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Original Article

Polymorphisms of OCT1 and metformin effects in Iraqi women with polycystic ovary syndrome in Karbala city

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Abstract: Objectives: The current study aimed to correlate OCT1 (organic cation transporter 1) polymorphisms with metformin response variability in Iraqi women with PCOS (polycystic ovarian syndrome) and determine the impact of OCT1 polymorphism. PCOS, an endocrine metabolic disorder, can seriously impact female health including infertility. Although its cause is unclear, it is usually known to be associated with hormonal imbalances. OCT1 is essential for metformin absorption in the liver. Recent research shown that OCT1 polymorphisms affects metformin responsiveness. Methods: In the present work, a prospective case-control study was conducted at Department of Infertility, Karbala Teaching Hospital for Obstetrics and Gynecology. 100 PCOS patients and 50 healthy controls aged 20-40 were enrolled. Consultant gynecologist diagnosed PCOS patients using Rotterdam criteria and recommended metformin 500 mg twice daily for 3 months. At the start of the trial and after 3 months, all patients and healthy controls underwent hormonal, biochemical and genetic tests. Results: The similar allelic frequencies of OCT1 polymorphism in PCOS and control groups was observed. Most patients with reference wild type alleles (C) showed considerable hormonal and metabolic responses to metformin, while those with mutant alleles (T) showed non-significant responses. Conclusion: FSH, prolactin and testosterone hormonal levels may be considered as candidate biomarkers for PCOS detection and metformin related biomedical respond.

Keywords: Metformin, OCT1 polymorphism, PCOS, polycystic ovary syndrome

Introduction

One of the most common reason for female infertility is polycystic ovary syndrome (PCOS) “an endocrine and metabolic disorder” that affects between 3 and 10 percent of women of reproductive age. PCOS is characterized by chronic anovulation, polycystic ovaries and hyperandrogenism [1]. There is compelling evidence that PCOS is largely a hereditary disease and the cause of the complex and multifaceted PCOS problem is unknown so far [2].

Metformin is a stable hydrophilic biguanide molecule that is highly polar, positively charged, and having low molecular weight that exhibits a wide variety of biological activities [3]. It may be found in a wide variety of tissues including skeletal muscle, liver, pancreas, adipose tissue, hypothalamus and pituitary as well as the gonads. Metformin is mostly found in the cytosol. According to several subcellular investigations conducted in rat liver, it has poor solubility [4]. As was shown by research conducted with mice, metformin may accumulate in specific tissues at larger amounts than it does in plasma [5].

The organic cation transporters (OCTs), are a kind of diffusional transporters that is polyspecifically and bidirectionally facilitates diffusion. These transporters play essential physiological functions in the clearance of metabolites and drugs [6]. Hepatocytes are where the gene for OCT1 (SLC22A1) is most often expressed in human beings [7]. Both the expression of the OCT1 gene and the levels of the OCT1 protein may be determined in adipose tissue, muscles of the skeleton, ovaries, and the intestines. Proximal tubular cells of the kidney are where the OCT2 gene is expressed. OCT3 is present in a wide variety of tissues and may be detected in

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astrocytes [8]. There is a wide range of possible outcomes in terms of the clinical response to metformin medication for PCOS patients. According to the findings of many studies, its potential to lower testosterone and insulin levels, normalize menstruation, and enhance body weight shown substantial variability [9]. It has been suggested that unknown or unmeasured factors influence the response to metformin treatment in PCOS. Even, polymorphisms in genes that are involved in the transport or effect of metformin are to blame for the variable responses to metformin as seen in type 2 diabetes [10]. It is widely known that the main transporter of metformin is organic cation transporter 1 encoded by the SLC22A1 gene located on the chromosome 6 and consisting of 11 exons spanning 37 kb [11]. The relationship between genetic variations, including those of OCT1 gene, and metformin response in PCOS is still an area of active research and not yet fully understood. Some reports suggest that OCT1 is the determinant of individual response to metformin treatment in PCOS. Different reactions to metformin treatment were caused through the differences in OCT1 gene polymorphisms. So this study aimed to determine OCT1 Polymorphism "rs12208357" in women who have polycystic ovary syndrome and find out possible biomarkers for the early detection and therapy during metformin intake for three months.

