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The Effect of *HNF1A* Gene Polymorphism on Risk of Polycystic Ovary Syndrome in Sample of Iraqi Women

Elham A. Toama, Hamsa A. Jasim

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

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Abstract: Polycystic ovary syndrome (PCOS) is one of the commonest causes of female infertility. Clinical features caused by high levels of androgens, oligo menorrhea, and polycystic ovarian morphology are necessary for diagnosis. This study aims to find a relationship between the genetic polymorphism of HNF1A on the risk of Polycystic Ovary Syndrome in a Sample of Iraqi Women. The study includes one hundred subjects of Iraqi women in Baghdad (15-35 years), Patients were divided to three groups. The first group was including Patients treated with metformin, while second group was including Patients without metformin, the third group was including apparently healthy. DNA was extracted, then the Genotyping polymorphism (rs2464196) of the HNF1A gene was done by RT-PCR. The AA genotype showed a higher frequency in the control (p=0.164) while the GG genotype showed a higher frequency in the patients (p= 0.006).

Keywords: Polycystic ovary syndrome (PCOS), genetic polymorphism, HNF1A protein, Hormones level.

Corresponding author: (Email: ahmedalham985@gmail.com).

Introduction

Polycystic ovarian syndrome (PCOS) is a common condition that affects hormones. It causes irregular menstrual periods, excess hair growth, acne and infertility(1). Treatment for PCOS depends on if you wish to become pregnant. People with PCOS may be at higher risk for certain health conditions like diabetes and high blood pressure (2). PCOS is caused by a combination of genetic, endocrine, metabolic, and environmental factors. Biochemically, the increased production of luteinizing hormone (LH) and normal or low amount of follicular stimulating hormone (FSH) by the anterior pituitary reveal ovarian dysfunction in PCOS (3). An important consideration for the measurement of androgen levels is the proper establishment of normal ranges or limits. These can be established by measuring androgens in a large population of well-characterized normal women, in whom the presence of menstrual/ovulatory dysfunction and hirsutism, among other factors, has been excluded (4). PCOS is associated with insulin resistance (IR), metabolic syndrome, and type 2 diabetes. IR can elevate serum insulin levels and increase the frequency of pulsatile gonadotropin-releasing hormone (GnRH) secretion, causing elevated serum LH levels and further promoting excess androgen production. A large

number of studies have confirmed that patients with PCOS have varying degrees of IR and compensatory hyperinsulinemia, including ovarian IR (5). Raised lipids is very common in the general population however, it's been shown that this risk increases if you have PCOS. One study suggested 70% of women with PCOS are affected by dyslipidemia, which is when you have unhealthy levels of one or more kinds of lipid – cholesterol and another type of fat called triglycerides – in your blood (6). Metformin is the only remaining member of the biguanide family that has been used for the treatment of diabetes for a long time (7). Several effects have been reported as related to metformin in PCOS patients including restoring ovulation, reducing weight, reducing circulating androgen levels, reducing the risk of miscarriage and reducing the risk of gestational diabetes mellitus (GDM) (8). The hepatocyte nuclear factor 1 alpha gene (*HNF1A*) located on human chromosome 12q24 and contains nine exons, the *HNF1A* gene provides instructions for making a protein called hepatocyte nuclear factor-1 alpha (HNF-1α). The HNF-1α protein acts as a transcription factor, which means it attaches to specific regions of DNA and helps control the activity of certain genes, HNF-1α protein is critical for the growth and development of beta cells in the pancreas. Beta cells produce and release the hormone insulin (9). Several mutations of *HNF1A* was found to be linked to hypertension and hypertriglyceridemia (10).

Materials and methods

One hundred volunteers (female) were taken in this study. Fifty with polycystic ovary syndrome and fifty apparently healthy, who randomly selected between October 2021 to April 2022 in a private clinic in the district of Mada'in, in Baghdad. The medical history from all PCOS patients were took from case-sheet records, and detected first by ultrasound scan, supported by an information recorded if they experienced from oligo menorrhea, amenorrhea or highly irregular menses, the age range for patients is between 15-35 years, In this study,100 volunteers were use and divided to three groups, The first group was include Patients treated with metformin, while second group was include Patients without metformin and the third group was include apparently healthy. Two ml of peripheral blood from all select subjects were collected and placed into sterile plain tube that contained EDTA and three ml of serum were collected and placed into sterile plain tube. The blood and serum were placed in a cool - box under aseptic conditions and transfer to the laboratory. Serum hormones were measured by AFIAS-6. Genotyping of polymorphism (rs2464196) of the *HNF1A* gene was done, by using HRM SNP Genotyping Assays. HRM analysis with ramping by 0.2 °C from 65 to 95 °C. Used master mixes were containing EVA-Green; HRM Master Mix Synthetic SNP sequences was tested using duplicates. the DNA was extracted, using DNA extraction kit EasyPure®Genomic (TransGen, biotech. EE101-01). primer sequences were designed according to their reference sequence (rs) in the database of National Center for Biotechnology Information (NCBI). The forward primer

GCCACCTGTGCAGAGCCATG and the Reverse primer CTCTGCAGCTGAGCCATGGT. The thermal cycling program was as follows: enzyme activation in 94 C° for 60 secs, (first one was denaturation 94 C° for 5 secs and second step of annealing 58C°for 15 secs (40 cycle) and extension 72 ºC for 20 sec).

