Original paper

Relation between the Genetic Variants of SLC47A1 (MATE1) and the Response to Metformin Therapy in Iraqi Women with Polycystic Ovarian Syndrome

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Abstract

Background: Metformin is widely used in polycystic ovary syndrome (PCOS), nevertheless, the treatment responsiveness reveals individual variation in patients with PCOS. Multidrug and toxins extrusion protein (MATE1) mediates metformin excretion in kidney and bile. MATE1 is encoded by SLC47A1 gene. In this study the single-nucleotide polymorphism (SNP) (rs1961669 A>G) was analyzed in an attempt to investigate the clinical, hormonal and biochemical effects of three months metformin treatment in women with polycystic ovary syndrome and to study the relation between MATE1 (SLC47A1) gene polymorphism and the response to metformin.

Patients and Methods: This study is a prospective group study; 231 women with PCOS were enrolled. All participated women with age range (18-40) were starting metformin tablet 500 mg per oral three times daily. Blood samples were taken from eligible patients to perform genetic analysis and estimation of follicle stimulating hormone, luteinizing hormone, total testosterone, fasting insulin, HbA1c, fasting blood glucose, sex hormone binding globulin and lipid profile.

Results: The study showed that SLC47A1 (rs1961669) (A>G) gene polymorphism has non-significant association with metformin response in PCOS. The comparison in demographic and biochemical parameters between pre and post metformin treatment results showed no improvement in clinical, hormonal and biochemical parameters.

Conclusion: SLC47A1 (rs1961669) (A>G) gene polymorphism had no association with clinical, hormonal and biochemical response to metformin in Iraqi women with polycystic ovarian syndrome.

Keywords: Polycystic Ovarian Syndrome, metformin, MATE1, SLC47A1, SNP.

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder which is characterized by oligo-amenorrhea and polycystic ovaries that is differently accompanied by acne, hirsutism, and obesity [(1)]. PCOS is a complex disease of unknown etiology, however there is strong indication that it can, to a large extent, be classified as a genetic disorder [(2, 3)]. women with disorder may exhibit hormonal imbalance, hyperinsulinemia, insulin resistance, dyslipidemia, weight gain and even may develop diabetes mellitus and cardiovascular disease due to obesity and insulin abnormalities [(4, 5)]. This syndrome has been diagnosed upon different criteria, where the most common one is Rotterdam criteria which specify that a woman to be PCOS must have 2 of the following 3 criteria; oligo-menorrhoea or amenorrhoea, biochemical or clinical evidence of hyper-androgenism (specifically hirsutism and

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acne), and typical ultrasound characteristics of a polycystic ovary (≥12 follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume >10 ml) [6]. Non pharmacological treatment includes weight loss through diet or exercise therapy and other lifestyle interventions that could improve clinical symptoms and restore the normal ovulation [7], while pharmacological treatment mostly involves using of insulin sensitizers such as metformin which is a therapeutic choice that objectives not merely insulin resistance current in this syndrome, however several other factors particularly reproductive abnormalities such as infertility, which is a predominant problem of challenge for females of reproductive age [8]. Metformin has been proven to improve PCOS-induced reproductive and metabolic defects, as well as it regulates menstrual cycle, improves hyperinsulinemia and hyperandrogenemia and restored ovulatory function [9], so, its use either on its personal or mixed with clomiphene citrate, could increase ovulation and conception rates in women with PCOS, in addition metformin may reduce first-trimester spontaneous abortions in women with PCOS, accordingly, it is highly recommended in ladies with this syndrome [10]. Metformin requires transporters for its absorption, distribution and excretion as it poorly diffuses across biological membranes, alteration in expression and function of these transporters contributes to individual variability in response to this drug [11]. DNA variants in genes encoding organic ion transporters that are mediated metformin transport may change metformin pharmacokinetics and could provide insight into the variability of the clinical response to metformin treatment [12], among these transporters is MATE 1 which is majorly expressed on the apical membrane of proximal tubule and bile canaliculi and mediates excretion of metformin and many other cations [13]. MATE 1 is encoded by SLC47A1 gene which is situated in the tandem on chromosome 17p11.2 and encode proteins of 570 [14]. genetic variants in SLC47A1 gene of MATE 1 have been demonstrated to influence metformin response in patients with Type 2 diabetes [15].

