1.98)	0.0002
AA	2 (3.33%)	15 (25.00%)	21.7	24.54 (4.84-124.35)	0.0001
Allele frequency					
G	78.33%	46.67%	--	Ref.	--
A	21.67%	53.4%	20.5	4.13 (2.35-7.25)	0.0001

[1] Apparently healthy subjects. [2] polycystic ovary syndrome.

No significant differences were found among different genotypes of rs7799039 polymorphism of leptin gene in PCOS patients and apparently healthy subjects as related with serum leptin levels and serum leptin receptor levels(Table 3). The rs7799039 polymorphism of leptin gene is located in the promoter region and influences the serum leptin expression (18), while, Marcello *et al*. (19) found a correlation between leptin gene genotypes and serum leptin concentrations. The present study show no significant differences in the leptin and leptin receptor levels between the three genotypes of leptin gene polymorphism rs7799039 in both PCOS patients group and apperantly healthy controls group. Also, these results are in disagreement with that of Marcello *et al*. (19) who found that the PCOS patients who presented the AA genotype of rs7799039 in *leptin* gene had lower serum levels of leptin than those with the AG genotype, also, in disagreement with Hoffsted *et al.* (18) who showed that individuals with the AA genotype of rs7799039 had higher serum leptin concentrations than the AG or GG genotypes carriers. Absence of significant differences between the different genotypes may be due to small sample size.

Table (3): Association of *leptin gene* polymorphism at rs7799039 SNP with serum leptin levels and serum leptin receptor levels in PCOS patients *versus* controls. (mean±SE)

Study groups	*Leptin gene* genotypes at rs7799039 SNP	Serum leptin receptor levels (pg/ml)	Serum leptin levels (ng/ml)
PCOS Patients	**GG**	122.57 ±13.79	4448.89 ±123.88
	GA	150.93 ±6.70	4316.51 ±86.82
	AA	144.38 ±11.87	4166.53 ±188.49
LSD (P-value)		30.406 NS (173)	402.86 NS (0.451)
Control	**GG**	131.67 ±11.38	3683.96 ±268.54
	GA	109.92 ±7.76	4062.24 ±381.74
	AA (No=1)	85.83 ±0.00	4649.38 ±0.00
LSD (P-value)		71.219 NS (0.248)	2159.50 NS (0.572)

NS mean no significant

As shown from table 4,the serum concentrations of LH, FSH, prolactin, testosterone, progesterone, TSH, T3 and T4 hormones were unaffected by leptin gene genotypes at rs 7799039.there is an A allele-related increase in serum estrogen levels in PCOS patients(37.29±7.53 and 23.60±3.96 for GA and AA genotypes *versus* 17.83±3.28 for GG genotype). In contrast, there is an A allele-related decrease in serum anti-mullarian hormone levels in PCOS patients (5.29±1.01 and 4.91±0.92 for GA and AA genotypes *versus* 8.23±1.51 for GG genotype. These results are agree with the those of Shilpa Shetty *et al* (17) who found no significant differences in the levels of FSH, LH, testesterone, estradiol and prolactin in PCOS patients in a study based on 150 PCOS patients.

Table (4): Association of *leptin gene* polymorphism at rs7799039 SNP with serum hormones levels in PCOS patients.. (mean±SE).

Serum hormones	*Leptin gene* polymorphsim rs7799039			LSD (P-value)
	GG	GA	AA	
LH	5.34 ±0.63	10.68 ±1.58	6.28 ±0.87	5.89 (0.095) NS
FSH	6.35 ±0.68	6.89 ±1.12	6.58 ±1.02	4.22 (0.954) NS
LH/ FSH ratio	0.884 ±0.18	1.67 ±0.29	1.108 ±0.34	1.127(0.260) NS
Prolactin	25.20 ±4.82	32.45 ±8.54	27.11 ±4.94	23.70 NS (0.774)
Testosterone	0.480 ±0.06	0.688 ±0.18	0.345 ±0.07	0.470 NS (0.267)
Estrogen	17.83 ±3.28 b	37.29 ±7.53 a	23.60 ±3.96 ab	18.63 * (0.049)
Progesterone	2.52 ±0.23	2.49 ±0.23	2.27 ±0.12	0.635 NS (0.647)
AMH	8.23 ±1.51 a	5.29 ±1.01 b	4.91 ±0.92 b	2.907 * (0.045)
TSH	2.99 ±0.31	2.74 ±0.65	2.69 ±0.40	1.43 NS (0.877)
T3	1.114 ±0.09	1.157 ±0.03	1.128 ±0.04	0.192 NS (0.862)
T4	7.36 ±0.57	37.13 ±19.21	8.79 ±0.21	32.40 NS (0.446)

