Research Article

The role of luteinizing hormone receptor gene (rs2293275) polymorphism on luteinizing hormone level in women with polycystic ovarian syndrome

Haitham Mohammed Saad[1]*, Maha Fadhal Smaism[2], Nadia Mudher Sulaiman[3]

ABSTRACT

Background: The aim of this study was to determine the genetic relation of luteinizing hormone/choriogonadotropin receptor gene (rs2293275) polymorphism on luteinizing hormone level in women polycystic ovarian syndrome (PCOS), which affects 5–10% of all women of reproductive age. **Materials and Methods:** A cross-sectional study was conducted on 88 Iraqi women diagnosed with PCOS. The females were divided into two groups 43 patients (Group A) with regular secretion of LH and 45 patients (Group B) with irregular secretion of LH. All females were assessed the status of obesity by body mass index (BMI) and blood samples were taken from these two groups for hormones and gene expression analysis. **Results:** LH receptors genotype and allele frequency was specified G935A polymorphism in the two group, Group A genotype, contain on 51.18% were wild genotype (GG), 32.55% were heterozygous (GA), and 16.27% were homozygous genotype (AA), while Group B appeared 42.22% were GG, 37.78% were GA, and 20.0% were AA. Regarding, hormones showed significant increase in LH level in Group B compared with Group A (18.91 ± 3.07 mIU/ml and 5.47 ± 0.49 mIU/ml, respectively). The mean value of other hormone levels was different in both study groups but remained within normal range. The age, BMI, and duration of infertility did not differ between Group A and B. **Conclusion:** From this study, we concluded the choriogonadotropin receptor gene (rs2293275) polymorphism effected on the LH level especially the women carrying GA genotype or A allele which has high risk to develop high LH level with PCOS.

KEY WORDS: Luteinizing hormone, Luteinizing hormone receptor gene, Polycystic ovary syndrome, rs2293275 polymorphism

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine defect of women in the reproductive age that characterized by increase androgens, oligomenorrhea, and\or amenorrhoea and is associated with a high risk to developing insulin resistance, obesity, and type 2 diabetes mellitus. The PCOS is a complex disorder with contributing factors as environmental and genetic.[1]

The PCOS characterized by ovulation failure, polycystic ovaries, and elevated androgen release, which leads to PCOS, is a complex syndrome involving endocrine and metabolic abnormalities.[2]

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

Hence, it could cause the most common endocrine problem in women of reproductive age, which affects 5–10% of all women of reproductive age.[3] Interesting studies mentioned the importance of this gene due to many factors, for example, it is present in the ovary on the theca cells and in the testis on the Leydig cells.[4,5]

Typical biochemical features are raised serum concentrations of gonadotropin hormone (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]), testosterone, and estradiol. The primary diagnosis depended on clinical criteria. In addition, a finding of raised serum testosterone and/or LH complements the clinical diagnosis.[6] However, the most interesting hypothesis was proposed by Franks *et al.*,[7] who defined PCOS as a genetically determined ovarian pathology characterized by overproduction of androgens and manifesting heterogeneously according to the interaction of this genetic "predisposition"

[1]Department of Medical Laboratory, Ministry of Health, Al-Anbar Health Directorate, Ramadi, Iraq, [2]Department of Clinical Biochemistry, College of Medicine, University of Babylon, Hillah, Iraq, [3]Department of Obstetrics and Gynecology, College of Medicine, University of Babylon, Hillah, Iraq

***Corresponding author:** Haitham Mohammed Saad, Department of Medical Laboratory, Ministry of Health, Al-Anbar Health Directorate, Ramadi, Iraq. E-mail: hms.stariraq@gmail.com

Received on: 19-07-2019; Revised on: 21-08-2019; Accepted on: 26-09-2019

with other genetic and environmental factors. The LH (rs2293275) gene polymorphism and its possible influence on reproductive performance in women with PCOS have not been documented. Therefore, this study was carried out with the objective of exploring LH (rs2293275) gene polymorphism and his association with LH in women with PCOS.

