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Metabolic outcomes in samples of PCOS reproductive aged Iraqi women reversed by oral combined metformin and spironolactone treatment

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SUMMARY

Background: One of the most critical parameters to diagnose Polycystic Ovarian Syndrome (PCOS) is prolactin (Pr), Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Luteinizing Hormone/Follicle-Stimulating Hormone (LH/FSH) ratio.

Objective: Assess the clinical metabolic outcomes value of LH/FSH ratio, LH, FSH and Pr in productive-age women having polycystic ovarian syndrome after an average treatment period of three months with combined Metformin and Spironolactone.

Design: A retrospective pilot study.

Setting: Private Gynaecological Clinics, Baghdad, Iraq.

Patients and methods: We selected reproductive-aged women with PCOS who underwent enzyme-linked immunosorbent assay tests of FSH, LH, and Pr before and after treatment. Women with hyperprolactinemia, thyroid disease, Cushing's syndrome, and adrenal hyperplasia were excluded. We investigated patients, Pre-treatment and Post-treatment regarding Metformin (MET) 500 mg q.i.d/day and Spironolactone (SPR) 50 mg/day treatment.

Main outcome measure: LH/FSH ratio, LH, FSH, Pr, menstrual cycle, hirsutism and acne.

Sample size: Thirty.

Results: LH and Pr showed a highly significant decreased after combined treatment with MET and SPR when compared with Pre-treatment (P=.0001, .0004), respectively, Menstrual Cycle/Year showed a significant increase in Post-treatment (P=.0001) and no significant change in body mass index and FSH parameter when compared Post-treatment with Pre-treatment (P=.297, .296) respectively, and a highly significant decreased and improvement in hirsutism and acne symptoms in Post-treatment when compared with Pre-treatment (P=.0001, .0073) respectively.

Conclusion: LH, LH/FSH ratio, Pr, menstrual irregularity, hirsutism, and acne as metabolic outcomes of PCOS can be reversed to normal by using combined MET and SPR as a complementary effect for three months.

Keywords: Follicle-stimulating hormone; Luteinizing hormone; Metformin; Polycystic ovary syndrome; Prolactin; Spironolactone

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INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is a complex endocrine condition that a large number of reproductive-aged women throughout the world suffer [1]. It affects (10%) of women before menopause, leading to various complications [2]. Root problems with the hypothalamic-pituitary axis, insulin production and activity, and ovarian function define PCOS [3].

The root causes of PCOS are an elevated Gonadotropin-Releasing Hormone (GnRH) secretion rate and a high Luteinizing Hormone (LH)/Follicle-Stimulating Hormone (FSH) ratio [4]. Because PCOS is characterized by an imbalance in GnRH production, the LH/FSH ratio is used in the diagnosis procedure. Usually, a healthy woman's LH/FSH ratio will be between 1 and 2. However, in PCOS women, this ratio turns upside down, and it can go up to 2 or 3 in some cases [5].

The Hypothalamic-Pituitary-Gonadal (HPG) axis is controlled by the hypothalamic hormone GnRH and the pituitary hormones FSH and LH [6]. Both the negative feedback from oestrogen and progesterone, which triggers the pulsatile secretion of these hormones and the positive feedback from the periovulatory stage, which triggers ovulation in female mammals, are necessary for ovarian maturity and cyclic function during puberty and adulthood [7]. The gonadotropic cells in the anterior pituitary receive GnRH upon secretion. Once there, it binds to its receptor and induces the production and release of LH and FSH into the bloodstream [8]. Gonadal steroidogenesis and gametogenesis are regulated by the secretion of LH and FSH, two gonadotropins, in asynchronous patterns in response to varying pulse frequencies of GnRH [9]. The process of steroidogenesis involves transforming precursor cholesterol into steroid hormones that have biological activity. Follicle formation and maturation are significantly impacted by the differentiation of theca and granulosa cells, mainly in the ovary [10].

Follicle development relies heavily on the theca cell, which is responsible for many of the androgens produced by the ovaries, including testosterone and androstenedione. The androgens are used as building blocks by the granulosa cells to make oestrogens. One mechanism in which LH affects theca cells is by boosting the androstenedione biosynthesis pathway. FSH regulates the enzyme aromatase's ability to convert androstenedione to oestradiol by acting on the granulosa cells. Elevated levels of LH in PCOS patients lead to hyperandrogenaemia and

infertility in two ways: first, by rerouting biosynthesis to produce androgens, and second, by promoting theca cell hyperplasia [11-13]. Direct symptoms associated with hirsutism, acne, and alopecia are associated with elevated androgen levels [14].