Materials and methods

Study designing

This study was considered as a prospective project on women with polycystic ovary syndrome when using metformin treatment for 3 months. Body mass index (BMI) was recorded for every women before and after treatment by metformin according to the formula [12].

Subjects

University of Karbala Institutional Review Board for the Protection of Human Subjects gave their stamp of approval to the study's protocol. Written informed consent forms were signed by all participants. Women with polycystic ovary syndrome were recruited from the obstetrics and gynecology departments at Al-Hassan Hospital in Karbala city. These women ranged in age from 20 to 40. Patients without menstrual cycle irregularities, clinical or biochemical hyperandrogenism, polycystic ovaries on ultrasound examination, or a history of systemic/endocrine disease were enrolled as controls from an infertility clinic prior to beginning an *in vitro* fertilization program.

PCOS criteria

The inclusion criteria were as follows: Iraqi women who confirmed diagnosis of polycystic ovary syndrome based on established diagnostic Rotterdam criteria. This diagnosis was using criteria such as the androgen excess and ages that should be ranged between 20 and 40 years old. Participants who provided informed consent to be part of the study and agreed to provide the necessary samples for research purposes. Included patients met two of three criteria in order to be classified as having PCOS: (1) polycystic ovaries (follicles measuring 2-9 mm in diameter, or ovarian volume > 10 mL in at least one ovary); (2) oligo-anovulation (a cycle length > 35 days or amenorrhea); (3) clinical hyperandrogenism (hirsutism with m-FG score 6 with/without acne or androgenic alopecia); and/or biochemical hyperandrogenism. The exclusion criteria were as follows: Women who were pregnant or planning to become pregnant during the study period. Excluded women who were undergoing hormonal treatments that might affect PCOS symptoms, such as oral contraceptive use, hormone replacement therapy, or anti-androgen medications. Women who had undergone significant surgical interventions related to PCOS or reproductive system.

Blood sample collection

Blood samples were collected from each patient via vacutanir safetr lok collection tubes and the blood were centrifuged to obtain serum for hematological and hormonal analysis including FSH (IU/L), LH (ng/ml), TSH (μIU/mL), Prolactin (ng/ml), Testosterone (ng/ml) and Progesterone (ng/ml).

DNA extraction

Using AddPrep Genomic DNA Extraction Kit (Korea), genomic DNA was isolated from whole-blood vials according to Manufactural protocol.

ARMS PCR assay

In the present investigation for ARMS PCR, Macrogen genomic company of korea synthesized the allele specific primers as indicated by Ali et al. [13]. In a total volume of 25 µl, 150 ng of genomic DNA was amplified by using 1 µl (10 mM) of each primer, 2.5 µl of Green Buffer (1), 2.5 µl of MgCl (20 mM), 5 µl of dNTP (2 mM; Thermo Fisher Scientific), and 0.150 µl of DreamTaq DNA Polymerase (5 U/L) for each PCR test tube.

Primers used were depend on OCT 1 Alleles C>T (forward common) 5'-CAGATGGCCACGTGCATTCTTC-3'; (reverse C allele) 5'-AGGGCTCCAGCCACAGCG-3'; other (reverse T allele) 5'-CAGGGCTCCAGCCACAGCA-3'. The PCR reaction was performed in the conventional thermocycler (Biobase company/China) and run under the following conditions: initial denaturation at 94°C for 5 min; 35 cycles at 94°C for 60 sec, annealing for 30 sec (testing 62°C, 64°C, or 66°C), extension at 72°C for 60 sec, followed by a final extension at 72°C for 10 min that was not cycled. As a result, one allelic amplicon was produced as a 407-bp fragment indicating the OCT 1 gene. The amplification products were resolved by electrophoresis in 1.5% (wt/vol) agarose gel in 600 mL of TBE (Tris/Borate/EDTA (ethylene diamine tetraacidic acid)). Electrophoresis was performed at a constant voltage (100 V) for 1 h at room temperature. The costs of genotyping per sample were calculated by considering the cost of reagents and consumables.

