European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 03, 2020

Association of genetic polymorphism of LKB/STK11 with therapeutic response of metformin in women with polycystic ovary syndrome.

Montaha R.Hussein[1], Dr. Mazin H.Ouda[2], Dr.Hameedah Hadi Abdulwahid[3], Dr.Abo Al-maali H.M[4]

[12]*Department of Pharmacology and Toxicology, college of Pharmacy, University of Kerbala/ Kerbela- Iraq*

[3]*Consultant Gynecologist, kerbala obstetric and gynecology teaching hospital, Iraq*

[4]*Department of Clinical Laboratory Science, College of Pharmacy, University of Kerbala / Kerbala - Iraq*

E-mail: muntaha.r@s.uokerbala.edu.iq

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is recognized as one of the most common endocrine abnormalities of human, it is characterized by hyperandrogenism, and metabolic derangement including glucose intolerance and hyperinsulinemia. Single Nucleotide Polymorphism in STK11 gene have been suggested to be associated with metformin efficacy in PCOS treated patients.

Purpose: To investigate the relation of STK11 gene polymorphism (rs741765 C/T) with the response to metformin therapy for a period of three months in PCOS treated women in Karbala city.

Patients and Methods: This is a prospective study for two hundred thirty-five Iraqi patients, who newly diagnosed with PCOS based on Rotterdam criteria by physician's diagnosis during their visit to Women's Hospital obstetrics education in Karbala /Iraq during the period from july,2019 till the end of april,2020.Patients with thyroid dysfunction, congenital adrenal hyperplasia and Cushing's syndrome were excluded,the matched age was (18-40years). DNA extraction kit and Allele-specific polymerase chain reaction (PCR) were used for STK11 gene polymorphism, PCR product was visualized by agarose gel electrophoresis, enzymatic methods were used for blood sugar, and lipid profile measurement, LH, FSH, TSH, prolactin, total testosterone, estradiol, F.insulin were all tested by cobas e 411 analyzer, ELISA kit was used to measure SHBG. LH/FSH ratio, FAI, and HOMA-IR were calculated, all these parameters

were measured for each patient and after 3-months therapy the patients had a medical checkup, identical to that before metformin giving.

Results: The clinical, metabolic and endocrinal characteristics among STK11 rs741765 genotypes showed a significant differences and metformin efficacy increase as following TT>CT>CC genotypes.

Conclusion: A polymorphism in STK11, a kinase gene expressed in liver and implicated in metformin action was an associated with metformin efficacy and percentage of responder women increase with number of T allele in rs741765 SNPs.

KEYWORDS: serine threonine kinase 11 gene (STK11) gene, polymerase chain reaction(PCR), fasting insulin(F.insulin), sex hormone binding globulin (SHBG), homeostasis model assessment for IR(HOMA-IR).

1. INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among infertile women. Its affected women during their reproductive ages (15 to 44). Despite of the exact cause of PCOS is not known, the syndrome can result from disturbance in the hypothalamic pituitary ovarian axis and hyperinsulinemia(1).

Prevalenceof PCOS related to the populationbeing assessed, because of ethnic differences in the clinical and biochemical features of PCOS the reported prevalence of PCOS ranges between (2.2 – 26)% in various countries(2). In Iraqi Women prevalence was found 25% of infertility associated with PCOS especially among younger age group (20-29)(3).

Signs and symptoms of PCOS include:irregular or no menstrual cycle, clinical or biochemical hyperandrogenism, difficulty to get pregnancy orinfertility, insulin resistance is a major symptom of PCOS results in hyperinsulinemia and alteration in metabolic profile(4).

In 2003 Rotterdam in Netherland presented new diagnostic criteria(5), based on two of the following three findings: no ovulation, high androgen levels, and ovarian cysts detectable by ultrasound(6).

Hyperinsulinemia contribute to or cause the abnormalities seen in the hypothalamic-pituitary-ovarian axis that lead to PCOS(7), this effect mediated by : increasing GnRH pulse frequency result in LH over FSH dominance,increase the sensitivity of pituitary cells to gonadotropin releasing hormone (GnRH) action, increase the number of the luteinizing hormone (LH) receptor that resulted in increasing the ovarian steroidogenic response to gonadotropins, and reducing sex hormone binding globulin (SHBG) synthesis in the liver, all these effects of hyperinsulinemia resulted in decreased follicular maturation and inhibition of ovulation(8)

Metformin as insulin sensitizer used in the treatment of PCOS through, Suppression of endogenous glucose production or gluconeogenesis, it has pleiotropic actions on several tissues sensitive to the primary effect of insulin or affected by insulin resistance, such as the liver, skeletal muscles, adipose tissue, endothelium and the ovaries , it also has a role in lipid metabolism by suppression of acetyl CoA carboxylase activity(9).