The present work aimed to investigate the clinical, hormonal and biochemical effects of three months metformin treatment in women with polycystic ovary syndrome (PCOS) and to study the relation between MATE1 (SLC47A1) gene polymorphism and response to metformin in these patients.

Patients and Methods

This study is prospective group study carried out at gynecological and obstetric teaching hospital, Kerbala Health Directorate/Kerbala–Iraq out clinic patients and college of pharmacy/University of Kerbala, during the period from July, 2019 to July, 2020 with age ranged between (18 to 40) years. The study was conducted on three hundred and forty-six woman with newly diagnosed polycystic ovary syndrome, the participated women were recruited by consultation of gynecologist and infertility according to the inclusion and exclusion criteria of the study, only two hundred and thirty-one woman returned after three months of treatment to follow up and enrolled in final data analysis, thirty female became pregnant during period of treatment so they were excluded from the study. All women enrolled in this study were newly diagnosed PCOS women according to Rotterdam criteria (two of three of hyperandrogenism, irregular an ovulatory periods or ultrasound polycystic ovary morphology), moreover, all of them were starting metformin tablet 500 mg per oral twice daily as standard therapy.

Women with Cushing syndrome, androgen secreting adrenal tumors, thyroid disorders, impaired kidney, function, hypertension, women taking oral hypoglycemic agents, any patients with HbA1c% more than 6.5 and women who become pregnant through the period of study were excluded from

study. Biochemical parameters were measured to all patients participated in this study before treatment and after the end of treatment period (3 months) for the returned patients only to demonstrate the response to metformin and this achieved according to continuous communication and follow up with patients through social media during period of treatment.

The protocol for study was approved by the ethical research committee of College of Pharmacy, University of Kerbala and Kerbala Health Directorate. Approval was taken from administration of gynecological and obstetric teaching hospital and consent was obtained from all patient after demonstrating the nature and aim of study.

Blood samples were obtained from eligible patients after taking the patient consent, 10 mL of venous blood were withdrawn from all women participated in this study, blood samples were drawn from the patients who are all fasting during the morning in follicular phase (cycle day 2), (3 ml) was placed in EDTA-tube for genetic testing then were analyzed directly to obtain high purity of DNA. (2 ml) was placed in another EDTA tube for HbA1c testing. (5ml) were placed in gel tube (EDTA-free tube) for serum analysis; serum was aspirated after centrifugation of the blood at 3000 rpm for 10 minutes where it used for measurement. Biochemical parameters were being measured for all patients before and after three months of starting with metformin which were: FSH, LH, total testosterone, sex hormone binding globulin (SHBG), free androgen index fasting insulin, HbA1c%, fasting blood glucose, homeostatic model assessment for insulin resistance (HOMA-IR ratio) and lipid profile [triglycerides, low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) and total cholesterol]. Free androgen index (FAI) was measured by multiplying total testosterone by 100 and divided by SHBG level(16), while HOMA-IR is equal to fasting blood glucose multiplying by insulin levels dividing by 405[17].

Genomic DNA was extracted from each blood sample using (G-DEX™ IIb / Intron, Korea) genomic DNA extraction kit for blood, according to the manufacturer's instructions. The DNA was stored at −20◦C until use. The SNP of MATE1 SLC47A1 (rs1961669) was genotyped using conventional genotyping assays using (Accupower® PCR PreMix–Bioneer Kit, Korea). The intron of MATE-1 (SLC47A1 gene) (rs1961669 **A>G**) was amplified by means of specific primers to study the SNP; PCR reaction was performed by using specific primers designed for SLC41 gene. Based on NCBI database, all genes information, sequence and SNP details were collected and by using specific software, primers were designed.