Statistical analysis

Study subjects' information was entered into a database, checked for accuracy and consistency, and then maintained, processed, and analyzed with the use of SPSS for Windows, Version 25, IBM, USA. The Kolmogorov Smirnov test and the histogram were used to check the normal distribution of all continuous (scale) variables. For continuous variables such as age, BMI (Body Mass Index), and number of children, their distribution was checked for normality using the Kolmogorov-Smirnov test and histograms. Descriptive statistics, including mean and standard deviation (SD), were used to present these scale variables. Parametric tests, specifically the Student t-test for two samples, were applied to compare the studied parameters between the different groups if the variables followed a normal distribution. Non-parametric tests, such as the Mann-Whitney U test for two independent samples, were used for variables that did not follow a normal distribution. Within the PCOS group, the Wilcoxon Signed Ranks Test was used to compare the studied parameters before and after treatment. A significance level of $P < 0.05$ was considered statistically significant. Nominal (categorical) variables' descriptive statistics are shown in terms of frequency (the number of people surveyed) and proportion (the percentage of those surveyed). Data given as mean and standard deviation (SD) on a scale.

Results

BMI values

By observing women during the metformin treatment period for three months, the results showed that just 96 (96%) women were committed to the treatment of metformin and 4 women (4%) were excluded due to the weight gain and non-compliance with the treatment for three months. **Table 1** showed that the most women with polycystic ovary syndrome were at a body mass index ranging between 30-34.9. It was also recorded that 35% of women with PCOS followed by the body mass index ranges between 25-29.9. The results of this study showed that there were some women who responded to treatment with metformin, as there were four women from the total of 28 women (14.28%) who responded to treatment via metformin uptake with body mass index ranging 18.5-24.9. On the other hand, data showed that there were two women out of 35 women (5.71%) who reached to BMI of 18.5-24.9 and two women (5.71%) who reached BMI of 25-29.9 in response to metformin treatment. Finally, we obtained four women (14.28%) who responded to treatment with metformin with a BMI value of more than 35.

Table 2 indicates that there are 15 women who have the ability to respond to treatment through the return of their natural hair and their lack of hair loss with significant differences ($P < 0.05$) when using metformin treatment for a period of 3 months. We noticed that there are 91 (91%) women who have hair loss, 21 (18.9%) women have responded to metformin treatment by observing the return of hair after its loss. We

Table 1. The body mass index of Iraqi women having PCOS before and after metformin treatment

BMI	No. of patients before metformin treatment	BMI	No. of patient after metformin treatment
> 18.5	0	0	0
18.5-24.9	12	18.5-24.9	9
		25-29.9	3
		30-34.9	0
		< 35	0
25-29.9	28	18.5-24.9	4
		25-29.9	23
		30-34.9	1
		< 35	0
30-34.9	35	18.5-24.9	2
		25-29.9	2
		30-34.9	30
		< 35	1
< 35	14	18.5-24.9	0
		25-29.9	0
		30-34.9	4
		< 35	10

Yellow color: no response to drugs and Red color: response to drugs.

also noticed that all women do not have a regular menstrual cycle, but after using metformin treatment, 13.5% of women obtained regularity with significant differences ($P < 0.05$). As for the rest of the women including 75 who still have no regulatory menstruation. Evaluation of vocal change values shows that there are about 64 changes in the tone of the voice, and after using metformin treatment, we have 48 women who have a change in the tone of the voice with non-significant differences ($P > 0.05$) (**Table 2**).