AMPK activation mediated by metformin resulted in suppression of gluconeogenesis and expression of several lipogenic genes (such as fatty acid synthase-FAS, etc.), participates in improvement of insulin

sensitivity through enhancement of peripheral glucose uptake, and regulation of lipogenesis by downregulating other genes required in lipid synthesize and by inhibiting proteolyticprocessing and

transcriptional activity upon AMPK-mediated phosphorylation at Serine 372(10).

Serine/threonine kinase 11 (STK11) is a proteinkinase that in humans is encoded by the *STK11*gene, LKB1 is a primary upstream kinase of adenosine monophosphate-activated protein kinase (AMPK) in the liver, enhance glucose uptake and fatty acid oxidation in muscle, and regulation of energy balance in the hypothalamus through serine-threonine-kinase(STK11)–adenosine monophosphate (AMP)- activated protein kinase (AMPK) signaling pathway, these functions of STK11 are thought to be achieved by direct phosphorylation of the AMPK family of proteins(11).

Aim of presented work was to investigate the possible association of single nucleotide polymorphisms (C/T) rs741765 of STK11 gene with response to metformin in Iraqi treated women with PCO

2. PATIENTS AND METHODS

It was a prospective study carried out at Women's Hospital and obstetrics education in Karbala during the period from July,2019 till the end of April, 2020. The study was conducted on two hundred thirty- five women with newly diagnosed polycystic ovary syndrome. Participated women were recruited by consultation of an infertility according to the inclusion and exclusion criteria of the study.

All women enrolled in this study starting metformin tablet 500mg twice daily as standard adjuvant therapy for three months.

Inclusion criteria carried out according to Rotterdam criteria when two of three of the following symptoms: hyperandrogenism, irregular anovalutory periods or ultrasound polycystic ovary (PCOS) morphology, ages of women should be (18-40) years.

Exclusion criteria included those with thyroid dysfunction, diabetes mellitus, Cushing's syndrome, congenital adrenal hyperplasia, all should be excluded before making diagnosis of PCOS.

Each patient was questioned about demographic parameters by history-taking, specifically for: Address, age, body mass index, blood pressure, Presence of other diseases, smoking, menstrual regularity, hirsutism, martial state, number of kids, and times of abortion.

Blood samples were obtained from all patients, (8ml) of venous blood were withdrawn from all women participated in this study.(5ml) was placed in EDTA-tube used for genetic testing and for measurement fasting glucose and HbA1C, remaining (3ml) was placed in gel tube(EDTA-free) tube used for serum separation after centrifugation of blood at 3000 rpm for 10 minutes for measurement of the following parameters: follicular stimulating hormone (FSH) ,(LH), (TSH), Prolactin,total testosterone,(SHBG), estradiol, F.insulin and Lipid Profile including: (TG),(LDL-C), (HDL-C), (TC).

Genomic DNA was extracted according to protocol G-DEX tmIIb Genomic DNA Extraction Kit, concentration and purity were measured by UV absorption at 260 and 280 nm (Bio Drop, U.K). Genotyping for STK11 SNPs rs741765 C\T SNP was done by ALLELE- SPESIFIC PCR.

Primers were taken in lyophilized state, units of lyophilized primers are known as mass in pico-moles, subsequent steps were done for reconstitution and delusion of primers,tube was centrifuged at 1000 rpm for 5-10 min before de-capping, chosen volume from nuclease free water were added according to the manufacturer to get 100 p-moles/µL (master stock).

Working stock prepared by re-mixing by vortex, 10 microliters were transported to 0.5ML Eppendorf tube containing 90 µL of nuclease free water to obtain 10 pmoles/ µL (working stock). master stock and working stock were kept at -20 ℃, working stock was warmed for each used in PCR, remaining quantity kept in ice then stored at -20.

The primers used in genetic study are; Forward (F) and Reveres (R1, R2), PCR reaction was performed using Go Taq ® green master mix (2X) of Promega (USA) instructions for a final volume of 25 µL (two tubes for each sample: one for F and R1 and second for F and R2, as following: 25 µL of 2X Go Taq ® green master mix, 1.5 µL of each primer (forward and reverse), 5µL of genomic DNA, and the volume was completed to 25 µL with 4.5 µL nuclease free water.

Two PCR reaction were performed, one with primer rs741765 C(R1) and rs741765(F) producing (PCR C allele) and second with primer rs741765 T(R2) and rs741765 (F) producing (PCR T allele), and run side by side on an agarose gel, rs741765 (177) bp band indicated the presence of the allele; if amplification failure indicated the absence of the allele. Reaction was carried out with Gene Amp ® PCR System 9700 thermocycler that used for the amplification analysis of STK11 SNP.

All data were analyzed using analysis system SPSS software X27, and represented by mean and stander deviation (M ±SD).

