Is polymorphism of the STK11 gene a predictor of response to metformin in polycystic ovarian syndrome?

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Abstract

Objectives: To evaluate possible associations between the genetic polymorphism of the STK 11 gene and response to metformin in women with polycystic ovary syndrome. Methods: This is a prospective longitudinal cohort study of 57 women with polycystic ovary syndrome. Baseline documentation of anthropometric measurements, menstrual history, hirsutism, hair loss, acne, and biochemical parameters, in addition to gene testing for STK11 polymorphism, were performed. Follow-up was arranged at 6 cycles following oral metformin therapy, 850 mg, twice daily. Results: Post-metformin therapy, there were statistically significant improvements in menstrual frequency, blood loss, acne, ultrasound findings, and a decrease in BMI, acne and hirsutism, but not in alopecia. Fasting insulin decreased significantly, but fasting blood sugar did not. Regarding Intron1 polymorphism, there was a significant response in the CC subgroup in menstrual regularity and blood loss. The CG subgroup showed a significant response in menstrual regularity and ultrasound findings. The GG subgroup showed a significant response in menstrual regularity, menstrual loss, acne and alopecia. Regarding Intron 6 polymorphism, there was a significant response in the CC subgroup in relation to menstrual regularity, blood loss, acne and ultrasound findings. The CT subgroup showed a significant response in menstrual regularity and ultrasound findings. The TT subgroup showed a significant response only in relation to alopecia. Conclusion: Polymorphism in STK11 is not predictive of response to metformin therapy at a dose of 850 mg, twice daily.

Date: March 6, 2021

Dear Editor-in-Chief

I am writing to submit our manuscript entitled "Is polymorphism of STK11 gene a predictor of response to metformin in polycystic ovarian syndrome?" The study addresses the issue of insulin resistance in patients with polycystic ovary syndrome, and that metformin is an insulin sensitizer and is commonly prescribed in cases of polycystic ovary syndrome. However, not all such women respond well to metformin. It has been suggested that the therapeutic response to metformin is associated with certain genetic polymorphism. Identifying such an association could be utilized as a prognostic tool in the selection of women that are likely to benefit from metformin therapy. The paper aimed at the evaluation of a possible associations between the genetic polymorphism of the STK 11 gene and response to metformin in women with polycystic ovary syndrome.

All of the authors have revised and approved the manuscript and contributed significantly to the study. The language has been edited by a professional, native English-speaking editor. Manuscript has not been published and is not under consideration for publication elsewhere. The authors have no conflicts of interest to disclose.

The authors would be grateful for your kind consideration and look forward to your response.

Sincerely,

Hamadneh

Title Page

Full title

Is polymorphism of STK11 gene a predictor of response to metformin in polycystic ovarian syndrome?

Short title

STK11 gene response to metformin in PCOS

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Methods: This is a prospective longitudinal cohort study of 57 women with polycystic ovary syndrome. Baseline documentation of anthropometric measurements, menstrual history, hirsutism, hair loss, acne, and biochemical parameters, in addition to gene testing for STK11 polymorphism, were performed. Follow-up was arranged at 6 cycles following oral metformin therapy, 850 mg, twice daily.

Results: Post-metformin therapy, there were statistically significant improvements in menstrual frequency, blood loss, acne, ultrasound findings, and a decrease in BMI, acne and hirsutism, but not in alopecia. Fasting insulin decreased significantly, but fasting blood sugar did not.

Regarding Intron1 polymorphism, there was a significant response in the CC subgroup in menstrual regularity and blood loss. The CG subgroup showed a significant response in menstrual regularity and ultrasound findings. The GG subgroup showed a significant response in menstrual regularity, menstrual loss, acne and alopecia. Regarding Intron 6 polymorphism, there was a significant response in the CC subgroup in relation to menstrual regularity, blood loss, acne and ultrasound findings. The CT subgroup showed a significant response in menstrual regularity and ultrasound findings. The TT subgroup showed a significant response only in relation to alopecia.

Conclusion: Polymorphism in STK11 is not predictive of response to metformin therapy at a dose of 850 mg, twice daily.

Keywords: Metformin, polycystic ovary syndrome, *STK11*gene.