Hormonal values

Our study was followed by measuring the level of hormones (including FSH, LH, TSH, prolactin, testosterone and progesterone) for Iraqi women with polycystic ovaries. **Table 3** showed that there was no significant relationship between hormonal concentrations before and after treatment with metformin, except for the hormones FSH, prolactin and testosterone. As the results show, the concentrations of these hormones are significantly decreased after treatment ($P < 0.05$). FSH concentration is decreased from 6.24±2.22 IU/L to 5.58±1.520 IU/L in C allele patients. While, in T allele patients there was no significant decrease in FSH concentration. The prolactin level is decreased in patient with C allele from 25.326±6.78 ng/ml to 18.98±6.13 ng/ml with significant differences ($P < 0.047$). Also the concentration of testosterone is significantly decreased from 0.61±0.15 ng/ml to 0.3±0.18 ng/ml in C allele patients. Data show that there are no significant decreases in hormonal levels in T allele patients before and after metformin treatments.

Genetic amplification

As the next and final step of our studies, the genetic variations was investigated on PCOS patients. The gene of OCT1 was amplified by using allele specific primers as described in materials and methods section. The amplification result of OCT1 gene (including C and T alleles) showed an amplicon of 407 bp as illustrated electrophoretically on 2% agarose gel (**Figure 1**).

Discussion

Metformin is the first insulin sensitizing drug that is used for PCOS pathogenesis and therapy. Previous reports show the significant improvement in menstrual regularity and reduction in androgen hormonal levels in PCOS patients treated with metformin [13]. Based on these reports, there are considerable inter-individual variabilities in response to metformin treatment. Therefore it was suggested that different genetic and non-genetic factors may determine and affect the metformin responses in different PCOS individuals [14]. In consistence with this, the results of our study showed that there were some women who responded to treatment with metformin and reduced their BMI values, as there were two women out of 35 women (5.71%) who reached to BMI of 18.5-24.9 and two women (5.71%) who reached BMI of 25-29.9. The action of metformin is anti-hyperglycemic medication that belongs to the biguanide class. It accomplishes this via lowering glucose synthesis in the liver, raising insulin sensitivity in the body's tissues, and elevating growth/differentiation factor 15 (GDF15) secretion, all of which lead to a decrease in hunger

Table 2. Clinical cases for women before and after treatment with metformin

Parameters		Before treatment	After treatment	*P* value
		Number N=100 N (%)	Number N=90 N (%)	
Hair loss	Yes	91 (91%)	69 (73%)	X^2=10.3 P=0.001
	No	9 (9%)	21 (18.9%)	
Menstrual cycle Regularity	Yes	0	15 (13.5%)	X^2=18.09 P=0.001
	No	100 (100%)	75 (67.5%)	
Vocal change	Yes	64 (64%)	48 (44.1%)	X^2=2.22 P=0.13
	No	36 (32.4%)	42 (37.8%)	

Table 3. Relationship between OCT1 polymorphisms and hormonal levels before and after metformin treatment

OCT1 polymorphism	Alleles	Pre	Post	*P* value
FSH (IU/L)	C	6.24±2.22	5.56±146	0.054
	T	5.58±1.520	5.48±1.67	0.814
LH (ng/ml)	C	9.28±2.322	8.64±2.13	0.312
	T	9.32±2.47	9.58±2.130	0.425
TSH (μIU/mL)	C	2.40±1.01	2.38±0.83	0.912
	T	2.323±0.905	2.323±0.88	0.917
Prolactin (ng/ml)	C	25.326±6.78	18.98±6.13	0.047
	T	27.94±94	26.85±5.32	0.76
Testosterone (ng/ml)	C	0.61±0.15	0.3±0.18	0.19
	T	0.66±0.16	0.73±0.17	0.50
Progesterone (ng/ml)	C	0.61±0.15	0.7±0.17	0.19
	T	0.66±0.16	0.73±0.17	0.50

