ARTICLE / INVESTIGACIÓN

Investigation of the Omentin-1 V109D gene polymorphism as a risk factor for the incidence of Polycystic Ovary Syndrome

Ahmed M. Mohsen[1], Rawaa S. A. AL-Azawi [2], Hawraa H. Naji [3], Hamzah H. Kzar[4*], Moaed E. Al-Gazally[5]

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[1,2] Collage of Science, Al-Qasim Green University, Iraq. Email: ahmed.mohsen@science.uoqasim.edu.iq. Email: rawaa1988@gmail.com.
[3,4] Collage of Veterinary Medicine, Al-Qasim Green University, Iraq. Email: hawraa1984@gmail.com., Email: hamza14shukri72@gmail.com.
[5] Collage of Medicine, University of Al-Ameed, Karbala, Iraq. Email: moaedalgazaly@gmail.com.
* Correspondence Author: hamza14shukri72@gmail.com

Abstract. This study was conducted to assess the omentin-1 (OME-1) V109D gene SNP in women with PCOS and healthy control and to investigate the possible relationship between this adipokine and metabolic profile. This study includes 50 women in the range of age (19-43 Y) who have been diagnosed with PCOC as a patient group and 40 healthy women in age (17-45 Y) as a control group. Five ml of peripheral blood were drawn from both groups to perform the biochemical and genetic study. Metabolic profiles were assessed by spectrophotometric methods, and PCR-RFLP investigated V109D of OME-1 SNP. The results suggest a statistical decrease in FBG, BMI, TG, TC, and LDL(p-value<0.05) in the control group compared to the PCOS group and a statistical increase in HDL levels in control compared to the PCOS group. Genotyping analysis shows statistically significant differences between DD and VV genotypes in PCOS and control groups (OR=4.66 (CI 95%,1.6-8.3), (OR=2.4 (CI95%,0.7-6.9) respectively. These results suggest statis-tically significant differences in three genotypes (DD, DV, and VV) of the PCOS group depending on age and BMI (p-value <0.05). DD and VV genotypes of OME-1 V109D SNP have more risk factors for PCOS incidence and changes in metabolic profile in Iraqi women.
Keywords: Polycystic ovary syndrome, Omentin-1, risk factor, polymorphism genetic

Introduction

It has been found that the most frequent endocrine disorder in women of reproductive age and, in some patients, presents with abdominal adiposity and dyslipidemia is called polycystic ovary syndrome (PCOS) [1]. The set of symptoms is here due to elevated androgens in females and is the main ovarian dysfunction in PCOS [2]. One of the well-known signs and symptoms of PCOS include irregular or no menstrual periods, heavy periods, excess body and facial hair, acne, pelvic pain, difficulty getting pregnant, and patches of thick, darker, velvety skin [3]. Associated conditions include T2DM, obesity, obstructive sleep apnea, heart disease, mood disorders, and endometrial cancer[4]. PCOS is the most common endocrine disorder among women between 18 and 44, affecting approximately 2% to 20% of this age group, [5-6]. Poor dietary choices and physical inactivity can exacerbate environmental factors implicated in PCOS, such as obesity; infectious agents and toxins may also play a role [7]. The reproductive and metabolic features of PCOS are sometimes reversible with lifestyle modifications such as weight loss and exercise [8]. Omentin-1 (OME-1) has been identified as a significant visceral (omental) fat secretory adipokine. This adipokine may act as an endocrine factor affecting muscles, liver, and omental adipose depot; it enhances insulin sensitivity and glucose metabolism [9]. It has been suggested that serum omentin-1 is elevated in patients with fatty liver diseases and represents an independent predictor for hepatocyte ballooning in these patients [10]. Omentin-1, also known as intelectin-1, is a recently identified novel adipocytokine of 313 amino acids, expressed in visceral (omental and epicardial) fat and mesothelial cells, vascular cells, airway goblet cells, small intestine, colon, ovary, and plasma [11]. This study aimed to determine OME-1 rs2274907 gene polymorphism in women with PCOS and healthy control and investigate the possible relationship between this adipokine and metabolic profile.

MATERIALS AND METHODS

Study group

This study includes 50 women in the range of age (19-43 Y) who have been diagnosed with PCOC as a patient group and 40 healthy women in age (17-45 Y) as a control group. Five ml of peripheral blood were drawn from both groups to perform of biochemical and genetic studies.

Measurement of metabolic profile

FBG, TG, TC, LDL, and HDL were performed by spectrophotometric methods depending on protocols provided by the manual kits.

Genetic analysis of OME-1 V109D

The genomic DNA Kit (Bioneer, Korea) provides AccuPrep®, an optimal protocol for extracting and purifying the genomic DNA from whole blood. Proteinase K and

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the specific buffers were used to lysate cells and destroy proteins. Amplification conditions of amplicon (471bp) up to 30 cycles of OME-1 gene were performed by PCR-RFLP depending on the method described by Khoshi et al. [12].