What's already known about this topic? It has been suggested that metformin induces a better response in controlled ovula

What does this article add? We conclude that metformin did improve periods regularity, ovarian appearance, acne, hirsutis

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a well-known disorder that affects the reproductive, metabolic, and cardiovascular health [1]. It affects an estimated 5-10% of women [2]. The reproductive effect of PCOS is characterized by oligomenorrhea or amenorrhea and chronic anovulation, hyperandrogenism and a characteristic polycystic appearance of the ovaries [3].

The etiology of PCOS is unknown. However, insulin resistance (IR) has been identified as a factor in the pathogenesis of PCOS, and the metabolic and cardiovascular consequences of the syndrome [4].

Insulin resistance and hyperinsulinemia are associated with ovarian hyper-androgenism and decrease in sex hormone-binding globulin (SHBG) leading to higher serum free testosterone levels (FT) [1]. This hyperandrogenic state leads to the characteristic anovulation, menstrual irregularities and hirsutism in (PCOS) [5].

Metformin is a biguanide that is used for type 2 diabetes mellitus. It has been found that it decreases basal, postprandial plasma glucose and IR in PCOS [6]. In contrast, Acbay et al. found that metformin does not decrease IR in PCOS, suggesting that the cellular mechanism of IR in PCOS is different from other IR states [7]. Several studies found that metformin increases the number of ovulatory cycles and improves hyperandrogenism, insulin sensitivity, menstrual regularity, and metabolic disorders in women with PCOS [8].

It has been suggested that metformin induces better response in controlled ovulation stimulation. A meta-analysis by Lord et al. showed that metformin had a significant effect on ovulation compared to placebo, but demonstrated no effect on weight, BMI or waist circumference [9].

The molecular mechanism behind metformin action seems to be related to its phosphorylation of AMP-activated protein kinase (AMPK), which inhibits glucagon-stimulated glucose production and causes an increase in glucose absorption in muscles and hepatic cells [10].

Pharmacogenomics address how genes affect an individual's response to certain drugs. This relatively new area combines pharmacology and genomics by mapping drug response phenotypes to individual genotypes. It has the potential for more advanced screening for disease, better vaccines, advanced drug discovery, quicker drug approval process, and a decrease in the overall cost of health care [11].

Serine–threonine kinase 11 (*STK11* /*LKB1*), which phosphorelates AMPK, has been reported to be linked to metformin effect [12-14]. Goldberg et al. in 2008, suggested an association between metformin, ovulation and polymorphism of *STK11* gene in PCOS [15]. A randomized clinical trial by Legro et al. found that polymorphism in *STK11* (rs8111699) is linked to the ovulatory response to metformin, and that the C allele was associated with a significantly decreased chance of ovulation in PCOS women treated with metformin [16].

Further benefits of pharmacogenomics include the use of more powerful medicines, accurate methods of determining appropriate dosages, making clinical decisions based on genetics by choosing a drug or adjusting the dosage, tailored to a person's genetic makeup [11].

The aims of this novel study were to determine the frequencies of different genetic *STK11* variations in women with PCOS, and to investigate the effect of *STK11* polymorphism on the response to metformin therapy in women with PCOS in the north of Jordan. The study was approved by the Institutional Review board of the Jordan University of Science and Technology.

METHODS

Study design : this is a prospective, longitudinal cohort study of women diagnosed with PCOS. The study group was selected from women that attended the gynecology clinics of the Jordan University of Science and Technology in the north of Jordan, between January 2016 and July 2019. Participants were fully counselled. Informed consents were obtained.

Intervention: eligible women received 6 months of oral metformin therapy. The initial dose was 850 mg orally once a day. The dose was titrated in 850 mg increments after 2 weeks as tolerated to a maintenance dose of 1700 mg/day in divided doses with meals.

Inclusion criteria: confirmed diagnosis of PCOS according to the Rotterdam criteria [17]. This is a combination of any two of the following three criteria:

1) chronic oligomenorrhea (<8 menstrual periods annually), or amenorrhea.

2) biochemical or clinical androgen excess.

3) polycystic ovaries on ultrasonography.