Specific primers of STK11 gen rs741765 and Allele specific –PCR program were shown in tables (1), (2).

Table (1). Specific primers of STK11 gen rs741765

Primer	Sequence (5´–3´)	Allele	Size (bp)	Company Country
Forward	TGTGCAGAAATGTAGGGTT		177	BIONEER/ Korea
Reverse 1	CCTCTGGGGTGGGAGTTCG	C	177	
Reverse 2	CCTCTGGGGTGGGAGTTCA	T	177	

Table (2). Allele specific –PCR program for detection of stk11 rs741765

No.	Stage	Cycle	Step	Temp.	Time
1	Initial Denaturation	1	1	92 °C	2min.
2	Denaturation	45	1	92 °C	30 sec.
3	Annealing	45	2	45 °C	30 sec.
4	Extension	45	3	72 °C	20 sec.
5	Final Extension	1	1	72 °C	5 min.
6	Hold Phase			10 °C	

3. RESULTS

Demographic features results

Mean age of patients was (27.1±5.21) years with a range of (18 – 40) years, and about (89.78 %) of patients age was less than 35 and only 8.5 % of the patients aged older than 35 years old, more response to treatment occurred at age group between (26-30) with percentages (86.4%) figure (1).

Overweight and obesity in current study were high approximately (31.4%, 44.6%) respectively, mean BMI before treatment was (28.8±5.62) kg/m2 and significantly decreased after treatment to (27.2± 5.66) kg/m2 ($p < 0.01$), and percentage of patients presented with obesity (BMI ≥ 25 kg/m2) was reduced from 76.1 to 42.5% upon treatment.

Residence investigation among the patients showed higher proportion was in urban population in comparison to rural one with more response is in urban residents about (51.9%) compared to (33.3%) in rural population, higher percentage of patients have school education 127(54.1%) and less with university education 108(45.9%) while more response was shown in university educated group 72(66%), compare to 44(34.6%) of school educated patients.

Majority of patients had their age at menarche in the 12 years old, Current study showed that response to treatment not correlated with age atmenarche.

Infertile patients' percentage reported in present PCOS study was (48.51%), and those divided into either primary or secondary infertile, with high number with secondarycompare with primary one. More response was noticed with secondary infertile patients, figure (2). About (28.36%) of patients were suffering from repeated miscarriages and menses irregularity was clear phenomena of all patients in the current study and improved in (41%) of patients upon therapy.

Hirsutism and alopecia are representable clinical feature of hyperandrogenism of in PCOS patients, so in current study revealed a high percentage of patients

with alopecia (hair thinning and hair loss) and hirsutism (thicker hair on their face and body) about (80.86 %, 91.96%) respectively, hirsutism and alopecia improvement were recorded in about (48.6%, 42.8 %), respectively after treatment.

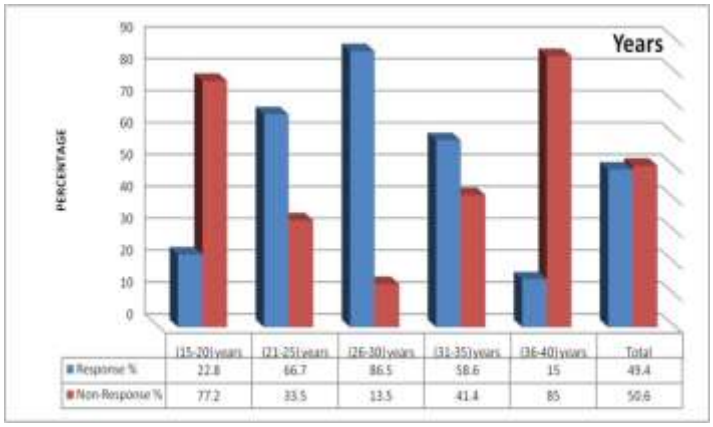

Figure (1). Response percentages among age groups in the study.

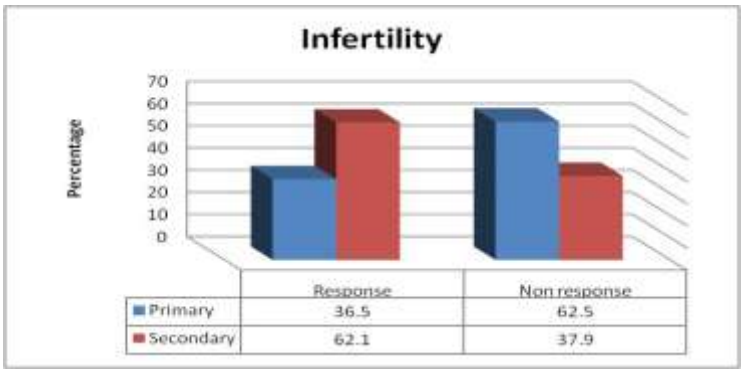

Figure (2) percentages of infertility types among the patients.