*** (P≤0.05), NS: Non-Significant.**

As shown from table 5,the serum concentrations of Cholesterol, Triglyceride, HDL, LDL, VLDL were unaffected by leptin gene genotypes at rs 7799039. So, there were no significant differences in lipid profile between the three genotypes of rs7799039 of leptin gene in the PCOS patients group and this may be attributed to small sample size in present study. These results disagree to some extent with results of Eda Becer *et al*(20) who study the association between leptin gene G-2548A polymorphism and BMI in both patients and control groups and found that obese subjects with the AA genotype had significantly higher serum total cholesterol than GA and GG genotypes. Their study included 110 obese and 90 non-obese subjects. also the present results disagree to some extent with that of Bouafi H et al(21)who compare the biochemical and clinical parameters between the genotypes of the rs7799039 polymorphism and showed a significant increased triglycerides levels in carriers of AA or GA genotypes.

Table (5): Association of *leptin gene* polymorphism at rs7799039 SNP with serum lipid profile in PCOS patients.. (mean±SE).

Parameters (mg/dl)	*Leptin gene* polymorphism at rs7799039			LSD (P-value)
	GG	GA	AA	
Cholesterol	160.48 ±6.58	162.83 ±14.74	159.00 ±13.80	47.86 NS 0.981
Triglyceride	88.96 ±12.33	78.67 ±15.20	107.00 ±21.32	42.44 NS 0.315
HDL	29.72 ±0.82	58.08 ±14.39	30.80 ±4.22	42.02 NS (0.229)
LDL	112.42 ±5.25	88.47 ±17.71	107.00 ±9.81	55.76 NS (0.578)
VLDL	17.65 ±2.57	9.56 ±3.01	21.20 ±4.21	11.98 NS (0.057)

NS: Non-Significant

Conclusion

The rs7799039 polymorphism of leptin gene is associated with PCOS. There was an A allele-related risk factor for PCOS in iraqi patients.there is significant increase in both serum leptin and leptin receptor hormones in PCOS patients but no significant differences were found among different genotypes of rs7799039 polymorphism of leptin gene in PCOS patients and apparently healthy subjects as related with serum leptin levels and serum leptin receptor levels and this results be in accordance with the lipid profile results. Also all studied hormones had no significant differences among the three genotypes of that polymorphism except estrogene and anti-mullarian hormone.

References

1. Azziz, R.; Carmina, E.; Chen, Z.; Dunaif, A.; Laven, J. S.; Legro, R. S.; *et al*. (2016). Polycystic ovary syndrome. Nature reviews Disease Primers, 2(1): 1-18.
2. Tehrani, F. R.; Rashidi, H.; Khomami, M. B.; Tohidi, M. and Azizi, F. (2014). The prevalence of metabolic disorders in various phenotypes of polycystic ovary syndrome: a community based study in Southwest of Iran. Reproductive Biology and Endocrinology: RB&E, 12, 89.
3. Macut, D.; Bjekić-Macut, J. and Savić-Radojević, A. (2013). Dyslipidemia and oxidative stress in PCOS. Frontiers of Hormone Research, 40: 51–63.
4. Hahn, S.; Haselhorst, U.; Quadbeck, B.; Tan, S.; Kimmig, R.; Mann, K.; *et al*. (2006).Decreased soluble leptin receptor levels in women with polycystic ovary syndrome. European Journal of Endocrinology, 154(2): 287-294.
5. Bruni, V.; Capozzi, A. and Lello, S. (2022). The role of genetics, epigenetics and lifestyle in polycystic ovary syndrome development: the state of the art. Reproductive Sciences, 1-12.
6. Barber, T. M.; Hanson, P.; Weickert, M. O. and Franks, S. (2019). Obesity and polycystic ovary syndrome: implications for pathogenesis and novel management strategies. Clinical Medicine Insights: Reproductive Health, 13, 1179558119874042.
7. Naeemah, H.; Sahsavari, Z. and Mohammed, M. T. (2021). Estimation of Reactive Oxygen Species and Total Antioxidant Capacity In Serum of Iraqi Females With Pcos. Turkish Journal of Physiotherapy and Rehabilitation, 32, 3.
8. Crespo, R. P.; Bachega, T. A.; Mendonça, B. B. and Gomes, L. G. (2018). An update of genetic basis of PCOS pathogenesis. Archives of Endocrinology and Metabolism, 62: 352-361.
9. Ahmed, S. and Abdulmaged, Ismail, A. (2022). Abdul-Hassan Association of Glutathione-S-Transferase Omega 2 (GSTO2) Gene Polymorphism with Incidence of Polycystic Ovary Syndrome in Iraqi Women. (21)2.
10. Jassim, T. S. and AlFaisal, A. H. M. (2020). Large Deletion of Sex Hormones Binding Globulin-SHBG Gene in Iraqi Women with Polycystic Ovary Syndrome-