Enrolled women with PCOS were confirmed to be non-menopausal, between 18-45 years of age, with a minimum of 3 years' post-menarche, not morbidly obese (BMI $>$ 35), with normal thyroid function and serum prolactin.

Exclusion Criteria: chronic medical illness including diabetes mellitus, abnormal kidney and liver function tests, current use of oral contraceptives or use of fertility drugs within 6 months of study, and ingestion of any investigational drug within 3 months prior to the study, including metformin.

Research application

A face-to-face interview was conducted. Demographic data included age, education, profession, income and marital status, in addition to medical and reproductive history, use of medications, menstrual history, and hyperandrogenic symptoms (hirsutism, acne, hair loss).

Pre-treatment clinical and biochemical parameters were obtained. Bilateral ovarian volumes, morphology and number of antral follicles, with a diameter of 2 to 9 mm were assessed by ultrasound during the 2nd or 3rd proliferative phase of the menstrual cycle. Ovarian morphology was defined as either PCO or not PCO. Women were asked to keep a menstrual calendar.

Hirsutism was assessed according to the modified Ferriman-Gallwey criteria at 9 different body sites that included upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, arm, and thigh. A score of 0 for absence of terminal hairs to 4 for extensive terminal hair growth was assigned as appropriate A score of [?] 8 was assigned as hirsutism [18].

Biochemical assays

Blood samples were taken between 8:00 a.m. and 9:00 a.m. after 12 hours of overnight fast. Hormonal evaluation consisted of assays of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH)), total testosterone (TT), dehydroepiandrosterone sulfate (DHEA-S) and 17-hydroxy progesterone (17-OHP), thyroid-stimulating hormone (TSH), prolactin (PRL) and estradiol (E2). Metabolic assessment included fasting glucose, insulin levels, liver and kidney function tests.

On the the sixth month of metformin therapy, serum progesterone (P) levels were assessed. Women with regular cycle were tested on day 6-9 prior to the expected menstruation. The test was repeated 3-4 days later if the level indicated anovulation. Women with irregular cycles were tested on a weekly basis. Ovulation was confirmed at a plasma progesterone of [?] 4 ng/ml.

A 3 ml venous blood sample to study *STK11* gene variation was taken. DNA was extracted from each sample under sterile conditions using the Puregene-Qiagen DNA extraction kit according to manufacturer instructions. The obtained DNA concentration and purity was determined using Nanodrop Spectrophotometer. Polymerase Chain Reaction (PCR).

Segment rs8111699 of the *STK11* gene was amplified using the Intron mastermix and 96-well Veriti Thermal Cycler (Applied Biosystems). PCR products were separated by 2% agarose electrophoresis. The gel was visualized under ultra-violet light using Gel-Doc (BioRad). Each sample was purified using bio spin columns, then sequenced separately by using the Big-Dye Terminator kit (Applied Biosystems). Each sample was

cleaned–up using Nucleo SEQ Columns (Macherey–Nagel), then run on the genetic analyzer 3130xl (Applied Biosystems) according to the manufacturer instructions. DNA sequences were blasted against reference sequence to determine the variations between samples and the reference sequence.

Statistical analysis

Collected data were double-entered on data sheets and analyzed using SPSS (version 22, 2013, IBM). Descriptive statistics were performed using frequency as well as means and standard deviations (SD) to describe the categorical and numeric data, respectively. The nonparametric Kolmogorov-Smirnov test was used to examine all numeric variables for normal distribution. The Pearson's Chi-square (χ^2) and Fisher's exact tests of independence were used to assess if there was a significant, dependent relationship between categorical variables. One-way analysis of variance (ANOVA) was used to examine the differences in the means of hirsutism scores in intron 1 and 6 of the *STK11*gene. A paired sample t-test was used to evaluate the difference between means of the normally distributed variables before and after the metformin therapy. The medians were compared when variables followed a significant skewed distribution using Wilcoxon Signed Rank test for paired samples. $P < 0.05$ was considered statistically significant.

RESULTS

For the purposes of this study, 120 women were interviewed, 88 women fulfilled the study criteria, 57 of these completed the study, another 31 women were excluded due to non-compliance or loss to follow-up.