Biochemical results

Significant differences in pre and post measurement of clinical, endocrinal and metabolic parameters were noticed upon a period of metformin treatment, as shown in table (3).

Table (3). Clinical characteristics of patients before and after treatment. Student t-test, p<0.05 statistically significant, p<0.01 very significant, SD=standard deviation, BMI = body mass index, LH luteinizing hormone, FSH follicular stimulating hormone, SHBG sex hormone binding globulin, FAI free androgen index, FBS fasting blood sugar, HbA1c glycated hemoglobin TG = triglycerides, TC = total cholesterol, HDL-C = high density lipoprotein-cholesterol, LDL-C = low density lipoprotein-cholesterol.

PARAMETERS	PRE. **(No. =235) Mean ± SD**	POST. **(No. =235) Mean ± SD**	P VALUE
AGE (years)	27.1±5.21		
BMI(Kg/m^2);	28.8±5.62	27.2±5.66	<0.05
FSH (m.Iu/ml)	5.73±1.996	6.49±2.51	<0,01
LH(m.Iu/ml)	10.01±5.92	8.55±4.86	<0.01
LH/FSH	1.807±0.96	1.4±0.86	<0.01
TSH(uI/Ml)	2.33±0.97	2.159±0.87	<0.01
PROLACTIN(ng/ml)	23.74±9.68	20.48±10.87	<0,01
TESTESTERONE(ng/ml)	0.576±0.35	0.456±0.326	<0.01
SHBG(nmol/L)	41.85±22.63	51.91±21.62	<0.01
FAI	8.053±10.39	3.6±7 .823	<0.01
ESTRADIOL(pg/L)	57.88±49.2	68.1±47.45	<0.01
FBS(mg/dl),	96.2±13.18	92.62±13.16	<0.05
F.INSULIN(mIu/L)	20.21±14.42	18.76±11.54	<0.05
HOMA IR	4.74±3,71	4.11±2.63	<0.01
HbA1c%	4.947±0.703	4.562±0.665	<0.01
TG(mg/dl),	124.38±45.58	111.28±37.08	<0.01
LDL(mg/dl),	91.76±26.14	83.37±21.67	<0.01
HDL(mg/dl),	44.69±9.42	43.37±21.67	<0.05
TC(mg/dl),	157.74±40.54	145.48±43	<0.01

Genetic results

Genetic analysis of the STK11 gen SNPs rs741765 C/T in PCOSpatients have indicated that STK11 gen polymorphism are associated with response to metformin therapy.

Three genotypes where recorded upon PCR reaction, (CC) genotype (homozygous wild type) represented in samples (three and five) (CT) (heterozygous) in samples (one, four, and seven) and (TT)(mutant) insamples (two, six and eight), figure (3).

Present study revealed that there was no strong association was found between genotypes variation with residence and age at menarche among the studied patients.

More improvement in menstrual cycle regularity recorded among the mutant alleles carriers after three-month period figure (4), the same group showed clear improvement inhirsutism and alopecia also.

Clinical, endocrinal, and metabolic parameters among the genotypes were also analyzed and significant differences ($p \leq 0.05$) were recorded after the period of therapy with response improved as following TT>CT>CC, variations were also adjusted for base line levels among genotypes, tables (4).

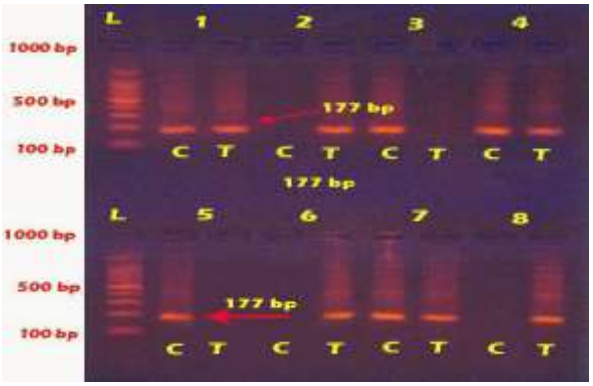

Figure (3). Amplification results of STK11 rs741765 SNP

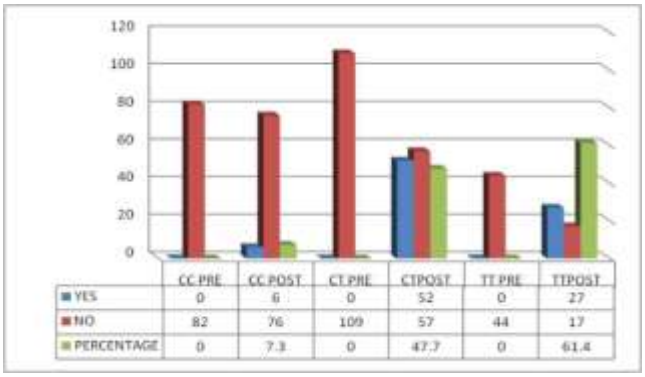

Figure (4). Regularity improvement among the STK11 rs741765

Table (4). Clinical, endocrinal and metabolic variables before and after 3 months on metformin therapy according toSTK11 rs741765 genotypes among the patients.