The data were expressed as mean ±SD, student T-test were used for calculating the probability using the (PAST version 3.09, 2004) that used for calculating probability value (*P* value) for response to metformin in biochemical parameters generally and across genotypes while chi- square (χ^2) were used to express the significance between the studied groups in demographical characteristics. In all statistical analysis the significant value is ($P \leq 0.05$) and the highly significant value is ($P \leq 0.01$).

Results

According to inclusion and exclusion criteria, 231 patients were enrolled in the final data analyses, the women which included in the study finally were with an average age ranged between (18-40) years and the mean ± SD of them were 27.2 ± 5.9 years. The results of the present study were shown in table (1) using Chi-Square statistical test.

There was a significant improvement in alopecia (hair loss) as (P value ≤ 0.01), as well as menstrual irregularity showed significant change where (P value ≤ 0.01).

Table 1. Comparison for the demographic parameters between pre- and post-metformin treatment as a response to metformin

Demographic parameter		Number (No.)	Pre-treatment	Post-treatment	*P* value
Hirsutism	Yes	N = 231	209 (90.48%)	120 (51.9%)	*P* value = 0.7 $\chi^2 = 0.13$
	No		22 (9.52%)	111 (48.1%)	
Alopecia	Yes	N = 231	186 (80.5%)	119 (51.5%)	*P* value ≤ 0.01 $\chi^2 = 43.31$
	No		45 (19.5%)	112 (48.5%)	
Regularity	Yes	N = 231	0 (0%)	95 (41.1%)	*P* value ≤ 0.01 $\chi^2 = 119.6$
	No		231 (100%)	136 (58.9%)	
Parameter	**Pre-metformin treatment**		**Post-metformin treatment**	**Mean difference ± S.D.**	***P* value**
BMI kg/m^2	29.12 ± 5.4		28.35 ± 5.3	0.77 ± 1.5	0.13

The results of the current study were shown in table (2) using statistical student T-test, there was a significant increase in mean ± SD of FSH level (*P* value ≤ 0.01) between pre- and post- metformin treatment while LH and LH/FSH ratio decreased significantly (*P* value ≤ 0.01) after three months of treatment with metformin.

Total testosterone demonstrated significant reduction from baseline (*P* value ≤ 0.01) while SHBG showed significant increase (*P* value ≤ 0.01) after metformin treatment as a result FAI indicated significant decrease (*P* value ≤ 0.01) after metformin therapy.

Fasting blood sugar decreased significantly between pre- and post-metformin treatment (*P* value ≤ 0.01). In contrast, mean ± SD level of fasting insulin indicated non-significant reduction from baseline (*P* = 0.23), so HOMA-IR showed non-significant reduction (*P* = 0.13) after treatment with metformin while HbA1c level reduced significantly (*P* value ≤ 0.01) after three months of metformin treatment.

Regarding lipid profile, the current study revealed significant reduction (*P* value ≤ 0.01) in mean ± SD level of triglycerides, LDL-C and total cholesterol. In contrast, HDL-C level indicated non-significant increase *(P* = 0.1) in HDL-C after the treatment period with metformin.

The subjects that enrolled in final data analysis of this study were classified into three genotypes, for SLC47A1(rs1961669) (A >G): one homozygous for the A allele (AA) wild type, one heterozygous (GA) and the last one was homozygous for the allele G(GG) mutant type. Out of 231 patients, there were 67 heterozygous (GA) genotypes (29%), 152(AA) genotypes (65.8%) and 12 (GG) genotypes (5.2%) of SNP rs1961669 in the SLC47A1gene, see fig.1 summarize genotyping of study subjects according to polymorphism of (A>G) (rs1961669) of the SLC47A1 gene.

Comparison of the Clinical Response to Metformin in Demographic Parameters across SLC47A1 (A> G) (rs1961669) Alleles and Genotypes

The results of the current study were shown in table (3) using Chi-Square statistical test, the table showed demographic parameters before and after initiation of metformin therapy in each genotype of SLC47A1(A> G) (rs1961669), there was a significant response to metformin in hirsutism for (AA) genotype which are the homozygous carriers of common A- allele (*P* value ≤ 0.01). The present study also demonstrated that there was a significant response to metformin in alopecia (*P* value ≤ 0.01) for both (AA) and (AG) genotype as well as menstrual regularity indicated significant

response to metformin treatment in both (AA) and (GA) genotypes (*P* value ≤ 0.01).