Characteristics of the study population

The descriptive statistics of the 57 participants revealed that, the mean and SD of age, weight, height, and BMI were 23.8 (5.7) years, 72.1 (15.1) kg, 159 (5.2) cm and 28.6 (6.1) kg/m2 respectively. The average monthly family income was 1000 JD ($ 1400), 70% were single and 28% were university graduates.

The average hirsutism score was 16.0±6.8, fasting blood glucose 5.1±0.5 mmol/L and the fasting insulin mean level was 13.5±7.0 mIU/L. FSH, LH and LH/FSH ratios were 5.4±1.2 IU/L, 8±4.3 and 1.5±0.74 IU/L respectively. TSH 2.0±0.9 ng/dl and prolactin 14.4±5.0 ng/dl, estradiol 111.1±55.7 pg/ml, DHES 238.2±109.1 µg/dl, 17OHP1.4±0.67 ng/dl, and total testosterone 0.3±0.2 ng/dl respectively. The average length of menstrual period was 5.6±2.2 days.

The distribution of the genotypes of intron 1 of STK11 gene were 26% CC, 44% CG and 30% GG. The frequency distribution of intron 6 of STK11 gene were 52% CC, 37% CT and 11% have TT genotype.

Comparing the frequencies of clinical characteristics, before and after metformin therapy, revealed statistically significant differences ($P < 0.01$) in the frequency of menstruation, amount of menstrual blood, acne and ultrasound findings but not in alopecia (P 0.134). . There was a significant difference in ovarian volume (Table 1a).

Post metformin therapy, there were statistically significant decrease in body weight (P 0.001), BMI (P 0.001), acne (P 0.001) and hirsutism score (P 0.001). Fasting insulin did significantly decrease after therapy (P 0.009), but fasting blood sugar did not. There was no significant statistical difference in relation to the other parameters (Table 1b).

Of the 25 women who underwent luteal progesterone tests, the level was >4 ng/ml in 24 women. Out of 17 women that were trying to conceive, 7 became pregnant during the study period.

As for SKT11 gene polymorphism, results before and after therapy in Intron1 showed significant response in the CC sub group regarding menstrual regularity and amount of menstrual blood loss (P 0.0169 and 0.017 respectively). The CG subgroup had significant response in relation to menstrual regularity and ultrasound findings (P 0.0001 and 0.0006 respectively). The GG subgroup had significant response in relation to menstrual regularity, amount of menstrual loss, acne and alopecia (P 0.0445, 0.044, 0.039, 0.032 respectively) (Table 2).

Regarding Intron 6 polymorphism, subgroup CC had significant response in relation to menstrual regularity, amount of menstrual blood loss, acne and ultrasound findings (P 0.0001, 0.0025 0.0384 and 0.0097 respectively). The CT sub group showed significant response in relation to menstrual regularity and ultrasound findings (P 0.0005 and 0.0038 respectively). The TT subgroup showed significant response only in relation to alopecia (P 0.0152) (Table 3).

Following therapy, Intron 1 and Intron 6 subgroups showed a difference in relation to alopecia in the Intron1 subgroup (P 0.024) (Table 4), and in relation to hirsutism score in the Intron 6 subgroup (P = 0.006) (Table 5).

The distribution of the 24 women with ovulatory levels of progesterone among the subgroups was CC 9, CG 7 and GG 8 in the Intron 1genotype subgroup, and CC 14, CT 6 and TT 4 in the Intron 6 genotype subgroup. Seven out of seventeen women trying to conceive got pregnant during the study period. Their distribution among the subgroups was CC 2/5, CG 2/5 and GG 3/7 in the Intron 1 sub-groups, and CC 3/7, CT 3/8 and TT 1/2 pregnancies in the Intron 6 genotype subgroup.

DISCUSSION

Polycystic ovary syndrome is one of the most common endocrine disorders in women of reproductive age, with no known definitive therapy. Metformin, well tolerated insulin sensitizer with minimal side effect, proved useful in correcting IR, hyperandrogenism, ovulation, and is relatively safe for the fetus (Category B) [19].

