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Article

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

Elham Ahmed Toama[1,], and Hamssa Ahmed Jasim[2]*
[1] Institute of Genetic Engineering and Biotechnology for Postgraduate, University of Baghdad, Iraq
[2] Institute of Genetic Engineering and Biotechnology for Postgraduate, University of Baghdad, Iraq
** Correspondence: ilham.ahmed1300a@ige.uobaghdad.edu.iq*
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ABSTRACT
Polycystic ovary syndrome (PCOS) is one of the most common causes of female infertility. Clinical features caused by high levels of androgens, oligomenorrhea, and polycystic ovarian morphology are necessary for diagnosis. This study aims to find a relationship between the genetic polymorphism of *HNF1A* and 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 into three groups. The first group included Patients treated with metformin, the second group included Patients without metformin, and the third group included healthy. DNA was extracted, then the Genotyping polymorphism (rs1169288) of the *HNF1A* gene was done by RT-PCR. The AA genotype showed a higher frequency in control (p=0.001), while the CC genotype showed a higher frequency in the patients (p= 0.0001).

Keywords: Polycystic ovary syndrome (PCOS), genetic polymorphism, *HNF1A* protein, Glucose and lipid profile.

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 genetic, endocrine, metabolic, and environmental factors. Biochemically, the anterior pituitary's increased production of luteinizing hormone (LH) and normal or low amount of follicular stimulating hormone (FSH) reveal ovarian dysfunction in PCOS [3]. An important consideration for measuring androgen levels is establishing normal ranges or limits properly. 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. Many studies have confirmed that patients with PCOS have varying degrees of IR and compensatory hyperinsulinemia, including ovarian IR. [5] Raised lipids are very common in the general population; however, it has been shown that this risk increases if you have PCOS. One study suggested that 70% of women with PCOS are affected by dyslipidemia, which is when you have unhealthy levels of one or more 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 treating 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*) is 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 were 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 healthy were randomly selected between October 2021 and April 2022 in a private clinic in Mada'in in Baghdad. The medical history of all PCOS patients was taken from case-sheet records and detected first by ultrasound scan, supported by information recorded if they experienced oligomenorrhea, amenorrhea or highly irregular menses; the age range for patients is between 15-35 years; in this study,100 volunteers were used and divided to three groups, The first group included Patients treated with metformin, while the second group included Patients without metformin. The third group was included healthy. Two ml of peripheral blood from all select subjects were collected and placed into a sterile plain tube that contained EDTA, and three ml of serum were collected and placed into a sterile plain tube. The blood and serum were placed in a cool - box under aseptic conditions and transferred to the laboratory. Serum glucose and lipid profile measured by Apel. Genotyping of polymorphism (rs1169288) 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 containing EVA-Green and HRM Master Mix Synthetic SNP sequences were tested using duplicates. The DNA was extracted using the DNA extraction kit EasyPure®Genomic (TransGen, biotech. EE101-01). Primer sequences were designed according to their reference sequence (rs) in the National Center for Biotechnology Information (NCBI) database. The forward primer GCTCGAGTCAGGGCTGAGCA and the Reverse primer TCCAGCCAGGAGGTAGGGCC The thermal cycling program was as follows: enzyme activation in 94 C° for 60 sec (the first one was denaturation 94 C° for 5 sec and second step of annealing 58C°for 15 sec(40 cycles) 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 different factors in study parameters. The least significant difference –LSD test (Analysis of Variation-ANOVA) was used to compare between means significantly. Pearson's Chi-square test evaluated SNP's allelic and genotype association, and odds ratio (OR) and 95 percent confidence intervals were determined. For a comparison of more than two groups, one-way ANOVA was used.

RESULT

Glucose tests and Lipid profile

The comparison of the mean value of selected (Glucose, Total cholesterol and Triglyceride) with significant differences (P≤0.05) when comparing between groups as shown in Table 2.