Data	Baseline				After 3 months of medication				Change 0-3 months			
	CC Mean ± SD	**CT** Mean ± SD	**TT** Mean ± SD	**P-VALUE**	**CC** Mean ± SD	**CT** Mean ± SD	**TT** Mean ± SD	**P-VALUE**	**CC**	**CT**	**TT**	**P-VALUE**
NO.	82	109	44		-	-	-		-	-	-	
BMI(kg/M2)	30.1+-5.49	29.72+-5.49	27.85+-5.18	<0.05	30.4+-4.66	28.56+-5.11	25.28+-4.29	<0.05	-0.39± 0.1	-1.05± 0.3	-2.19± 0.6	<0.05
FSH(m.Iu/ml	5.93± 1.93	5.73± 1.99	5.16 ± 2.13	<0.05	5.51± 1.53	6.36± 2.53	7.01 ± 2.87	<0.05	-0.39± 0.6	0.63± 0.4	1.821± 0.2	<0.05
LH(m.Iu/ml)	9.1± 4.84	10.48± 6.24	8.99 ± 5.68	<0.05	8.67± 4.87	8.5± 4.95	6.69 ± 4.45	<0.05	-0.41± 0.5	-1.92± 0.3	-2.12± 0.6	<0.05
LH/FSH	1.64± 0.9	1.91± 1.06	1.78 ± 0.81	>0.05	1.59± 0.9	1.36± 0.73	1.06 ± 0.64	>0.05	0.049± 0.3	-0.53± 0.6	-0.72± 0.6	>0.05
TSH(uI/Ml)	2.25± 0.87	2.3± 1	2.52 ± 0.96	>0.05	2.25± 0.79	2.41± 2.54	2.13 ± 0.9	>0.05	0.09± 0.02	0.12± 0.1	-0.41± 0.2	<0.05
Prolactin(ng/ml)	26.12± 14.3	23.86± 12.82	22.04 ± 12.35	>0.05	25.91± 10.63	20.34± 10.99	17.48 ± 10.14	<0.05	-0.210± 0.3	-3.52± 0.2	-4.66± 0.1	<0.05
T.testosterone(ng/ml)	0.65± 0.36	0.55± 0.34	0.72 ± 0.4	>0.05	0.71± 0.39	0.4± 0.27	0.32 ± 0.23	<0.05	0.059± 0.6	-0.15± 0.6	-0.37± 0.1	<0.05
SHBG(nmol/L)	50.48± 19.82	41.43± 23.64	41.26 ± 18.99	>0.05	46.63± 19.77	50.62± 22.55	57.74 ± 17.89	<0.05	-3.84± 0.3	9.19± 0.5	16.21± 0.2	<0.05
FAI	6.92± 9.21	8.82± 14.22	6.1 ± 3.35	>0.05	5.26± 6.61	3.34± 6.28	1.95 ± 1.73	<0.05	-1.66± 0.1	-5.48± 0.6	-4.095± 0.4	<0.05
Estradiol(pg/ml)	49.87± 29.44	61.23± 56.64	58.79 ± 58.04	<0.05	49.77± 29.07	71.39± 47.6	78.01 ± 62.51	<0.05	-0.099± 0.2	9.41± 0.3	19.15± 0.6	<0.05
FBS(mg/dL)	99.83± 11.44	95.98± 13.78	91 ± 13.96	>0.05	97.83± 11.92	90.33± 15.68	88.81 ± 10.4	<0.05	-2± 0.6	-5.65± 0.5	-2.319± 0.1	>0.05
F.Insulin (mIu/L)	24.35± 13.8	19.63± 14.11	21.11 ± 16.61	>0.05	24.5± 13.58	17.96± 10.43	16.15 ± 10.02	<0.05	0.14± 0.5	-1.67± 0.3	-4.96± 0.3	<0.05
HOMA IR	6.01± 3.62	4.73± 3.64	4.46± 3.43	>0.05	5.78± 3.4	4.04± 2.61	2.15 ± 0.93	<0.05	-0.23± 0.1	-0.69± 0.1	-2.31± 0.1	<0.05
HbA1C%	4.82± 0.58	4.9± 0.73	5.21 ± 0.732	>0.05	4.62± 0.63	4.77± 0.61	4.51± 0.76	>0.05	-0.2± 0.3	-0.131± 0.3	-0.7± 0.6	>0.05
TG(mg/dL)	118.87± 43.26	124.25 ± 44.19	123.37 ± 37.49	>0.05	116.66± 37.43	111.32± 39.14	103.73 ± 33.2	<0.05	-2.21± 0.6	-12.21± 0.6	-19.64± 0.4	<0.05
LDL(mg/dL)	101.94± 104.47	91.79± 30.36	96.87 ± 34.05	<0.05	85.09± 25.86	83.9± 25.99	80.72 ± 22.47	<0.05	-16.85± 0.4	-7.11± 0.6	-16.15± 0.4	>0.05
HDL(mg/dL)	47.85± 9.51	44.6± 9.56	43.81 ± 11.71	<0.05	47.4± 10.13	45.93± 9.26	47.81 ± 11.09	>0.05	-0.43± 0.6	0.88± 0.4	4.21± 0.1	>0.05
T.Cholestrol(	170.65±	156.7±	152.4 ±	<0.05	167.45±	142.3±	132.4 ±	<0.05	-0.39±	-0.63±	-1.821±	<0.05