MATERIALS AND METHODS

Study Design and Collection of Samples

This study was located in the Infertility Center in Maternity and Children Hospital in Babylon province. Eighty-eight female (average live weight 56.00 ± 0.59 kg) were assigned into two groups, the Group A includes 43 female has PCOS with normal level of LH and the Group B includes 45 female has PCOS with high level of LH according to Rotterdam criteria.[8] The blood samples were collected from patients started in September 2018 until March 2019. Verbal approval was taken from all patients included in the study before sampling, the objectives, and methodology of this study explained to all participants in the current study to gain their verbal acceptance.

All patients were exposed to clinical examination for assessing the status of obesity by the body mass index (BMI), ultrasound is done to assess the polycystic ovary morphology, and venous blood sample drawn from all subjects using disposable syringe (5 ml) in the sitting position. Five ml of blood was obtained from each patient by vein puncture and pushed slowly into two tubes (2 ml blood in ethylenediaminetetraacetic acid tube for genetic study and 3 mL blood in plan tube). Plan tube of blood was centrifuged at 10000 g for approximately 10–15 min, then the serum was divided into three parts and stored at −20°C until analysis.

Extraction of DNA and Genotype Analysis

The genomic DNA was isolated from the peripheral blood leukocytes according to the method provided by the kit (Favorgen FavorPrep Genomic DNA Mini Kit/Taiwan). Isolated DNA stored at −20°C until it was analysis. The LH receptor (LHR) gene G935A, amplified by polymerase chain reaction (PCR) with specific design primers was designed from Primer3web (http://primer3.ut.ee/) forward primer 5'- GAACGAGATGCTGGTGTAATAG -3' LHR gene reverse primer 5'- CCATAGGGACACTGGGATTA -3'. The PCR carried out in a total volume of 25 µl reaction mixture, containing 12.5 µl Master Mix, 5 µl DNA product, 1.5 µl of forward primer and 1.5 µl reverse primer, and 4.5 µl nuclease-free distal water. The amplification reaction is done in Bio-Rad T100 thermal cycler (Thermo Fisher Scientific, Korea). Programmed condition as follows: First step denaturation at 94°C for 4 min, 32 cycles of 30 s denaturation at 94°C, 1 min primer annealing at 60°C, 2-min template elongation at 72°C, and a 5 min final extension at 72°C 1 cycle.

The PCR product of The LHR gene (G935A) has digested with RsaI restriction enzyme, then electrophoresis through agarose gel 1.5%. In wild genotype (GG) generates tow bands of 525 bp. and 291 bp., while in the presence homozygous mutation AA only show one band of 816 bp., and in heterozygous variant (GA) generates three bands of 816 bp. 525 bp. and 291 bp. Serum testosterone, estradiol, FSH, and LH were collected in 3rd day of menstrual cycle and the samples were tested with enzyme-linked immunosorbent assay according to manufacturer instructions (Biotech/USA).

Data Analysis

Statistical analysis was carried out using SPSS version 24. Continuous variables presented as (means ± standard deviation). The correlation coefficient (r) was used to find the relationship between two continuous variables. $P \leq 0.05$ was considered as significant.

RESULTS

Genotyping and Allele Frequency

The genotyping and allele frequency has studied LHR gene G935A polymorphism in the two groups. The results showed that Group A with the presence 51.18% was wild genotype (GG), 32.55% was heterozygous (GA), and 16.27% was homozygous genotype (AA), while in Group B, 42.22% was wild genotype, 37.78% was heterozygous, and 20.0% homozygous genotype Table 1.

Allele frequency in the Group A, 32% was carried A allele but 68% was carried G allele as compared with Group B which was carrying 39% of A allele and 61% G allele [Table 1].