Statistical Analysis

Statistical analysis was performed using SPSS 22 software. Observed and expected genotype frequencies using the Hardy-Weinberg equation by odds ratio (OR) and 95% confidence intervals (CI 95%). The association of OME-1 rs2274907 and metabolic profile was estimated by t-test.

Results

Genetic analysis of OME-1 V109D:

The following pairs of primes (forward and reverse) were used in PCR analysis (Table1):

Primer sequence (5⟶3)	Amplicon length	RE bands
F: GAGCCTTTAGGCCATGTCTCT	471 bp	471, 274, 197 bp
R: CTCTCCTTCTTCTCCAGCCCAT		

Table 1. Primers of OME-1 V109D method of PCR-RFLP that used in genotyping analysis

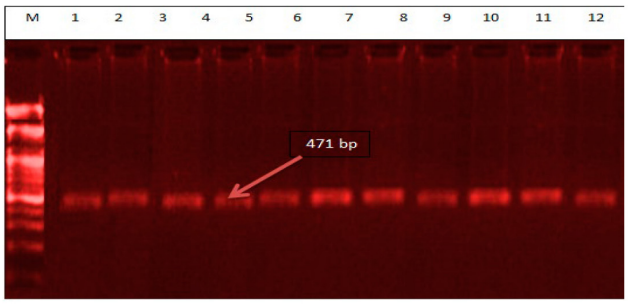

Figure 1. PCR product (471 bp) of OME-1 V109D gene. Lane M: DNA marker, lanes 1-12 represented samples of PCOS and control

The PCR product (471 bp) is shown in figure (1). PCR-RFLP investigates the V109D of the OME-1 gene in patients with PCOS and control groups involved in this study by using XmiI as a restriction enzyme (RE). The PCR was performed by an Exicycler 96^{R} (Bioneer, Korea), in which the DNA templates were denatured at 95 °C for 4 min. The amplification step consisted of 30 cycles of 30 sec at 95 °C, 45 sec at 58.5 °C, 72 °C for 1 min s, with a final extension of 5 min at 72 °C. Gel electrophoresis was running the final products on a 2%, non-digested by RE represented 471 bp of D allele (DD genotype), (274 and 197 bp) were identified as homozygotes for V allele (genotype VV). Moreover, both digested and undigested fragments (471, 274, and 197 bp) were detected as heterozygotes (genotype DV), as shown in figure (2).

Table 2 shows the metabolic profile in both PCOS and control groups. The results suggest a statistical decrease in FBG, BMI, TG, TC, and LDL(p-value<0.05) in the control group compared to the PCOS group and a statistical increase in HDL levels in control compared to the PCOS group.

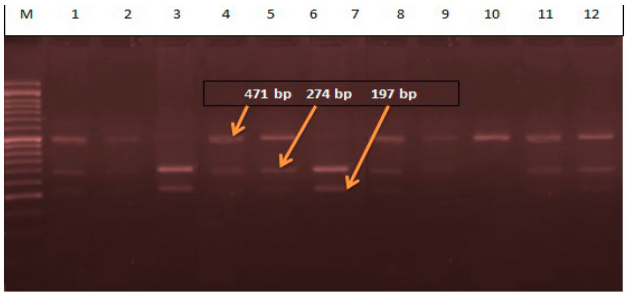

Figure 2. XmiI restriction enzyme of the PCR product of OME-1 V109D gel electrophoresis. Lane M: DNA marker, lanes 1,4,5,8,9, 11 and 12) represented DV genotype, lanes 2 and 10 represented DD genotype, and lanes 3 and 7 represented VV genotype

Parameters groups	FBG mg/dl	BMI Kg/m^2	TG mg/dl	TC mg/dl	LDL mg/dl	HDL mg/dl
PCOS N=115	109±16	27±3.1	167±17	223±22	108±16	33±3
Control N=100	98±11	22±1.1	123±19	189±29	98±19	37±7
p-value	0.042	0.012	0.001	0.001	0.021	0.045

Table 2. Metabolic profile in study groups

Table 3. Shows the distribution of genotyping and allele frequencies of OME-1 V109D SNP in PCOS and control groups. Genotyping analysis shows statistically significant differences between DD and VV genotypes in PCOS and control groups (OR=4.66(CI 95%,1.6-8.3), (OR=2.4(-CI95%,0.7-6.9) respectively. The results also show the significant differences between D and V alleles in PCOS and control groups (OR=3.2(CI 95%,0.8-6.2).

Data is represented as a number (%) *p< 0.05 is considered statistically significant.