In this study of the effect of metformin in 57 women with PCOS, results showed statistically significant improvement in cycle regularity, amount of blood loss, acne and ovarian ultrasound morphology. This is consistent with the findings of other investigators [20,21].

Unfortunately, women with oligomenorrhea failed to comply with weekly progesterone tests. Whereas, women with normal cycles had a 96% ovulatory progesterone levels, with 41% positive beta hCG in women trying to get pregnant. These results are consistent with the results of other studies [9].

In agreement with the findings of other studies, metformin therapy in this study demonstrated a significant decrease in hirsutism in women with PCOS [22, 23]. (Table 1b).

As stated by Palomba et al, the results of this study suggest that it would be reasonable to use Metformin as first line therapy in women, with relative contraindication to the long term use of the combined oral contraceptive pill (COC), who wish to have regular menstruation, where "COC menstruation" is simply a "withdrawal bleed" [24]. This might be more pertinent in communities where young and unmarried women are reluctant to use hormonal contraceptives for menstrual cycle regulation.

The study of Intron 1 and Intron 6 revealed that their subgroups responded to metformin differently in relation to various parameters. The Intron1 subgroups' response to metformin revealed no statistically significant difference in all parameters except for alopecia. Study of same parameters in Intron 6 showed no significant difference in response in all sub-groups in response to metformin therapy except for hirsutism. The ovulatory response was similar, and pregnancies were reported in all subgroups.

One of the limitations of this study is the small sample size to thoroughly assess statistical significance. Secondly, this study is lacking the important free testosterone and SHBG tests which are considered essential to evaluate whether the hyperandrogenic therapeutic goals are being met in response to therapy [25].

We conclude that metformin did improve periods regularity, ovarian appearance, acne, hirsutism and ovulatory response. SKT11 polymorphism both Intron1 and Intron6 may have some effect on alopecia and hirsutism but no conclusion could be drawn regarding ovulatory response because of the small sample size.

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Conflict of Interest: No author has any potential conflict of interest.

Ethical approval : All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee. Informed consent: Informed consent was obtained from all individual participants included in the study.

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Tables

Table 1a : Frequencies of the clinical characteristics of PCOS before and after metformin therapy (N =57).

Variable	**Before, *n* (%)**	**After, *n* (%)**	***P* value**
Frequency of period			0.001
Regular	0 (0.0)	33 (57.9)	
Irregular	57 (100.0)	24 (42.1)	
Amount of menstrual blood			0.001
Low	17 (29.8)	01 (1.8)	
Normal	40 (70.2)	56 (98.2)	
Acne			0.001
No	21 (36.8)	42 (73.7)	
Yes	36 (63.2)	15 (26.3)	
Alopecia			0.134
No	25 (43.9)	33 (57.9)	
Yes	32 (56.1)	24 (42.1)	
Ultrasound PCOS picture			0.001
No	16 (28.1)	37 (64.9)	
Yes	41 (71.9)	20 (35.1)	
Serum progesterone (ng/dL)			NA
[?] 4	NA	1 (4.0)	
>4	NA	24 (96.0)	

*Only 25 of women performed progesterone. NA: Not applicable**.**

Table 1b: Means of characteristics of PCOS before and after metformin therapy (N =57).

Variable	**Before, mean ± SD**	**After, mean ± SD**	***P* value**
Weight (kg)	72.1 ± 15.5	69.7 ± 14.2	0.001
BMI (kg/m^2)	28.6 ± 6.1	27.6 ± 5.6	0.001
Hirsutism score	16.0 ± 6.8	13.8 ± 5.5	0.001
Fasting blood glucose	5.1 ± 0.5	5.0 ± 0.5	0.238
Fasting insulin	13.5 ± 7.0	11.7 ± 6.3	0.009
LH/FSH	1.5 ± 0.74	1.4 ± 0.78	0.608
E2	111.1 ± 55.7	123.2 ± 54.1	0.219
DHES	238.2 ± 109.1	262.2 ± 139.5	0.045
Total testosterone	0.3 ± 0.21	0.3 ± 0.20	0.218
17OHP	1.4 ± 0.7	1.4 ± 0.7	0.429
Length of period (days)	5.6 ± 2.2	5.8 ± 1.4	0.333

Table 2: Frequencies of clinical characteristics of PCOS before and after therapy according to intron 1 (*N* =57).