When compare the parameter distribution with genotype showed, the heterozygous variant of LHR polymorphism (GA) of Group B patients was

Table 1: Genotype distribution, allele frequency, allele distribution between the two study groups

Genotype of luteinizing hormone receptor	Group A	Group B
	n (%)	Luteinizing hormone (%)
GG	22 (51.18)	19 (42.22)
GA	14 (32.55)	17 (37.78)
AA	7 (16.27)	9 (20.0)
Allele frequency		
G	0.68	0.61
A	0.32	0.39

increased LH and testosterone level when compared with other genotypes, the prevalence of FSH was significantly higher in PCOS AA carrier in comparison to GG carrier. The homozygous (GG) carrier reported enlarge the right ovarian with high-level E2 and LHR concentration more than other genotyping as shown in Table 2, this result highlights the contribution of this polymorphism to the phenotype of PCOS and helps in better understanding the role of genetic variation.

Hormonal Concentration

This study has shown increase in LH level in-Group B compared with Group A; the result was 18.91±3.07mlU/mland5.47±0.49mlU/ml,respectively. The FSH in-Group B was higher than Group A which was 6.05 ± 0.34 and 5.06 ± 0.31 mlU/ml, respectively. Testosterone in Group B was 41.19 ± 5.16 ng/dl, while in Group A was 39.53 ± 4.98 ng/dl. Estradiol in Group B was 72.65 ± 7.16 pg/ml, while in-Group A was 79.45 ± 8.17 pg/ml as shown in Table 3.

Demographic and clinical data presented in Table 4 compared between differences of the study variables including age, BMI, and duration of infertility, which showed there were no significant differences between the groups.

DISCUSSION

Impact of LHR Genes Polymorphisms on Hormonal Characteristics

The study showed that homozygous 935A subject was at higher risk to develop high LH level 1.35 fold (odds ratio 1.35, 95% Confidence interval: 0.75–2.42, P = 0.30). A statistically was insignificant association when comparing either genotypes or allele frequency of the studied G935A polymorphism between two groups. The risk factor between the PCOS phenotype was similar to the Egyptian population.[9] In case of the homozygous variant, the risk of high LH level in our population is 1.4-fold compared to other genotypes, which was lower than the risk of the Mediterranean population 2.7-folds.[10] In addition, this study disagrees with Almawi *et al.*,[11] in his study, shows that the polymorphism was not associated with PCOS in Bahrain Arab and Dutch.

In this study determined whether LHR overexpressed in PCOS with high LH level and LHR that the overexpression of LHR may be due to polymorphism result in insensitive to the LH and increases the LH due to lack of the negative feedback. This finding is similar to Arnhold *et al.*[12] and El-Shal *et al.*,[13] who stated the PCOS individuals feedback is altered, and the control of LHR on hormonal levels is lost, and consequently also the correlation between genotype and LH level, there for serum LH levels high in those with single nucleotide polymorphisms (SNPs). The PCOS group (which Group A or B or both) showed a more intense band, indicating overexpression of LHR protein in PCOS. The LHR protein expression in PCOS women is ~150% higher than in normal cases.[2] The LHR is overexpressed in theca cells and granulose cell from PCOS patients; a number of functional SNPs have been described in LH and LHR genes which may affect the LH and LHR function.[14]

Hormonal Change

In this study, the mean value of FSH and LH in patients was 5.06 mIU/ml and 5.47 mIU/ml, compared with Group B mean that equal to 6.05 mlU/ml and 18.91 mlU/ml, respectively. There was highly significant difference between two groups, the increase LH level may be due to lack of negative feedback because the receptor (LHR) cannot recognize the LH