Statistical analysis

Difference between groups was tested using The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference–LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. The allelic and genotype association of SNP were evaluated by Pearson's Chi-square test; and odds ratio (OR) and 95 per cent confidence intervals were determined. For comparison of more than two groups, one-way ANOVA was used.

Results and discussion

The comparison of the mean value of the selected hormonal profile between PCOS patient's groups and controls group-containing (Prolactin, Estradiol, FSH, Testosterone, TSH, LH) as shown in table (1).

Table (1): Comparison between PCOS patient's groups and controls according to the selected Hormonal profile

Group	Mean ± SE					
	Prolactin	Estradiol	FSH	Testosterone	TSH	LH
PCOS with metformin	10.21 ±1.56 a	33.43 ±3.73 ab	8.71 ±1.03	0.680 ±0.09 a	2.11 ±0.24	8.00a ±2.03
PCOS	10.19 ±1.68 a	37.13 ±5.27 a	8.76 ±1.27	0.795 ±0.19 a	2.33 ±0.24	8.73 ±0.99a
Control	6.51 ±0.33 b	25.42 ±1.90 b	8.62 ±0.37	0.372 ±0.03 b	2.68 ±0.17	7.95 ±0.37b
LSD value	3.058 **	9.605 *	2.310 NS	0.287 **	0.615 NS	2.383 NS
P-value	0.010	0.0285	0.991	0.0055	0.138	0.701

Means having with the different letters in same column differed significantly.
* ($P \leq 0.05$), ** ($P \leq 0.01$).

The results show serum prolactin in PCOS patients with metformin was (10.21 ±1.56 ng/mL), while PCOS patients was (10.19 ±1.68 ng/mL) compared with the mean of controls (6.51 ±0.33 ng/mL) with significant differences ($P<0.01$) as is shown in table (1). The results of the current study agree with the results of previous study, (11) they have found that PRL levels are significantly higher in PCOS patients when compared to controls over all age groups ($p < 0.05$). At the same time (12) found that PRL levels are significantly lower in PCOS patients when compared to controls over all age groups ($p < 0.05$). On other hand, the results of serum Estradiol in PCOS patients with metformin was time (33.43±3.73ab ng/mL) while for PCOS patients (37.13 ±5.27 ng/mL) compared to the mean of control (37.13 ±5.27 a pg/mL) table (1) with significant differences ($P \leq 0.05$) when comparing between groups. The results of serum Estradiol are in agreement with (13) who had found that women diagnosed with PCOS may present increased levels of estradiol when compared with eumenorrheic ovulatory women. At the same time the serum FSH in PCOS patients with metformin (8.71 ±1.03 ng/mL), PCOS patients (8.76 ±1.27 ng/mL) compared with the mean of controls (8.62 ±0.37 ng/mL), with no significant differences as in table (1). The results of the present study agree with the results of previous study (14)

since they have observed that the mean values of patients serum FSH are significantly lower than those of controls. In usual menstruation, the elevation of plasma FSH through the (luteal-follicular change) and consequently ovulation (15). At the same time the result of testosterone in was PCOS patients with metformin 0.680 ±0.09 ng/mL while for PCOS patients was 0.795 ±0.19 ng/mL compared with the mean of control (0.372 ±0.03 b ng/mL with highly significant differences (P<0.01) as in table (1). The results of serum testosterone agree with previous studies (16,17) since they concluded that mean values of patients serum testosterone were significantly higher than those of controls. testosterone and estradiol coordinate to maintain the balance of female reproductive endocrine system. In the peripheral organs, androgen helps to increase muscle mass, bone growth, and calcium deposition. The appearance of pubic hair and axillary hair, an indication of puberty, also relies on the secretion of androgens from adrenal gland, known as the adrenarche. However, excessive androgen can cause follicular dysplasia, which may impair ovulation and would result in menstrual disorders. In addition, androgen is an important hormone for maintaining the female's sexual desire (18). The results show serum TSH in PCOS patients with metformin was (2.11 ±0.24ng/mL), PCOS patients (2.33 ±0.24 ng/mL) compared with the mean of controls (2.68 ±0.17 ng/mL) with no significant differences when comparing between groups as in table (1). The results show serum LH in PCOS patients with metformin was (8.00 ±2.03ng/mL), PCOS patients (8.73 ±0.99ng/mL) compared with the mean of controls (7.95 ±0.37ng/mL). This study showed that LH mean has no significant differences when comparing the studies groups as s in table (1). (19) found that LH levels are significantly elevated in PCOS patients in contrast to control group (p <0.05), also the most evident neuroendocrine feature regulating abnormal ovarian follicle development in PCOS is increased luteinizing hormone (LH) pulsatility regarding both frequency and amplitude, with relatively low FSH secretion. Increased LH pulse frequency increases theca cell production of androgens, while the lower FSH levels impairs follicle maturation and consequently ovulation.