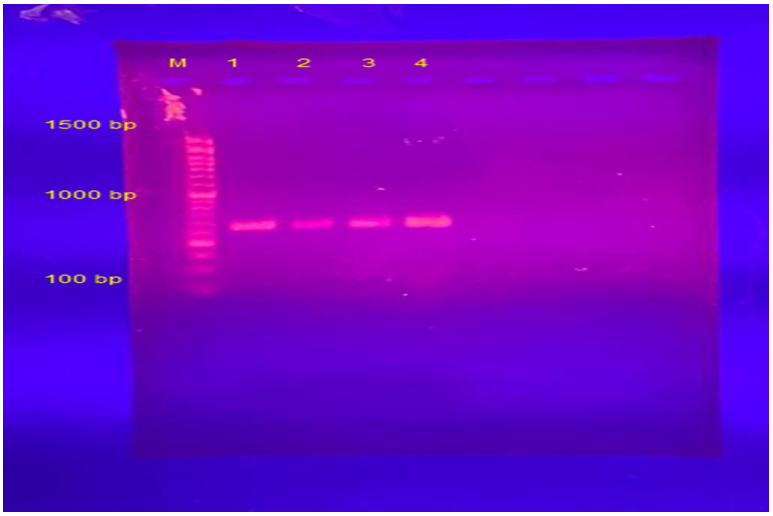

Figure 1. The electrophotography of OCT1 gene PCR product loaded in each well on 2% agarose gel electrophoresis (70 V, 120 min, and 5 μL). Lanes 1-4: represented to PCR products of C and T alleles; Lanes 5-8: represented to negative results; Lane M: Ladder DNA (100-1500 bp).

as well as a lower in total calorie consumption [15]. This does not mean that the treatment is not effective in our current study, but it is due to the short period of treatment, which is a period of a few months, as well as the lack of complete knowledge of how women should follow up and take treatment.

Similarly, the results showed that out of 91 women with hair loss, 21 (18.9%) exhibit hair growth after metformin treatment. PCOS is associated with higher than usual amounts of testosterone, which is known to inhibit hair growth. Hence, PCOS is linked to hair loss in certain patients. Therefore metformin treatment reduces the concentration of testosterone and so causes hair growth in women who have polycystic ovaries [16]. Through the current

study, we only included 15 (15%) women whose menstrual cycle was regulated. PCOS women typically experience fewer than six to eight menstrual periods throughout the course of the year. Some girls go through puberty with regular menstrual periods, however such cycles can change to become erratic if the girl is overweight. PCOS is related with gradual weight increase and obesity in roughly one-half of women, which occurs in approximately one-third of women with PCOS [17]. The results of the present study showed no significant differences in the vocal voice after treatment with metformin.

In this study, we investigated the association between the polymorphisms of *OCT1* gene and the treatment effectiveness of metformin in PCOS patients. OCT1 cation transporter has been reported to mediate metformin transport in the liver. Its polymorphism may affect the activity of metformin transport and thereby influence the treatment response of metformin in PCOS patients [18]. The rs316019 polymorphism of *OCT1* gene, has been widely studied in PCOS patients [19]. The results are based on the Genome Wide Association Studies (GWAS), which have been the main studies in the genetic search for PCOS. *OCT1* gene located on chromosome 1q25.3 and it is predominantly expressed in the hepatocytes. Its expression and protein levels are also detectable in adipose tissue, skeletal muscles, ovaries, intestine and is associated with the reproductive and metabolic phenotypes in PCOS [20]. It is especially associated with insulin resistance in women with PCOS and betapancreatic dysfunction in their siblings. This gene has also been related to the prevalence of autoimmune thyroid disease (ATD) in patients with PCOS [21]. In a different genetic polymorphic study, PCOS patients with the G allele of *OCT1* rs683369 and/or with the A allele of *OCT1* rs628031 had increased insulin sensitivity compared to those with wild-type genotype after receiving metformin treatment. Taking all these together, genetic polymorphisms of *OCT1* gene contribute to different metformin treatment responses in PCOS individuals.