Variable	**CC (*n*=15)**	**CC (*n*=15)**	**CG (*n*=25)**	**CG (*n*=25)**	**GG (*n*=17)**	**GG (*n*=17)**
	Before	**After**	**Before**	**After**	**Before**	**After**
Frequency of period						
Regular	0 (0.0)	9 (60.0)	0 (0.0)	12 (48.0)	0 (0.0)	12 (70.6)
Irregular	15 (100.0)	6 (40.0)	25 (100.0)	13 (52.0)	17 (100.0)	5 (29.4)
P value	**0.0169**	**0.0169**	**0.0001**	**0.0001**	**0.0445**	**0.0445**
Menstrual blood loss						
Low	6 (40.0)	0 (0.0)	6 (24.0)	1 (4.0)	5 (29.4)	0 (0.0)
Normal	9 (60.0)	15 (100.0)	19 (76.0)	24 (96.0)	12 (70.6)	17 (100.0)
P value	**0.017**	**0.017**	0.098	0.098	**0.044**	**0.044**
Acne						
No	6 (40.0)	12 (80.0)	9 (36.0)	15 (60.0)	6 (35.3)	12 (70.6)
Yes	9 (60.0)	3 (20.0)	16 (64.0)	10 (40.0)	11 (64.7)	5 (29.4)
P value	0.060	0.060	0.089	0.089	**0.039**	**0.039**
Alopecia						
No	7 (46.7)	9 (60.0)	11 (44.0)	10 (40.0)	7 (41.2)	14 (82.4)
Yes	8 (53.3)	6 (40.0)	14 (56.0)	15 (60.0)	10 (58.8)	3 (17.6)
P value	0.464	0.464	0.774	0.774	**0.032**	**0.032**
Ultrasound picture						
No	6 (40.0)	10 (66.7)	5 (20.0)	17 (68.0)	5 (29.4)	10 (58.8)
Yes	9 (60.0)	5 (33.3)	20 (80.0)	8 (32.0)	12 (70.6)	7 (41.2)
P value	0.143	0.143	**0.0006**	**0.0006**	0.0841	0.0841
Hirsutism score	17.3 ± 6.2	14.9 ± 5.2	15.5 ± 6.7	13.9 ± 5.6	15.6 ± 7.6	12.6 ± 6.1
P value	0.2604	0.2604	0.3642	0.3642	0.2135	0.2135

Categorical data are presented as *n* (%) and continuous data are presented as mean ± SD.

Table 3 : Frequencies of clinical characteristics of PCOS before and after therapy according to intron 6 (*N*

=57).

Variable	**CC, *n*=30**	**CC, *n*=30**	**CT, *n*=21**	**CT, *n*=21**	**TT, *n*=6**	**TT, *n*=6**
	Before	**After**	**Before**	**After**	**Before**	**After**
Frequency of period						
Regular	0 (0.0)	17 (56.7)	0 (0.0)	11 (52.4)	0 (0.0)	5 (83.3)
Irregular	30 (100.0)	13 (43.3)	21 (100.0)	10 (47.6)	6 (100.0)	1 (16.7)
P value	**< 0.0001**	**< 0.0001**	**0.0005**	**0.0005**	1.000	1.000
Menstrual blood loss						
Low	11 (36.7)	01 (3.3)	3 (14.3)	0 (0.0)	3 (50.0)	0 (0.0)
Normal	19 (63.3)	29 (96.7)	18 (85.7)	21 (100.0)	3 (50.0)	6 (100.0)
P value	**0.0025**	**0.0025**	0.2317	0.2317	0.1818	0.1818
Acne						
No	12 (40.0)	20 (66.7)	9 (42.9)	15 (71.4)	0 (0.0)	4 (66.7)
Yes	18 (60.0)	10 (33.3)	12 (57.1)	6 (28.6)	6 (100.0)	2 (33.3)
P value	**0.0384**	**0.0384**	0.0614	0.0614	0.0606	0.0606
Alopecia						
No	13 (43.3)	17 (56.7)	11 (52.4)	10 (47.6)	1 (16.7)	6 (100.0)
Yes	17 (56.7)	13 (43.3)	10 (47.6)	11 (52.4)	5 (83.3)	0 (0.0)
P value	0.3017	0.3017	0.7576	0.7576	**0.0152**	**0.0152**
Ultrasound picture						
No	11 (36.7)	21 (70.0)	3 (14.3)	12 (57.1)	2 (33.3)	4 (66.7)
Yes	19 (63.3)	9 (30.0)	18 (85.6)	9 (42.9)	4 (66.7)	2 (33.3)
P value	**0.0097**	**0.0097**	**0.0038**	**0.0038**	0.5671	0.5671
Hirsutism score	17.7 ± 6.7	15.1 ± 5.6	12.1 ± 4.3	11.0 ± 3.8	21.2 ± 8.2	18.0 ± 7.2
P value	0.1083	0.1083	0.3850	0.3850	0.4890	0.4890