Comparison of the Clinical Response to Metformin in Biochemical Parameters across SLC47A1 (A> G) (rs1961669) Alleles and Genotypes

The results of the present study are shown in table (4) using student t-test, the table showed biochemical parameters before and after initiation of metformin therapy in each genotype of SLC47A1 (A> G) (rs1961669).

The results of current study demonstrated that the reductions in LH level between pre- and post- metformin treatment were significant in patients of heterozygous and homozygous carriers of SLC47A1 of (A> G) (rs1961669) [(*P* value ≤ 0.01) and (*P* value≤ 0.05)] respectively. A significant increment in FSH in both GA and GG genotypes between pre- and post-metformin treatment [(*P* value≤ 0.01) and (*P* value≤ 0.05)] respectively as a result LH/FSH ratio also indicated significant reduction in GA and GG genotypes (*P* value ≤ 0.01) between pre- and post-metformin treatment.

The data of the current study revealed a significant reduction in total testosterone level (*P* value ≤ 0.01) between pre- and post- metformin therapy in AG and AA genotype while SHBG level indicated significant increment (P value ≤ 0.01) in both GA and AA genotype between pre- and post- metformin therapy so FAI in this study demonstrated significant reduction (*P* value ≤ 0.01) between pre- and post-treatment metformin in both GA and AA genotypes.

Table 2. Comparison between biochemical parameters in PCOS patients pre- and post-metformin treatment as response to metformin

Parameters	Pre–treatment Mean ± SD	Post–treatment Mean ± SD	Mean difference ± SD	*P* value
FSH (mIU/mL)	5.7 ± 1.99	6.5 ± 2.4	-0.8 ± 2.2	≤ 0.01
LH (mIU/mL)	10.13 ± 6.19	8.5 ± 4.8	1.6 ± 4.5	≤ 0.01
LH / FSH	1.85 ± 1.08	1.4 ± 0.8	0.1 ± 0.55	≤ 0.01
T. testosterone (ng/mL)	0.6 ± 0.35	0.45 ± 0.3	0.14 ± 0.25	≤ 0.01
SHBG (nmol/L)	41.84 ± 22.6	51.3 ± 21.8	-9.4 ± 15.1	≤ 0.01
FAI	9.6 ± 17.7	4.5 ± 6.7	5.06 ± 15.6	≤ 0.01
FBS (mg/dL)	96.03 ± 12.5	92.45 ± 12.97	3.6 ± 11.3	≤ 0.01
F. Insulin level (µUl/mL)	20.02 ± 14.5	18.6 ± 11.7	1.46 ± 9.09	0.23
HOMA-IR	4.8 ± 3.7	4.3 ± 2.96	0.48 ± 2.48	0.13
HbA1c%	4.95 ± 0.69	4.6 ± 0.65	0.36 ± 0.56	≤ 0.01
TG (mg/dL)	127.6 ± 49.4	113.2 ± 39	14.4 ± 25.99	≤ 0.01
LDL-C (mg/dL)	98.06 ± 66.4	85.1 ± 25.5	12.96 ± 59.5	≤ 0.01
HDL-C (mg/dL)	44.8 ± 9.96	46.28 ± 9.46	-1.48 ± 6.25	0.1
T. Cholesterol (mg/dL)	160.7 ± 43.8	146.6 ± 44.03	14.08 ± 28.4	≤ 0.01