Group	Mean ± SE		
	Glucose	Cholesterol	Triglyceride
Pcos with metformin	83.00 ±2.03 b	157.05 ±9.69	125.75 ±15.22 a
Pcos	95.35 ±3.32 a	139.15 ±5.58	100.80 ±6.01 b
Control	90.55 ±2.79 ab	139.22 ±4.31	97.72 ±2.16 b
LSD value	8.777 *	18.124 NS	21.632 *
P-value	0.0402	0.0971	0.0242
Having the different letters in the same column differed Means significantly. * (P≤0.05).			

Table 1. Comparison between PCOS patients and control groups according to the selected lipid profile and Glucose

The results of serum Glucose in PCOS patients with metformin (83.00 ±2.03 pg/mL), PCOS patients (95.35 ±3.32 pg/mL) compared to the mean of control (90.55 ±2.79 pg/mL) as in table (1) with P-value of (≤0.05).

Estimation of Serum HNF1A Level in PCOS Patients and Control

The comparison of patients with PCOS treated with metformin, PCOS patients, and control was applied according to the serum level of HNF1A, as in Table 2.

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
This means that having different letters in the same column differed significantly. ** (P≤0.01).	

Table 2. Comparison between different groups in HNF1A ELISA

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 (3.96 ±0.17 pg/mL) as compared with its mean in un treated PCOS patients and control (3.98 ±0.18 pg/mL, 2.30 ±0.12 pg/mL) as shown in table 2, 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 [16]. 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 hyperinsulinemic 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 [17]. HNF1A regulates several genes involved in lipoprotein metabolism, such as apolipoproteins, cholesterol-synthesizing enzymes, bile acid transporters, and glucose-stimulated insulin secretion.

Genotypes and alleles frequency of HNF1A gene (rs1169288) polymorphism C>A

The genotypes and allele frequency distributions of rs1169288 SNP polymorphism are presented in Table 3, which reveals the genotype and allele frequency of HNF1A gene (rs1169288) polymorphism C>A in apparently healthy women versus women with polycystic ovary syndrome. The percentage of AA genotype in healthy women was higher than that of apparently PCOS patients (26% versus 13.7%, respectively OR = 0.45 not significant). In contrast, the Frequency of AC genotype in apparent control was significantly P≤0.01 higher than that of PCOS patients (46% versus 21.56%, respectively OR = 0.32). At the same time, the frequency of CC genotype was significantly P≤0.01 higher in PCOS patients than in apparently healthy subjects (64.71% versus 28%, respectively OR = 4.71) while at A allele level frequency values were 49 for apparently healthy subjects and 25 for PCOS patients. Also, C allele frequency values were 51 for apparently healthy subjects and 77 for PCOS patients, respectively Table 3.

Genotypes for rs1169288	Patients no. 50(%)	Control no. 50(%)	Odd ratio	CI.	P-value
CC	33 (64.71%)	14 (28%)	4.71^{**}	2.02-10.95	0.0003
AC	10 (21.56%)	23 (46%)	0.32^{**}	0.13-0.76	0.010
AA	7 (13.73%)	13 (26%)	0.45^{ns}	0.16-1.25	0.12
Allele Frequency					
C	77	51	2.96^{**}	1.62-5.37	0.0004
A	25	49	0.34^{**}	0.18-0.61	0.0004
**** (P≤0.01), NS: Non-Significant**					

Table 3. Genotypes distribution and Allele frequency of rs1169288 genotype in PCOS patients and control group.

The effect of rs1169288 on HNF1A Serum Level

The serum HNF1A level and its association with the HNF1A C>A (rs1169288) genotypes between the studied groups (patients and control) were illustrated in Table 2. However, when the PCOS patients were compared within these genotypes according to HNF1A, There was a significant increase of CC genotype in PCOS patients compared with apparently healthy subjects (3.87 ±0.14 pg/mL, 2.15 ±0.21pg/mL). At the same time, the frequency of the AC genotype was significantly P≤0.01 higher in PCOS patients than in apparently healthy subjects (3.90 ±0.21 vs.. 48±0.16, respectively T-test=5.08), while the frequency of AA genotype was significantly P≤0.01 higher in PCOS patients than in apparently healthy subjects (4.31 ±0.33 versus 2.60 ±0.22, respectively T-test=4.25) (table 4).