Categorical data are presented as *n* (%) and continuous data are presented as mean ± SD.

Table 4 : Frequencies of clinical characteristics of PCOS before and after therapy in intron 1 subgroups (*N* =57).

Variable	**Before**	**Before**	**Before**	**After**	**After**	**After**
	CC, *n*=15	**CG, *n*=25**	**GG, *n*=17**	**CC, *n*=15**	**CG, *n*=25**	**GG, *n*=17**
Age (years)						
[?] 35	13 (86.7)	25 (100.0)	15 (88.2)	NA	NA	NA
> 35	2 (13.3)	0 (0.0)	2 (11.8)	NA	NA	NA
P value	0.184	0.184	0.184	NA	NA	NA
BMI (kg/m^2)						
[?] 24.99	6 (40.0)	9 (36.0)	5 (29.4)	06 (40.0)	9 (36.0)	5 (29.4)
> 24.99	9 (60.0)	16 (64.0)	12 (70.6)	09 (60.0)	16 (64.0)	12 (70.6)
P value	0.815	0.815	0.815	0.815	0.815	0.815
Frequency of period						
Regular	0 (0.0)	0 (0.0)	0 (0.0)	09 (60.0)	12 (48.0)	12 (70.6)

Variable	Before	Before	Before	After	After	After
Irregular	15 (100.0)	25 (100.0)	17 (100.0)	06 (40.0)	13 (52.0)	5 (29.4)
P value	0.927	0.927	0.927	0.340	0.340	0.340
Menstrual blood loss						
Low	6 (40.0)	6 (24.0)	5 (29.4)	0 (0.0)	1 (4.0)	0 (0.0)
Normal	9 (60.0)	19 (76.0)	12 (70.6)	15 (100.0)	24 (96.0)	17 (100.0)
P value	0.563	0.563	0.563	0.521	0.521	0.521
Acne						
No	6 (40.0)	9 (36.0)	6 (35.3)	12 (80)	15 (60.0)	12 (70.6)
Yes	9 (60.0)	16 (64.0)	11 (64.7)	3 (20)	10 (40.0)	5 (29.4)
P value	0.956	0.956	0.956	0.409	0.409	0.409
Alopecia						
No	7 (46.7)	11 (44.0)	7 (41.2)	9 (60)	10 (40.0)	14 (82.4)
Yes	8 (53.3)	14 (56.0)	10 (58.8)	6 (40)	15 (60.0)	3 (17.6)
P value	0.952	0.952	0.952	0.024	0.024	0.024
PCOS by U/S						
No	6 (40.0)	5 (20.0)	5 (29.4)	10 (66.7)	17 (68.0)	10 (58.8)
Yes	9 (60.0)	20 (80.0)	12 (70.6)	5 (33.3)	8 (32.0)	7 (41.2)
P value	0.391	0.391	0.391	0.818	0.818	0.818
Progesterone (ng/dL)						
[?] 4	NA	NA	NA	0 (0.0)	0 (0.0)	1 (12.5)
> 4	NA	NA	NA	9 (100.0)	7 (100.0)	8 (87.5)
P value	NA	NA	NA	0.396	0.396	0.396
Pregnancies						
No	NA	NA	NA	3 (60)	3 (60)	4 (57.1)
Yes	NA	NA	NA	2 (40)	2 (40)	3 (42.9}
P value	NA	NA	NA	NA	NA	NA
Hirsutism score	17.3 ± 6.2	15.5 ± 6.7	15.6 ± 7.6	14.9 ± 5.2	13.9 ± 5.6	12.6 ± 6.1
P value	0.708	0.708	0.708	0.552	0.552	0.552

Categorical data are presented as n (%) and continuous data are presented as mean ± SD.