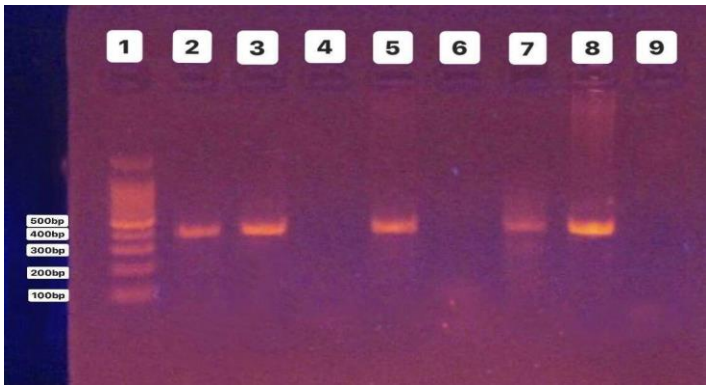

Figure 1. Allele Specific PCR of SNP of MATE1: SLC47A1 (A> G) (rs1961669) gene polymorphisms

In present study there is significant decline in FBS (P value≤ 0.05) in both GA and AA genotypes between pre- and post-metformin therapy while fasting insulin level revealed non-significant results after metformin therapy in all genotypes AA, GA and GG [*P* value = 0.6, *P* value=0.15 and *P* value=0.6] respectively, accordingly, HOMA-IR indicated non-significant reduction the same as fasting insulin in all genotypes : AA, GA and GG after three months treatment [*P* value = 0.48, *P* value = 0.076 and *P* value= 0.6] respectively. In contrast, HbA1c showed significant reduction (*P* value ≤ 0.01) in both GA and AA genotypes between pre- and post-metformin therapy.

Regarding lipid profile, there was highly significant lowering (*P* value ≤ 0.01) in TG and total cholesterol level between pre- and post- metformin treatment in GA and AA genotypes, moreover, LDL-C revealed significant reduction (*P* value ≤ 0.05) after three month treatment with metformin while HDL-C level displayed significant increment (*P* value ≤ 0.05) only in heterozygous group of SLC47A1 Of (A> G) (rs1961669) between pre- and post-metformin treatment.

Table 3. Association of genetic polymorphism of SLC47A1 Of (A>G) (rs1961669) with demographic parameters in Iraqi PCOS women No.=(231)

Parameter	**Genotype**	**No.**	**Response**	**Pre-metformin treatment**	**Post -metformin treatment**	**Chi square Test**
Hirsutism	**AA**	**152**	**Yes**	138 (90.8%)	56 (36.8%)	P value ≤ 0.01 $\chi^2 = 95.79$
			No	14 (9.2%)	96 (63.2%)	
	GA	**67**	**Yes**	60 (89.5%)	54 (80.6%)	P value = 0.15 $\chi^2 = 2.1$
			No	7 (10.5%)	13 (19.4%)	
	GG	**12**	**Yes**	11 (91.7%)	10 (83.3%)	P value = 0.5 $\chi^2 = 0.38$
			No	1 (8.3%)	2 (16.7%)	
Parameter	**Genotype**	**No.**	**Response**	**Pre-metformin treatment**	**Post -metformin treatment**	**Chi square Test**
Alopecia	**AA**	**152**	**Yes**	128 (84.2%)	54 (35.6%)	P value ≤ 0.01 $\chi^2 = 74.97$
			No	24 (15.8%)	98 (64.4%)	
	GA	**67**	**Yes**	48 (71.7%)	56 (83.6%)	P value = 0.097 $\chi^2 = 2.75$
			No	19 (28.3%)	11 (16.4%)	
	GG	**12**	**Yes**	10 (83.3%)	9 (75%)	P value = 0.6 $\chi^2 = 0.25$
			No	2 (16.7%)	3 (25%)	
Parameter	**Genotype**	**No.**	**Response**	**Pre-metformin treatment**	**Post -metformin treatment**	**Chi square Test**
Regularity	**AA**	**152**	**Yes**	0 (0%)	79 (52%)	P value ≤ 0.01 $\chi^2 = 106.7$
			No	152 (100%)	73 (48%)	
	GA	**67**	**Yes**	0 (0%)	13 (19.4%)	P value ≤ 0.01 $\chi^2 = 14.4$
			No	67 (100%)	54 (80.6%)	
	GG	**12**	**Yes**	0 (0%)	3 (25%)	P value = 0.06 $\chi^2 = 3.4$
			No	12 (100%)	9 (75%)	
Parameter	**Genotype**			**Pre-metformin treatment**	**Post -metformin treatment**	**P value**
BMI(kg/m$^{2)}$	**AA**			29.8 ± 5.6	27.5 ± 5.4	≤ 0.01
	GA			28.2 ± 4.8	27.1 ± 4.6	0.2
	GG			26.1 ± 5.6	24.99 ± 5.2	0.6