Estimation of serum *HNF1A* Level in PCOS patients and control

The Comparison of patients PCOS treated with metformin, PCOS patients as well as control were applied according to the serum level of HNF1A, as in table (3).

Table (3): Comparison between difference groups in HNF1A ELISA

Group	Mean ± SE of HNF1A ELISA
PCOS with metformin	3.96 ±0.17 a
PCOS	3.98 ±0.18 a
Control	2.30 ±0.12 b
LSD value	0.455 **
P-value	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).	

The results indicated highly significant differences (P≤0.01) between studied groups in the serum level of HNF1A, there was a significant increase in HNF1A serum level in PCOS patients treated with metformin was (3.96 ±0.17 a) as compared with its mean in un treated PCOS patients and control (3.98 ±0.18 a, 2.30 ±0.12 b) as shown in table (3), respectively. Also women with T2DM had a higher mean waist-to-hip ratio when compared to those with HNF1A-MODY (P < 0.0001) despite matching for BMI in both groups (20). It now appears to be part of a multifaceted metabolic disease closely associated with insulin resistance and hyperinsulinemia, Approximately 75% of obese patients with PCOS are insulin resistant and hyperinsulinemia and demonstrate an increased incidence of diabetes, hypertension, dyslipidemia, and atherosclerosis, not only are defects in insulin homeostasis strongly correlated with endocrine abnormalities in PCOS, they appear to play a causal role in the pathogenesis of this syndrome (21). *HNF1A* a regulates several genes involved in lipoprotein metabolism, such as Apo lipoproteins, cholesterol synthesizing enzymes, and bile acid transporters as well as glucose-stimulated insulin secretion. Hence, we deemed it necessary to evaluate its potential influence on lipid profiles in our study population (22).

Genotypes and alleles frequency of *HNF1A* gene (rs2464196) polymorphism G>A

The percentage of AA genotype was not significant, control higher than that of PCOS patients (27.50% versus 22.50%, respectively, X2=1.043), while, the percentage of GA genotype in control was significantly (P≤0.01) higher than that of PCOS patients while there was highly significant difference (P≤0.01) in CC genotype percentage between two groups related with rs2464196 at HNF1A gene. The A allele frequency values were 0.60 for apparently healthy subjects and 0.33 for PCOS patients Also, G allele frequency values were 0.40 for healthy subjects and 0.67 for PCOS patient's healthy subjects, respectively (table 4).

Table (4): Genotypes distribution and Allele frequency of rs2464196 SNP in difference groups

Genotype rs2464196	Control group No. (%)	PCOS group No. (%)	Chi-Square (χ^2)	P-value
GG	6 (12.50%)	26 (55.00%)	11.623 **	0.0006
GA	28 (60.00%)	15 (22.50%)	10.075 **	0.0008
AA	16 (27.50%)	9 (22.50%)	1.043 NS	0.164
Allele	**Frequency**			
G	0.40	0.67	-	-
A	0.60	0.33	-	-
** (P≤0.01).				

The previous results could be explained that G allele carriers who were not diagnosed with PCOS had an increased risk of developing PCOS and GG genotype carrier was responsible for PCOS occurrence.Also, *HNF1A* single nucleotide polymorphisms rs2464196 had been associated with serum lipid traits in several previous genome-wide association studies. It had

been associated with levels of total cholesterol (TC), triglyceride (TG) and Apo lipoprotein (Apo) (23). Also genotype analysis of the selected SNP showed that the *HNF1A* gene have a direct effect on the PCOS phenotype, and the allele frequency analysis showed that SNP rs2464196 was contribute to the occurrence of PCOS because the frequency of the risk allele G at rs2464196 SNP locus was significantly higher in PCOS cases compared to control group as shown in table (4) which may be due to previous results showing that the GG genotype was a risk factor and AA genotype protective factor.

Conclusion

PRL, Estradiol, LH, are significantly higher in polycystic ovary syndrome (PCOS) patients than healthy women group during early follicular phase of menstrual cycle. While TSH, FSH were no significant change in these groups. The concentration of serum HNF1A observed a significant difference ($P< 0.01$) between women with PCOS and apparently healthy controls group. The genotypes and allele frequency of *HNF1A* gene distribution frequencies at (rs2464196) G>A SNP polymorphism, the percentage of AA genotype was not significant, control higher than that of PCOS patients, while, the percentage of GA genotype in control was significantly ($P\leq0.01$) higher than that of PCOS patients while there was highly significant difference ($P\leq0.01$) in GG genotype percentage between two groups related with rs2464196 at *HNF1A* gene.

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