Result of allele frequency revealed that better metformin efficacy was found in the mutant T allele 140 (60%), OR 4.9 95% CI = (3.3-7.2) compare with wild C allele 92 (40%) in responding group for rs741765 a table (5). In the dominant model, when the STK11 rs741765, CC genotype was made to be the reference versus the CC+CT, the dominant genotype was linked to a significantly better therapeutic efficacy of metformin (CC versus CC+CT: adjusted OR 7.2 95% CI = (3.3-15.9), in the recessive model CT+TT, however, there was no difference between the CT+TT genotype and TT genotype adjusted OR 1.1 95% CI = (3.3-15.9).

Table (5) Association between STKII rs741765 genetic polymorphism and metformin efficacy in PCOS patients adjusted for clinical, endocrinal and metabolic parameters.

Genotypes	Non response N=119	Response N=116	OR(95%CI)	P
CC	74(62.2%)	8 (6.9%)	1 reference	-
CT	33 (27.8%)	76 (65.6%)	21.3(9.3-49.2)	<0.0001
TT	12 (10.09%)	32 (27.6%)	24.7(9.2-66.1)	<0.0001
ALLELS				
C	181 (76%)	92 (40%)	1	
T	57 (24%)	140 (60%)	4.9 (3.3-7.2)	<0.0001
CC versus CC+CT dominant model	107	84	7.2 (3.3-15.9)	<0.0001
CT+TTrecessive model versus TT	45	108	1.1 (0.5-2.3)	=0.7

4. DISCUSSION

PCOS is the most common endocrine disorder among infertile women (12) and recent studyhave identified wide variation among PCOS patients and it is complicated by epigenetic and environmental factors, several demographic and biochemical parameters were analyzed among them and revealed significant variation.

Ages less than 35 years old were more prevalence among the patients, this finding consisting with(13)who reported that 73% of Iraqi PCOS women were young, because In Iraq, diagnosis of PCOS often associated with infertility and mostly start in the first year after the marriage and

majority of the patients in the current study were married, even though some symptoms may start at menarche. High proportion was reported from urban population in comparison to rural counterparts, the same finding reported by (14), it is likely that prevalence of PCOS varies significantly between urban and rural regions owing to different environments, lifestyles, incidence of obesity, and dietary variation.

Elevated percent of patients with secondary infertility resulted from hormonal imbalance that interfere with growth and release of eggs from the ovaries and result in secondary infertility lead to increase the interval between pregnancies and reduces the number of kids in women with PCOS, this compatible with (15)

Iraqi environment is suffered from pollution that might causes women infertile, because hormonal system in females is more sensitive to these effects. in addition, the Iraqi society suffered from psychological difficulties that affect women's behavior which effect female hormonal system that might be lead to PCOS this in agreement with(16).

Obesity is common among the patients in present study, increase BMI in PCOS patients due to high insulin levels that raise the production of androgens and weight gain typically in the abdomen (17). Metformin reduces BMI by decreasing insulin resistance and modulating the level of various peptides involved in controlling appetite like ghrelin, neuropeptide YY and adipokines, via hypothalamic adenosine 5′- monophosphate-activated kinase (AMP Kinase)(18).

Irregular mensesthat reported in all patient due to hormonal imbalance that result in high levels of androgens and excess insulin leading to disruption the monthly cycle of ovulation and menstruation(19), irregular bleeding is a consequence of excessive endometrial proliferation due to unopposed chronic estrogen secretion. Restoration of regular menstrual cycle with metformin was reported in (41%) of the patients upon metformin therapy, this agree with (36) and (37), this previously studies were indicated that thepercentage of regularity improvement not exceed 60% among the PCOS patients.