Discussion

This is the first study was done to demonstrate and assess the effect of MATE1 (SLC47A1) (A>G) (rs1961669) genetic polymorphism on metformin response in Iraqi population with PCOS. In this prospective study, the most common clinical manifestations of PCOS are hirsutism, alopecia and menstrual irregularity which occur as a result of abnormal steroidogenesis that included excessive LH secretion and hyperandrogenism [18]. The study showed a significant improvement following metformin treatment and the women exhibited more regular cycle and less hair loss where this improvement was due to the fact that metformin improves insulin resistance which lead to decrease theca cells hypertrophy, consequently decrease LH production from these cells and in turn decrease ovarian androgen production and ovarian (cytochrome P450c17α) enzyme activity with a consequent decline in the serum testosterone which is the main responsible factor of hair loss [19]. Similar results were obtained from some studies **(Ou HT *et al.*, 2016 and Velija-Ašimi *et al.*, 2013)** which showed that metformin reduce hair loss in women with PCOS and improve cyclicity [20, 21].

Regarding the effect of metformin on the hormonal profile in PCOS women, it is best represented in table (2). The study demonstrated that metformin treatment resulted in a significant reduction in serum level of LH, total testosterone along with significant decrease in LH/FSH ratio, this study also clarified a significant increase in FSH, SHBG level, the observed data indicated a significant increase in FSH level after 3 months of treatment. In PCOS, an increase in GnRH pulse frequency results in decrease FSH production and reduction in stimulation of granulosa cell, this contributes to poor egg development and inability or difficulty of ovulation that improved by metformin treatment which reduce exaggerated GnRH pulse frequency, rebalanced the hormones level, consequently, induces the ovulation[22].

These results are in agreement with **(Kazerooni *et al.*, 2003 and Aruna *et al.*, 2004)** that showed a significant increase in FSH level [23, 24].

Serum LH levels decreased significantly as women with PCOS exhibit increased expression of cytochrome P450c17α (CYP 17), which results in increased activity of 17α-hydroxylase and 17,20-lyase in ovarian theca cells where this dysregulation of CYP 17 enhances the production of 17α-hydroxyprogesterone, androstenedione and testosterone which increased by insulin so metformin decreases ovarian cytochrome P450c17α activity secondary to a reduction in insulin [25]. These findings were compatible with **(Kurzthaler *et al.*, 2014)** that showed a similar result [25], while **(De Leo *et al.*, 2000)** showed that the reduction in LH was more limited or non-significant [26]. The reduction in values of LH/FSH ratio after metformin treatment period was highly significant as LH decreased significantly and there is significant change in FSH, so LH / FSH ratio is also decreased significantly.

Data observed in table (2) described a significant reduction in total testosterone levels and significant increase in SHBG levels due to that metformin induces insulin level reduction which is associated with an increase in SHBG, IGFBP-I availability to ovaries and a reduced IGF-I/IGFBP-I ratio, which may be partly responsible for the reduction of plasma androgen levels in PCOS patients [26, 27]. Similar results were obtained from other studies that showed a significant reduction in total testosterone and significant increment in SHBG after metformin therapy. Free androgen index (FAI) in current study showed a significant reduction after three months of metformin treatment as there was a significant decrease in total testosterone and significant increase in SHBG, accordingly FAI decreased significantly [25, 28, 29].