Table 5 : Frequencies of clinical characteristics of PCOS before and after therapy in intron 6 subgroups (N =57).

Variable	Before	Before	Before	After	After	After
	CC, n=30	**CT, n=21**	**TT, n=6**	**CC, n=30**	**CT, n=21**	**TT, n=6**
Age in years						
[?] 35	27 (90.0)	20 (95.2)	6 (100.0)	NA	NA	NA
> 35	3 (10.0)	1 (4.8)	0 (0.0)	NA	NA	NA
P value	NA	NA	NA	NA	NA	NA
BMI (kg/m^2)						
[?] 24.99	12 (40)	5 (23.8)	3 (50)	11 (36.7)	6 (28.6)	3 (50.0)
> 24.99	18 (60)	16 (76.2)	3 (50)	19 (63.3)	15 (71.4)	3 (50.0)
P value	0.354	0.354	0.354	0.603	0.603	0.603

Variable	**Before**	**Before**	**Before**	**After**	**After**	**After**
Frequency of period						
Regular	0 (0.0)	0 (0.0)	0 (0.0)	17 56.7)	11(53.4)	5 (83.3)
Irregular	30 (100.0)	21 (100.0)	6 (100.0)	13 (43.3)	10 (47.6)	1 (16.7)
P value	0.407	0.407	0.407	0.392	0.392	0.392
Menstrual blood loss						
Low	11 (36.7)	3 (14.3)	3 (50.0)	1 (3.3)	0 (0.0)	0 (0.0)
Normal	19 (63.3)	18 (85.7)	3 (50.0)	29 (96.7)	21 (100.0)	6 (100.0)
P value	0.119	0.119	0.119	0.633	0.633	0.633
Acne						
No	12 (40.0)	9 (42.9)	0 (0.0)	20 (66.7)	15 (71.4)	04 (66.7)
Yes	18 (60.0)	12 (57.1)	06 (100.0)	10 (33.3)	06 (28.6)	02 (33.3)
P value	0.138	0.138	0.138	0.933	0.933	0.933
Alopecia						
No	13 (43.3)	11 (52.4)	1 (16.7)	17 (56.7)	10 (47.6)	6 (100.0)
Yes	17 (56.7)	10 (47.6)	5 (83.3)	13 (43.3)	11 (53.4)	0 (0.0)
P value	0.298	0.298	0.298	0.071	0.071	0.071
Ultrasound picture						
No	11 (36.7)	03 (14.3)	02 (33.6)	21 (70)	12 (57.1)	04 (66.7)
Yes	19 (63.3)	18 (85.7)	04 (66.7)	09 (30)	09 (42.9)	02 (33.3)
P value	0.206	0.206	0.206	0.636	0.636	0.636
Progesterone						
[?] 4	NA	NA	NA	0 (0.0)	01 (14.3)	0 (0.0)
> 4	NA	NA	NA	14 (100.0)	06 (85.7)	04 (100.0)
P value	NA	NA	NA	0.262	0.262	0.262
Pregnancies						
No	NA	NA	NA	4 (57.1)	5 (62.5)	1 (50.0)
Yes	NA	NA	NA	3 (42.9)	3 (37.5)	1 (50.5)
P value	NA	NA	NA	NA		
Hirsutism score	17.7 ± 6.7	12.1 ± 4.3	21.2 ± 8.2	15.1 ± 5.6	11.0 ± 3.8	18.0 ± 7.2
P value	0.001	0.001	0.001	0.006	0.006	0.006

Categorical data are presented as n (%) and continuous data are presented as mean ± SD.