Genotypes for rs1169288	Mean ± SE of HNF1A concentration		T-test	P-value
	Patients	Control		
CC	3.87 ±0.14 a	2.15 ±0.21	6.52**	**0.0001**
AC	3.90 ±0.21b	2.48±0.16	5.08**	**0.0001**
AA	4.31 ±0.33 ab	2.60 ±0.22	4.25**	**0.001**
This means that having different letters in the same column differed significantly. ** (P≤0.01), NS: Non-Significant.				

Table 4. Relationship between Genotypes for rs1169288 with HNF1A concentration in control and patients group.

DISCUSSION

The results of the current study agree with the results of the previous [11] in that they have found that Glucose levels are significantly higher in PCOS patients in contrast to controls (P <0.05); women with PCOS frequently demonstrate intrinsic insulin resistance (IR), which inherent to PCOS, IR is suggested as one of the main pathophysiological features contributing to both reproductive and metabolic disturbances in PCOS. These results agreed with [12,13], who found no significant differences between PCOS patients with metformin and PCOS patients in the level of serum total cholesterol. A study aimed at evaluating the genetic and environmental factors affecting lipids among twins found no significant differences between women with and without PCOS in serum total cholesterol; at the same time, The results of serum Triglyceride in PCOS patients with metformin (125.75 ±15.22 pg/mL) PCOS patients (100.80 ±6.01 pg/mL) compared to the mean of control (97.72 ±2.16 pg/mL). This study showed Triglyceride that mean showed significant differences (P≤0.05). The results of the current study agree with the results of the previous [14] in that they have found that Triglyceride levels are significantly higher in PCOS patients in contrast to controls (p <0.05); previous studies evaluating the effect of metformin on lipid profile in women with PCOS produced conflicting results; some reports demonstrated an improvement characterized by the decline of LDL cholesterol and triglycerides, while other found no significant change in these parameters [15].

The previous results could explain that C allele carriers not diagnosed with PCOS had an increased risk of developing PCOS, and CC genotype carrier was responsible for PCOS occurrence. HNF1A, which is involved in lipoprotein metabolism, is a new susceptibility gene for PCOS [18]. The results of the present study disagreed with the results of [19]. Also, genotype analysis of the selected SNP showed that the HNF1A gene has a direct effect on the PCOS phenotype, and the allele frequency analysis showed that rs1169288 polymorphism contributed to the occurrence of PCOS because the frequency of the risk allele C at this SNP locus was significantly higher in PCOS cases compared to control groups. HNF1A plays an important role in dyslipidemia. Dyslipidemia is the most common metabolic abnormality in PCOS, and previous research has shown that PCOS patients have a 4–5 times greater risk of suffering from dyslipidemia than healthy women [20]. HNF1A encodes a transcription factor (TF) that binds to promoters of a variety of genes expressed predominantly in the liver and also in pancreatic islet cells [21]; an association between polymorphism rs1169288 of HNF1A and the risk of T2DM has been identified in a Chinese population [22]. Also, mutations found in HNF1A could cause maturity-onset diabetes in youth type 3, primarily through impaired insulin secretion [23]. In addition, rs1169288 of HNF1A was found to be linked to hypertriglyceridemia [24] and linked to hypertension [25]; all of these results suggest that the HNF1A gene might be involved in the etiology of PCOS [18].

CONCLUSIONS

Glucose and triglycerides are significantly higher in polycystic ovary syndrome (PCOS) patients than healthy women group. In comparison, cholesterol had no significant change in these groups. The concentration of serum HNF1A observed a significant difference ($P< 0.01$) between women with PCOS and the healthy control group. The genotypes and allele frequency of HNF1A gene distribution frequencies at (rs1169288) C>A SNP polymorphism, the percentage of AA genotype was not significant, control higher than that of PCOS patients. In contrast, the percentage of AC genotype in control was significantly ($P\leq0.01$) higher than that of PCOS patients. At the same time, there was a highly significant difference ($P\leq0.0003$) in CC genotype percentage between two groups related with rs21169288 at HNF1A gene.

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