OR: odds ratio, CI: confidence interval

Genotype	Control n=100 (%)	PCOS n= 115(%)	Total	OR (CI 95%)	p-value
DD	34 (34)	53 (46)	87	**4.667(1.64-8.33)***	**0.0001***
DV	51 (51)	42 (36)	93	1.0	**0.0001***
VV	15 (15)	20 (18)	35	**2.432(0.77-6.91)***	
Total	100	115	215		
D allele	54%	64%		**3.22(0.81-6.29)***	**0.0001***
V allele	46%	36%			

Data is represented as a number (%) *p< 0.05 is considered statistically significant. OR: odds ratio, CI: confidence interval

Table 3. Distribution of genotyping and alleles frequencies of OME-1 V109D SNP in PCOS and control groups

Variables		Genotypes n =115(%)			P-value
		DD 53 (46%)	DV 42 (36%)	VV 20 (18%)	
Age	<25 n (%)	30(26)	23(20)	12(10)	**0.0001***
	≥25 n (%)	23(20)	19(16)	8(8)	
BMI	<25 n (%)	22(16)	19(28)	7(6)	**0.0001***
	≥25 n (%)	31(13)	23(27)	13(12)	

Table 4. Effects of age and BMI on OME-1 V109D SNP in PCOS group

Parameters PCOS genotype	FBG mg/dl	BMI Kg/m^2	TG mg/dl	TC mg/dl	LDL mg/dl	HDL mg/dl
DD N=53	108±13	27±4.1	166±17	221±22	108±17	31±6
DV N=42	99±12	22±1.7	149±13	210±29	104±15	33±5
VV N=20	103±12	24±1.9	153±14	220±17	103±12	32±4
p-value	0.012	0.009	0.065	0.055	0.056	0.061

Table 5. Metabolic profile in DD, DV, and VV genotypes in PCOS group

Table 4 shows the effects of age and BMI on OME-1 V109D SNP in the PCOS group. These results suggest statistically significant differences in three genotypes (DD, DV, and VV) of the PCOS group depending on age and BMI (p-value <0.05):

Table 5, listed the distribution of metabolic profile of the PCOS group depending on different genotypes DD, DV, and VV. The results showed statistically significant differences in FBG and BMI in different genotypes of the PCOS group (p-value <0.05) but no statistical difference in TG, TC, LDL, and HDL levels (p-value> 0.05).

DISCUSSION

One of the well-known, most frequent endocrine disorders in women of reproductive age and, in some patients, presents with abdominal adiposity and dyslipidemia is called polycystic ovary syndrome (PCOS). Although genetic, environmental, and behavioral factors are related to PCOS development, its etiology remains to be elucidated [13]. OME is a newly identified adipokine that is highly and selectively expressed in visceral adipose tissue relative to subcutaneous adipose tissue, and there are two highly homologous isoforms of OME, OME-1 and OME-2; however, omentin-1 is the major circulating form in human plasma. OME-1 has been identified in other tissues at lower expression levels and is also named OME, intelectin, intestinal lactoferrin receptor and endothelial lectin [14]. This study aimed to assess OME-1 V109D gene SNP in women with PCOS and healthy control and investigate the possible relationship between this genetic variation of adipokine and metabolic profile. The results suggest a statistical decrease in FBG, BMI, TG, TC, and LDL(p-value<0.05) in the control group compared to the PCOS group and a statistical increase in HDL levels in control compared to the PCOS group. Many studies have reported that in PCOS patients, there is a positive correlation between other adipokines called chemerin and their correlation with BMI, TG, HOMA-IR, LDL, and an inverse correlation between chemerin and HDL-C [15-18]. Genotyping analysis shows statistically significant differences between DD and VV genotypes in PCOS and control groups (OR=4.66(CI 95%,1.6-8.3), (OR=2.4(CI95%,0.7-6.9) respectively. Kohan et al. (2016) suggested that OME-1 rs2274907 polymorphisms might be a candidate genetic factor for nonalcoholic fatty liver disease [19]. The results also show the significant differences between D and V alleles in PCOS and control groups (OR=3.2(CI 95%,0.8-6.2). These results suggest statistically significant differences in three genotypes (DD, DV, and VV) of the PCOS group depending on age and BMI (p-value <0.05). In line with these results, our data indicated that DD homozygous genotype was more frequent, 53% in PCOS compared to healthy women 34%. Bahadori et al. (2015) concluded that Iranian women with VV genotype had a greater risk of obesity complications than those with DD genotypes [20]. Many studies demonstrated that A326T rs2274907 SNP (miss-sense in the exon-4 of the OME-1 gene, which substitutes valine instead of aspartate at position 109 (Val109Asp or V109D), could be accompanied by T2DM and obesity [21,22].

Conclusions

This study confirms the presence of an interesting correlation between the polymorphism of the OME-1 V109D SNP and increasing risk factors for incidence of PCOS and changes in metabolic profile in Iraqi women.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interest.

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Author contributions

Conceptualization, HHK and MEA; methodology, RAA and HHK.; software, HHK; validation, AMM, HKK and RAA.; formal analysis, HHK; investigation, HHK; resources, HHK; data curation, AMM; writing—original draft preparation, HHK; writing—review and editing, HHK; visualization, MEA; supervision, MEA; project administration, MEA; funding acquisition, MEA. All authors have read and agreed to the published version of the manuscript.

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