Table 2: Association between LHR genotype and parameters study in Group B

Parameter	GG (n=19)	GA (n=17)	AA (n=9)	P-value
LH (mlU/ml)	14.77±0.91	24.90±8.18	17.44±4.37	0.3
FSH (mlU/ml)	3.8710±0.40	5.73±0.39	6.37±0.57	0.01*
E2 (pg/ml)	108.22±18.11	69.83±7.80	56.19±5.38	0.04*
Testo. (ng/dl)	34.20±6.48	48.47±8.84	40.45±11.37	0.4
Rt. ovarian vol. (cc)	11.79±1.03	10.03±0.70	10.98±1.66	0.4
Lt. ovarian vol. (cc)	9.67±0.66	10.11±1.2	10.94±1.01	0.7
LHR (ng/ml)	7.83±1.98	4.84±1.21	3.11±0.61	0.1

LHR: Luteinizing hormone receptor, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, Testo.: Testosterone, E2: Estradiol, Rt.: Right, Lt.: Left, *P<0.05

Table 3: Compare between Group A and Group B in hormones

Group	LH (mlU/ml)	FSH (mlU/ml)	Testo. (ng/dl)	E2 (pg/ml)
Group A	5.47±0.49	5.06±0.31	39.53±4.98	79.45±8.17
Group B	18.91±3.07	6.05±0.34	41.19±5.16	72.65±7.16
P-value	0.0001**	0.038	0.81	0.53

LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, Testo.: Testosterone, E2: Estradiol, **P<0.01

Table 4: Compare between Group A and Group B in difference variables

Group	BMI (kg/m²)	Age (year)	Infertility (month)
Group A	31.66±1.16	24.25±0.78	35±6.07
Group B	29.82±2.12	23.51±0.57	29±4.27
P-value	0.45	0.44	0.48

BMI: Body mass index

even if it is high in the circulating blood. This study agreement with Moran and Teede,[15] which explains the high level of LH and low level of FSH may be due to enhanced pituitary sensitivity to gonadotropin-releasing hormone (GnRH) stimulation or to increase pulse frequency of GnRH secretion. Moreover, this study shows that the difference between testosterone and E2 hormone level was insignificant in the two groups. The result of this study was in agreement with the finding of previous studies like Meek *et al.*,[16] which suggests that ovarian theca cells in affected women with PCOS are more efficient at converting androgenic precursors to testosterone than the normal theca cells.

Demographic and Clinical Change

There was no significant difference in BMI and the age between Group A and Group B (P = 0.45 and 0.44), respectively, may be due to the lifestyle of population and eat behaviors and all women in reproductive age. This study agreement with Alnakash and Al-Tae'e,[17] the PCOS reported to be more prevalent in younger ages (<35) than among older women, proposing that due to a physiological decline of the follicular leading to a normalized ovarian ultrasonographic appearance with advancing age. Furthermore, recent research has indicated that age can also influence both the clinical presentation and metabolic manifestations of PCOS.[18]

There was no significant difference in duration of infertility between two groups in the duration of infertility (P = 0.489), due to both group has PCOS and maybe need to increase the sample size. The results of this study have showed the duration of Group A was 35 months, while Group B 29 months. This study agreement with Brassard *et al.*,[19] with increasing age of the female partner and increasing duration of infertility (>3 years), conception is even less likely.

CONCLUSION

The LH concentration was influenced by the role of LHR gene (G935A) polymorphism. However, the expression pattern of LHR gene (G935A) polymorphism under different conditions would explore information that is more valuable on the specific isoforms of LHR gene (G935A) polymorphism function in relation with LH level in women with PCOS.

ACKNOWLEDGMENTS

The authors would like to thank the team of infertility center in maternity and children hospital of Babylon for their valuable support. We would also like to mention the staff of the biochemistry department; I say a big thank you for your cooperation and assistance to me while carrying out my experiment and all the volunteers and the patients.