The results of our study "based on rs12208357 OCT1 polymorphisms as a different OCT1 SNPs" showed only three hormones respond to treatment with metformin for three months, which include FSH, testosterone and prolactin hormones just in patient with C alleles. The cause related to the ability of patient to metabolism of metformin and its activity via normal alleles of OCT1. OCT1 acts as a mediator for the transport of Type I organic cations, which are protonated molecules such as dopamine and choline. It also facilitates the transport of Type II cations, that are bigger and heavier molecules such as metformin and quinidine. OCT1-mediated organic cation transport is an electrogenic and sensitive to membrane potential organic cation transport mechanism [22, 23]. On the other hand, the study found that patients with T alleles not reduce testosterone hormonal level in their blood. Ovaries affected by polycystic ovary syndrome do not function normally and as a result create an excessive amount of testosterone (hyperandrogenism). The ovaries generally create very modest levels of testosterone, but with PCOS, they make more. This can lead to acne, increased hair growth, and sometimes hair loss from the scalp [24]. Based on the obtained results, FSH, prolactin and testosterone hormonal levels may be used as candidate biomarkers for PCOS detection and metformin related medical respond.

Dealing with patients in taking blood samples, as well as the difficulty of taking metformin treatment were the limitations of the present study.

Conclusion

The present findings suggest that a change in the OCT1 gene structure/genetic variations might play a role in PCOS. We noticed a change in some physio-biochemical characteristics (specially including FSH, prolactin and testosterone levels) of women with polycystic ovaries after metformin treatment that might be used as valuable indicators for early diagnosis and medical therapy of C allele containing PCOS patients. More large-scale and functional investigations are recommended to confirm the correlations between OCT1 polymorphism and the clinical properties of PCOS.

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Disclosure of conflict of interest

None.

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References

[1] Armanini D, Boscaro M, Bordin L and Sabbadin C. Controversies in the pathogenesis, diagnosis and treatment of PCOS: focus on insulin resistance, inflammation, and hyperandrogenism. Int J Mol Sci 2022; 23: 4110.

[2] Zeng X, Xie YJ, Liu YT, Long SL and Mo ZC. Polycystic ovarian syndrome: correlation between hyperandrogenism, insulin resistance and obesity. Clin Chim Acta 2020; 502: 214-221.

[3] Glossmann HH and Lutz OMD. Pharmacology of metformin - an update. Eur J Pharmacol 2019; 865: 172782.

[4] Kim HS, Ren G, Kim T, Bhatnagar S, Yang Q, Bahk YY and Kim JA. Metformin reduces saturated fatty acid-induced lipid accumulation and inflammatory response by restoration of autophagic flux in endothelial cells. Sci Rep 2020; 10: 13523.

[5] Zhang W, Xu JH, Yu T and Chen QK. Effects of berberine and metformin on intestinal inflammation and gut microbiome composition in db/db mice. Biomed Pharmacother 2019; 118: 109131.

[6] Brosseau N and Ramotar D. The human organic cation transporter OCT1 and its role as a target for drug responses. Drug Metab Rev 2019; 51: 389-407.

[7] Whisenant TC and Nigam SK. Organic anion transporters (OAT) and other SLC22 transporters in progression of renal cell carcinoma. Cancers (Basel) 2022; 14: 4772.

[8] Zhang P, Azad P, Engelhart DC, Haddad GG and Nigam SK. SLC22 transporters in the fly renal system regulate response to oxidative stress in vivo. Int J Mol Sci 2021; 22: 13407.

[9] Wabitsch S, McCallen JD, Kamenyeva O, Ruf B, McVey JC, Kabat J, Walz JS, Rotman Y, Bauer KC, Craig AJ, Pouzolles M, Phadke I, Catania V, Green BL, Fu C, Diggs LP, Heinrich B, Wang XW, Ma C and Greten TF. Metformin treatment rescues CD8+ T-cell response to immune checkpoint inhibitor therapy in mice with NAFLD. J Hepatol 2022; 77: 748-760.