Table 4. Association of genetic polymorphism of SLC47A1 of (A>G) (rs1961669) with biochemical parameters in Iraqi PCOS women (No.=231)

Genotypes	AA N = 152			GA N = 67			GG N = 12		
Parameters	**Pre metformin treatment**	**Post metformin treatment**	***P* value**	**Pre metformin treatment**	**Post metformin treatment**	***P* value**	**Pre metformin treatment**	**Post metformin treatment**	***P* value**
LH (mIU/mL)	9.4 ± 5.9	8.8 ± 5.1	0.33	10.4 ± 5.3	7.6 ± 3.7	≤ 0.01	17.9 ± 9.3	10.3 ± 5.1	≤ 0.05
FSH (mIU/mL)	5.7 ± 1.98	6.2 ± 2.4	0.08	5.7 ± 2	7.1 ± 2.2	≤ 0.01	5.6 ± 2.1	7.6 ± 2.5	≤ 0.05
LH/FSH Ratio	1.7 ± 1.1	1.5 ± 0.9	0.087	1.9 ± 0.8	1.1 ± 0.6	≤ 0.01	3.3 ± 1.2	1.4 ± 0.6	≤ 0.01
T. testosterone (ng/mL)	0.6 ± 0.35	0.5 ± 0.3	≤ 0.01	0.5 ± 0.3	0.4 ± 0.2	≤ 0.01	0.6 ± 0.4	0.4 ± 0.2	0.09
SHBG (nmol/L)	43.2 ± 22.97	49.95 ± 21.3	≤ 0.01	38.4 ± 20.6	52.2 ± 22.1	≤ 0.01	44.1 ± 27.9	62.4 ± 24.4	0.1
FAI	10.2 ± 20.4	5.1 ± 7.4	≤ 0.01	7.95 ± 9.8	3.4 ± 5.1	≤ 0.01	10.6 ± 14.6	2.4 ± 2.01	0.066
FBS (mg/dL)	96.5 ± 12.6	93.4 ± 14	≤ 0.05	94.96 ± 12.5	90 ± 10.95	≤ 0.05	95.6 ± 10.3	94.3 ± 7.4	0.7
F. Insulin (µUl/mL)	21.1 ± 15.4	20.3 ± 12.3	0.6	18.1 ± 12.4	15.2 ± 9.5	0.15	19.7 ± 13.5	15.3 ± 9.95	0.6
HOMA-IR	5.1 ± 3.9	4.8 ± 3.1	0.48	4.4 ± 3.3	3.5 ± 2.4	0.076	4.3 ± 3.5	3.6 ± 2.5	0.6
HbA1c%	4.9 ± 0.7	4.6 ± 0.7	≤ 0.01	5.01 ± 0.6	4.5 ± 0.6	≤ 0.01	5.1 ± 0.5	4.7 ± 0.7	0.1
TG (mg/dL)	126.6 ± 40.4	116.4 ± 36.2	≤ 0.05	131.9 ± 63.7	108.6 ± 43.1	≤ 0.01	116.3 ± 62.9	97.8 ± 46.5	0.4
LDL-C (mg/dL)	98.4 ± 77.3	85.4 ± 22.5	≤ 0.05	96.7 ± 37.8	83.6 ± 30.7	≤ 0.05	101.9 ± 38.3	89.7 ± 30.9	0.4
HDL-C (mg/dL)	45.6 ± 9.3	46.3 ± 9.5	0.5	42.2 ± 10.8	45.9 ± 9.98	≤ 0.05	49.2 ± 11.1	48.9 ± 5.5	0.9
Total cholesterol (mg/dL)	162.5 ± 41.8	152.2 ± 42.4	≤ 0.05	154.5 ± 46.9	131.9 ± 43.6	≤ 0.01	172.7 ± 49.5	157.8 ± 51.02	0.47