Abortion is one of the PCOS complication (20), so in current study repeated miscarriages were reported in high percentage, and previous studies like(21)showed that PCOS patients suffering from repeated miscarriage three times higher than normal due to hormonal disturbance, Progesterone synthesis deficiency of luteal phase and/or action is the major cause of spontaneous abortion due to elevation of both LH and insulin, studies like (22) revealed that elevated plasminogen-activator inhibitor-1 (PAI-1) levels in PCOS have been found to be an independent reversible risk factor for early spontaneous abortion.

Increase in progesterone concentration was found to be increased in the metformin-treated PCOS patients because it improves both LH and insulin concentrations and result in decreasing probability of abortion (23) .

Hirsutism and alopecia may be occurring simultaneously due to androgen production and elevated insulin levels. Insulin has direct and indirect influence on increasing serum androgen levels (hyperandrogenism) in PCOS, so, it has important role in PCOS pathogenesis, direct insulin effect by stimulation both ovarian and adrenal androgen secretion, while indirect effect by decreasing SHBG that lead to increasing FAI, metformin act by amelioration of their hyperandrogenemia and possibly by reducing circulating insulin levels result in improvement of insulin resistance(24).

Dyslipidemia in presented study was mostly correlated with BMI in women with PCOS this correlation due more insulin resistance in obese women result in increased fatty acid synthesis through fatty acid oxidation because of decreasing the activity of AMPK that act by phosphorylation or inactivation of Acetyl-CoA-Carboxylase(ACC) (25).

Metformin in addition to the main effect as insulin sensitizer, it is improve clinical, endocrinal and metabolic parameters by improvement of endocrinological and morphological response of the ovariesthrough its direct effect on ovaries by reducing CYP17α, which is a key enzyme in androgen synthesis and related to increased activity of 17α-hydroxylase and 17,20-lyase in ovarian theca cells(26).

Pronounced differences in response to metformin was found in this study according to the STK11 rs741765 variation, as reflected by a robust response in the main endocrine, metabolic and clinical parameters. Mutant allele carrierswere improved well than wild allele one, predicted that significant influence of STK11 rs741765 SNP on metformin efficacy was reported.

Metformin is frequently used in research as an AMPK agonist through phosphorylation by STK11, then activation of necessary element in cell metabolism that is required for maintaining energy homeostasis(27) , although STK11 and AMPK are not direct targets of metformin, butthey are necessary for metformin actions (28). The STK11-AMPK signaling pathway is one of the key regulators of insulin sensitivity and glucose homeostasis, playing critical roles in metabolic processes such as fatty acid synthesis and gluconeogenesis not only via an allosteric mechanism by CAMP, but also through phosphorylation of a key threonine residue (Thr172) on the a-catalytic subunit, the last is catalyzed by STK11.

Studies on diabetic patients revealed that polymorphisms in the STK11 gene have been associated with different metabolic disorders (29). Results of present study consistent with(30), that studied the candidate genes polymorphism that may play an important role in PCOS improvement after period of treatment with metformin, this included STK11, estrogen receptor 1 (ESR1), CYP genes (CYP2C9 and CYP2D6), and concluded that in STK11 gene polymorphism was associated with a response to metformin in PCOS women. The present study was the first study that investigate the relationship between STK11 rs741765 genetic polymorphism and metformin therapeutic efficacy in Iraqi population specifically in Kerbala city.

5. Conclusion

Polymorphism in STK11, a kinase gene expressed in liver and implicated in metformin action is associated with response to treatment with metformin alone and the percentage of women who improved was increased with the number of (T) alleles carriers.

ACKNOWLEDGMENT

The authors wish to think all the staff members at theWomen's Hospital obstetrics education in Karbala and Pharmacology and Toxicology Department at College of Pharmacy for their scientific support and help.

ETHICALAPPROVAL

Ethical Committee in College of Pharmacy, Karbala University was approved the study protocol.

6. References

1. Padmapriya B, Kesavamurthy T, editors. Diagnostic tool for PCOS classification. 7th WACBE World Congress on Bioengineering 2015; 2015: Springer.
2. Tabassum R, Imtiaz F, Sharafat S. Prevalence and clinical profile of insulin resistance in young women of poly cystic ovary syndrome: A study from Pakistan. Pakistan journal of medical sciences. 2013;29(2):593.