[10] Xu J, Liu LQ, Xu LL, Xing Y and Ye S. Metformin alleviates renal injury in diabetic rats by inducing Sirt1/FoxO1 autophagic signal axis. Clin Exp Pharmacol Physiol 2020; 47: 599-608.

[11] Mukminatin AA, Ningrum VDA and Istikharah R. PCR primer design for detection of SNPs in SLC22A1 rs683369 encoding OCT1 as the main transporter of metformin. In: Unity in Diversity and the Standardisation of Clinical Pharmacy Services: Proceedings of the 17th Asian Conference on Clinical Pharmacy (ACCP). Yogyakarta, Indonesia: CRC Press; 2017. pp. 161.

[12] Rahman M and Berenson AB. Accuracy of current body mass index obesity classification for white, black, and Hispanic reproductive-age women. Obstet Gynecol 2010; 115: 982-988.

[13] Velazquez EM, Mendoza S, Hamer T, Sosa F and Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism 1994; 43: 647-654.

[14] Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, Furlong TJ, Greenfield JR, Greenup LC, Kirkpatrick CM, Ray JE, Timmins P and Williams KM. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 2011; 50: 81-98.

[15] Day EA, Ford RJ, Smith BK, Mohammadi-Shemirani P, Morrow MR, Gutgesell RM, Lu R, Raphenya AR, Kabiri M, McArthur AG, McInnes N, Hess S, Pare G, Gerstein HC and Steinberg GR. Metformin-induced increases in GDF15 are important for suppressing appetite and promoting weight loss. Nat Metab 2019; 1: 1202-1208.

[16] Valdimarsdottir R, Wikström AK, Kallak TK, Elenis E, Axelsson O, Preissl H, Ubhayasekera SJKA, Bergquist J and Poromaa IS. Pregnancy outcome in women with polycystic ovary syndrome in relation to second-trimester testosterone levels. Reprod Biomed Online 2021; 42: 217-225.

[17] Jacob J, Vijayakumar N and Olickal JJ. Effect of satapushpa churnam with tila tailam in oligomenorrhea associated with polycystic ovarian syndrome. J Ayurveda Integr Med 2021; 12: 695-699.

[18] Zolk O. Disposition of metformin: variability due to polymorphisms of organic cation transporters. Ann Med 2012; 44: 119-129.

[19] Sekar N, Samak R, Patil S, Ghorpade N and Abilash VG. Association of methylenetetrahydrofolate reductase C677T gene polymorphism and polycystic ovary syndrome in the

South Indian cohort. Indian J Exp Biol 2022; 58: 493-497.
[20] Aflatounian A, Edwards MC, Rodriguez Paris V, Bertoldo MJ, Desai R, Gilchrist RB, Ledger WL, Handelsman DJ and Walters KA. Androgen signaling pathways driving reproductive and metabolic phenotypes in a PCOS mouse model. J Endocrinol 2020; 245: 381-395.
[21] Alattabi AS and Aljabery H. Evaluation of follicular fluid CXCL10 in patients with polycystic ovary syndrome. Biochem Cell Arch 2021; 21: 3013.
[22] Ali BH, Khalaf BH, Mahmoud H, Aboalmaali M and Ali BH. Effect of organic cation transporter 1 (OCT1) polymorphism on metabolic response of metformin in Iraqi women with polycystic ovary syndrome. Medico-legal Update 2021; 21: 236-240.
[23] Ali BH, Abo Almaali HM and Abdulwahid HH. Association of genetic polymorphism of organic cation transporter (OCT1) with the therapeutic response of metformin in women with polycystic ovary syndrome in Iraq. Biochem Cell Arch 2020; 20: 4497-4501.
[24] Carmina E. Cutaneous manifestations of polycystic ovary syndrome. Curr Opin Endocr Metab Res 2019; 12: 49-52.