Results of current study indicated that metformin treatment also improved biochemical profile (i.e.) there was a significant decrease in fasting blood sugar level (FBS), HbA1c% and lipid profile. The effect of metformin on FBS is well represented in table (2) which indicated that fasting blood sugar (FBS) in current study demonstrated a significant reduction after metformin therapy as the drug suppresses liver production of glucose, increases the sensitivity of liver, muscle, fat and cells to insulin and decrease the absorption of carbohydrates[23], therefore, current results agreed with **(Kazerooni *et al.*, 2003)**. Present study showed a significant reduction in HbA1c% level after metformin treatment where HbA1c% level reflects the average blood glucose levels from the last 3 months because once glucose has attached to hemoglobin in a red blood cell, it stays there for the life of the red blood cell, which is around 3 months which were the same period of treatment of present study so metformin decrease glucose production from liver and absorption of glucose from intestine and result in decrease HbA1c% after three months treatment [30], these outcomes are compatible with **(Jakubowska *et al.*, 2008)** and interfere with **(Bredella *et al.*, 2013)** that indicted a non-significant reduction in HbA1c% after metformin treatment [31].

Regarding lipid profiles data that are observed in table(2) which showed a significant reduction of TG, LDL-C and total cholesterol after three months of metformin treatment as the treatment led to a reduction in the activity and expression of several products or enzymes that involved in the lipid synthesis, such as acetyl-CoA carboxylase, SREBP-1, fatty acid synthase, and HMG-CoA reductase so metformin improved lipid profiles by its glucose lowering effects, these outcomes are in agreement with **(Singh *et al.*, 2010 and**

Kocer *et al.*, 2014) which showed similar results **(32, 33)**.

Table (3) revealed a significant response in both hirsutism and alopecia in homozygous wild (AA) group after three months treatment with metformin in comparison with homozygous and heterozygous carriers of SLC47A1 (A>G) (rs1961669), it means that AA genotype had better response to metformin in hirsutism and alopecia than GG and AG genotypes. Regularity of menstrual cycle in current study indicated significant response to metformin after three months of treatment in AA and GA genotypes. Present study demonstrated significant reduction in BMI after three months of metformin therapy in AA genotypes in comparison with GA and GG genotypes of PCOS women, so the conclusion that G-allele variant of SLC47A1 (A>G) (rs1961669) had no association with demographic parameters in Iraqi PCOS patients.

Regarding the association of the study genotypes of SLC47A1 (A> G) (rs1961669) and the biochemical parameters which was best represented in table (4), there was a significant reduction in LH level among homozygous and heterozygous carriers of SLC47A1 (A> G) (rs1961669) after metformin therapy, FSH showed significant increment in GG and AG genotype in response to metformin in comparison with common wild A-allele, as a result LH/FSH ratio indicated significant reduction in GG and AG genotype. In present study, while total testosterone level indicated significant reduction in GA and AA genotypes. The current study demonstrated that SHBG had significant increase in GA and AA genotypes as a result FAI showed significant decrease in GA and AA genotypes after metformin treatment. Lipid profile included TG, LDL-C and TC showed significant reduction in GA and AA.

Fasting blood glucose and HbA1c% demonstrated significant reduction in GA and AA genotypes as compared with GG genotypes so GA and AA had better response to metformin than GG genotype. All these results indicated that there was no association of G-allele variant of SLC47A1 (A>G) (rs1961669) with biochemical parameters in Iraqi PCOS populations, these results were in line with the findings of the **(Becker. *et al.*, 2009)** which involve studying the association of SLC47A1 (A>G) (rs1961669) gene polymorphism and other SNPs of SLC47A1 gene with HbA1c change in diabetic Caucasian populations **(34).**

The SNP rs1961669 ($r^2 = 0.85$, $D' = 0.96$) was in linkage disequilibrium, minor or G-allele frequency is 19.7% (i.e.) approximately (0.2) and major or A-allele frequency was approximately (0.8). The frequency of minor or G- allele of this SNP is approximately similar to minor allele frequency of G-allele variant in study **(Becker. *et al.*, 2009)** which was 17%, so due to this low frequency of minor allele and being an intronic one, therefore, it is no coded for amino acids changes **(34)**.

Conclusion

The current study is the first published study performed to assess the role of SLC47A1 (A>G) (rs1961669) gene polymorphism in metformin response in Iraqi PCOS populations till the time of writing this paper, this SNP had no association with clinical, hormonal and biochemical response to metformin.

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