3. Dumitrescu R, Mehedintu C, Briceag I, Purcarea V, Hudita D. The polycystic ovary syndrome: an update on metabolic and hormonal mechanisms. Journal of medicine and life. 2015;8(2):142.
4. Nahar K. Polycystic Ovary Syndrome in Teenage and Young Women. Journal of Bangladesh College of Physicians and Surgeons. 2019;37(2):78-82.
5. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocrine reviews. 2015;36(5):487-525.
6. Kabel AM. Polycystic ovarian syndrome: insights into pathogenesis, diagnosis, prognosis, pharmacological and non-pharmacological treatment. J Pharma Reports. 2016;1(103):2.
7. Baskind NE, Balen AH. Hypothalamic–pituitary, ovarian and adrenal contributions to polycystic ovary syndrome. Best Practice & Research Clinical Obstetrics & Gynaecology. 2016;37:80-97.
8. De Leo V, Musacchio M, Cappelli V, Massaro M, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. Reproductive Biology and Endocrinology. 2016;14(1):38.
9. Khan A, Karim N, Ainuddin JA. The Triad Of PCOS, Infertility And Metformin. 2019.
10. Cernea S, Cahn A, Raz I. Pharmacological management of nonalcoholic fatty liver disease in type 2 diabetes. Expert review of clinical pharmacology. 2017;10(5):535-47.
11. Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell metabolism. 2005;1(1):15-25.
12. Schmid J, Kirchengast S, Vytiska-Binstorfer E, Huber J. Infertility caused by PCOS—health-related quality of life among Austrian and Moslem immigrant women in Austria. Human reproduction. 2004;19(10):2251-7.
13. Nabag WO, Farajalla EA, El Sheikh MA. Insulin resistance in infertile Sudanese patients with poly cystic ovarian syndrome (PCOS) at laparoscopy. Journal of Advances in Medicine and Medical Research. 2014:889-97.
14. Balaji S, Amadi C, Prasad S, Bala Kasav J, Upadhyay V, Singh AK, et al. Urban rural comparisons of polycystic ovary syndrome burden among adolescent girls in a hospital setting in India. BioMed research international. 2015;2015.
15. Deepika M, Ranjith K, Usha Rani V, Ishaq M, Jahan P. Familial background of complex diseases in PCOS probands of South Indian population. Asian J Epidemiol. 2012;5(2):50-5.
16. Al-Shattawi SS, Al-Jumili EF, Al-Azzam MA. The relationship between obesity and polycystic ovary syndrome in a sample of Iraqi infertile women. Iraqi journal of biotechnology. 2018;17(3).
17. Wright C, Zborowski J, Talbott E, McHugh-Pemu K, Youk A. Dietary intake, physical activity, and obesity in women with polycystic ovary syndrome. International Journal of Obesity. 2004;28(8):1026-32.
18. Palomba S, Falbo A, Zullo F, Orio Jr F. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocrine Reviews. 2009;30(1):1-50.
19. Carmina E, Oberfield SE, Lobo RA. The diagnosis of polycystic ovary syndrome in adolescents. American journal of obstetrics and gynecology. 2010;203(3):201. e1-. e5.
20. Joham AE, Palomba S, Hart R, editors. Polycystic ovary syndrome, obesity, and pregnancy. Seminars in reproductive medicine; 2016: Thieme Medical Publishers.

21. Giudice LC. Endometrium in PCOS: implantation and predisposition to endocrine CA. Best practice & research Clinical endocrinology & metabolism. 2006;20(2):235-44.
22. Homburg R. Pregnancy complications in PCOS. Best Practice & Research Clinical Endocrinology & Metabolism. 2006;20(2):281-92.
23. KACHHAWA G, SINGH A. Evaluation of the Infertile Female. Indian Journal of Clinical Practice. 2019;30(2).
24. Malini N, George KR. Evaluation of different ranges of LH: FSH ratios in polycystic ovarian syndrome (PCOS)–Clinical based case control study. General and comparative endocrinology. 2018;260:51-7.
25. Lath R, Shendye R, Jibhkate A. Insulin resistance and lipid profile in polycystic ovary syndrome. Asian Journal of Biomedical and Pharmaceutical Sciences. 2015;5(47):30.
26. Luque-Ramírez M, F Escobar-Morreale H. Adrenal hyperandrogenism and polycystic ovary syndrome. Current pharmaceutical design. 2016;22(36):5588-602.
27. Rahimi A, Khosravifar M, Peirosabet M, Chekini Z, Shiva M, Afsharian P. Study of genetic alterations of STK11 in patients with polycystic ovary syndrome (PCOS) and their response to ovarian stimulation. Iranian Journal of Reproductive Medicine. 2015.
28. Hardie DG. Neither LKB1 nor AMPK are the direct targets of metformin. Gastroenterology. 2006;131(3):973.
29. Shaw RJ, Lamia KA, Vasquez D, Koo S-H, Bardeesy N, DePinho RA, et al. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science. 2005;310(5754):1642-6.
30. Goldenberg N, Glueck CJ. Is pharmacogenomics our future? Metformin, ovulation and polymorphism of the STK11 gene in polycystic ovary syndrome. 2008.