PCOS. Iraqi Journal of Biotechnology, 3(19).
11. SAS (2018). Conference Proceedings: SAS Global Forum 2018. April 8-11, Denver, Colorado, 471 papers.
12. Peng, Y.; Yang, H.; Song, J.; Feng, D.; Na, Z.; Jiang, H.; *et al*. (2022). Elevated serum leptin levels as a predictive marker for polycystic ovary syndrome. Frontiers in Endocrinology, 13, 845165.
13. Abdul, A. S. A. I. A. (2022). Association of Glutathione-S-Transferase Omega 2 (GSTO2) Gene Polymorphism with Incidence of Polycystic Ovary Syndrome in Iraqi Women. Iraqi Journal of Biotechnology, *21*(2).
14. Sepilian, V. P.; Crochet, J. R. and Nagamani, M. (2006). Serum soluble leptin receptor levels and free leptin index in women with polycystic ovary syndrome: relationship to insulin resistance and androgens. Fertility and Sterility, 85(5): 1441-1447.
15. Tu, X.; Yu, C.; Gao, M.; Zhang, Y.; Zhang, Z.; He, Y.; et al (2017).LE.PR gene polymorphism and plasma soluble leptin receptor levels are associated with polycystic ovary syndrome in Han Chinese women. Personalized Medicine, 14(4): 299-307.
16. Kargasheh, F. B.; Ansaripour, S.; Borumandnia, N.; Moradi, N.; Zandieh, Z.; Maleki, M.; *et al*. (2021). Association of leptin G2548A and leptin receptor Q223R polymorphisms and their serum levels with infertility and recurrent pregnancy loss in Iranian women with polycystic ovary syndrome. PloS one, 16(8): e0255920.
17. Al-Shattawi, S. S., Al-Jumili, E. F., & Al-Azzam, M. A. (2018). The relationship between obesity and polycystic ovary syndrome in a sample of Iraqi infertile women. Iraqi Journal of Biotechnology, 17(3).
18. Hoffstedt, J.; Eriksson, P.; Mottagui-Tabar, S. and Arner, P. (2002). A polymorphism in the leptin promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin. Hormone and Metabolic Research, 34(07): 355-359.
19. Marcello, M. A.; Calixto, A. R.; De Almeida, J. F. M.; Martins, M. B.; Cunha, L. L.; Cavalari, C. A.; *et al*. (2015). Polymorphism in LEP and LEPR may modify leptin levels and represent risk factors for thyroid cancer. International Journal of Endocrinology, 2015.
20. Becer, E.; Kızılkanat, M.; Tınazlı, M. and Serakıncı, N. (2016). Association between leptin G-2548A gene polymorphism, plasma leptin levels and lipid profiles in Turkish Cypriot obese subjects/Kıbrıslı Türk obez kişilerde leptin G-2548A gen polimorfizmi ile plazma leptin seviyeleri ve lipid profili arasındaki ilişki. Turkish Journal of Biochemistry, 41(1): 1-7.
21. Bouafi, H.; Krami, A. M.; Morjane, I.; Slaoui, K.; Harmak, H.; Charoute, H., *et al*. (2023). Genetic Association of LEP Gene Polymorphisms with Obesity in Moroccan Individuals: Case–Control Study and Updated Meta-analysis. Biochemical genetics, 1-17.