REFERENCES

1. Goodarzi MO, Jones MR, Li X, Chua AK, Garcia OA, Chen YD, *et al.* Replication of association of DENND1A and THADA variants with polycystic ovary syndrome in European cohorts. J Med Genet 2012;49:90-5.
2. Kanamarlapudi V, Gordon UD, Bernal AL. Luteinizing hormone/chorionic gonadotrophin receptor overexpressed in granulosa cells from polycystic ovary syndrome ovaries is functionally active. Reprod Biomed Online 2016;32:635-41.
3. Rzaij ZF, AL-Ani NK. Evaluation of cancer antigens (CA125 and CA153-) in some Iraqi women with polycystic ovarian syndrome. Iraqi J Embryos Infertil Res 2013;3:11-4.
4. Mutharasan P, Galdones E, Bernabé BP, Garcia OA, Jafari N, Shea LD, *et al.* Evidence for chromosome 2p16. 3 polycystic ovary syndrome susceptibility locus in affected women of European ancestry. J Clin Endocrinol Metab 2013;98:E185-90.
5. Dos Santos E, Dieudonné MN, Leneveu MC, Pecquery R, Serazin V, Giudicelli Y. *In vitro* effects of chorionic gonadotropin hormone on human adipose development. J Endocrinol 2007;194:313-26.
6. Franks S. Polycystic ovary syndrome. Medicine 2017;45:527-31.
7. Franks S, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: Involvement of genetic and environmental factors. Int J Androl 2006;29:278-85.
8. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-7.
9. Bassiouny YA, Rabie WA, Hassan AA, Darwish RK. Association of the luteinizing hormone/choriogonadotropin receptor gene polymorphism with polycystic ovary syndrome. Gynecol Endocrinol 2014;30:428-30.
10. Capalbo A, Sagnella F, Apa R, Fulghesu A, Lanzone A, Morciano A, *et al.* The 312N variant of the luteinizing hormone/choriogonadotropin receptor gene (LHCGR) confers up to 2 7-fold increased risk of polycystic ovary syndrome in a Sardinian population. Clin Endocrinol 2012;77:113-9.
11. Almawi WY, Hubail B, Arekat DZ, Al-Farsi SM, Al-Kindi SK, Arekat MR, *et al.* Leutinizing hormone/choriogonadotropin receptor and follicle stimulating hormone receptor gene variants in polycystic ovary syndrome. J Assist Reprod Genet 2015;32:607-14.
12. Arnhold IJ, Lofrano-Porto A, Latronico AC. Inactivating mutations of luteinizing hormone β-subunit or luteinizing hormone receptor cause oligo-amenorrhea and infertility in women. Horm Res Paediatr 2009;71:75-82.
13. El-Shal AS, Zidan HE, Rashad NM, Abdelaziz AM, Harira MM. Association between genes encoding components of the leutinizing hormone/luteinizing hormone-choriogonadotrophin receptor pathway and polycystic ovary syndrome in Egyptian women. IUBMB life 2016;68:23-36.
14. Liu N, Ma Y, Wang S, Zhang X, Zhang Q, Zhang X, *et al.* Association of the genetic variants of luteinizing hormone, luteinizing hormone receptor and polycystic ovary syndrome. Reprod Biol Endocrinol 2012;10:36.
15. Moran L, Teede H. Metabolic features of the reproductive phenotypes of polycystic ovary syndrome. Hum Reprod Update 2009;15:477-88.
16. Meek CL, Bravis V, Don A, Kaplan F. Polycystic ovary

syndrome and the differential diagnosis of hyperandrogenism. Obstet Gynaecol 2013;15:171-6.
17. Alnakash AH, Al-Tae'e NK. Polycystic ovarian syndrome: The correlation between the LH/FSH ratio and disease manifestations. Middle East Fertil Soc J 2007;12:35.
18. Alsibyani NA, Malibary MA, Derham AA, Almnabri AA, Jazzar NK, Melibari RW, *et al.* Clinical presentation of polycystic ovary syndrome among Saudi Arabian women Jeddah, Saudi Arabia. Egypt J Hosp Med 2017;31:1-5.
19. Brassard M, AinMelk Y, Baillargeon JP. Basic infertility including polycystic ovary syndrome. Med Clin North Am 2008;92:1163-92.

Source of support: Nil; Conflict of interest: None Declared