This study compared with others that looked at PCOS women over longer periods (six months and a year) and found that anovulation, caused by an elevated LH, LH/FSH ratio and Prolactin (Pr) necessitates medical intervention, lowering androgen levels, and increasing the likelihood of pregnancy within an average of three months.

It has been demonstrated that the benefits of combination therapy who, Mazza, et al. [15] found that MET at dose 1700 mg/day if combined with SPR at dose of 25mg/day for six months improved hirsutism scores and reduced hyperandrogenism symptoms, along with metabolic parameters like LH and FSH.

However, in another study they used the same combination but in higher dose [16] of MET 1500 mg/day and SPR 40 mg/day but for short period of three month which improved insulin resistance and decline in testosterone levels in PCOS. The presence study will investigate the effect of treatment using a regimen (MET 2000 mg/day, SPR50 mg/day) for short period (three months). This approach allows for a more comprehensive assessment of hormonal changes, involving LH/FSH ratio, FSH, LH, and Pr, which are crucial for understanding PCOS pathophysiology. In addition, this study will have impact on clinically relevant symptoms like hirsutism and acne, providing insights into patient-centred outcomes.

We hope that this regimen will have an effective and safe treatment strategy that improves menstrual regularity and reduces hyperandrogenic symptoms, potentially enhancing fertility prospects for women with PCOS.

PATIENTS AND METHODS

A retrospective pilot study was conducted on 30 reproductive-aged women aged 17 to 35 years suffering from PCOS with a Body Mass Index (BMI) mean of 30.7 kg/m^2 who attended private Gynaecological clinics in Baghdad – Iraq between September 2022 to February 2023 were enrolled in the study. The participant flowchart is shown in Figure 1. A diagnosis of PCOS was made when a patient exhibited two or more of the following symptoms: (anovulation, oligomenorrhea, biochemical signs of hyperandrogenism (hirsutism or acne or both), positive ultrasound PCOS was around ten to twelve or more ovarian follicles measuring two to ten mm, with a pearl necklace appearance) [5] Inclusion criteria were (1) women need to be pregnant and suffer from infertility due to PCOS (2) age of $\geq$ 17 years. The exclusion criteria (1) hyperprolactinemia (2) thyroid disease (3) Cushing's syndrome, and (4) Adrenal hyperplasia should be excluded to establish a differential diagnosis for PCOS because they affect LH, FSH and androgen levels [17]

The study protocol was approved by the research ethics committee of University of Al-Esraa, College of Pharmacy (institutional registration number 1). The requirement for written informed consent was waived given the retrospective nature of the study.

As certain women are unable to tolerate the long-term effects of MET due to their gastrointestinal problem [18], subjects were undergone Metformin (MET) (Glucophage, Merck) 500mg q.i.d/day and Spironolactone (SPR) (Spironolactone, accord) 50 mg/day treatment for an average duration (3 months).(16) They were encouraged to follow their routine with a low-glucose diet and to increase physical activity for weight reduction.

The study collected laboratory data from 30 women suffering from PCOS who visited private Gynaecological clinics and were referred for further laboratory investigation in Almaamoon private laboratory in Baghdad – Iraq. Their samples were placed into a gel tube and followed by centrifugation at 4000 rpm (1252 X) g for 10 minutes; the separated sera were divided into tiny aliquots and stored at (-20 °C) until analyzed for the evaluation of LH, FSH and Pr. The biomarkers (LH, FSH and Pr) were measured before and after treatment using Enzyme-Linked Immunosorbent Assay (ELISA) kits obtained from the Cobas E411 automated analyser (Roche Diagnostics GmbH, Mannheim, Germany).

The Statistical Analysis System - SAS (2018) program detects the effect of treatment on study parameters. The student T-test compared the group for normally distributed continuous variables. The chi-square test was employed to compare categorical variables between Pre-treatment and Post-treatment. A P-value between (0.05 and 0.01) is considered for statistical significance.

RESULTS

Parameters comparison in reproductive-aged women between Pre- and Post-Combination Treatment shows in Post-Combination Treatment a highly significant reduction in Menstrual Cycle number/year, LH, LH/FSH ratio and Pr (P=.0001, .0001, .0001 and .0004) respectively, but there was no difference between Pre- and Post-Combination Treatment in BMI and FSH (P=.297 and .296) respectively **(Tab. 1.)**.

Distribution of sample study according to hirsutism and acne with different groups in reproductive-aged women before treatment, the percentage of hirsutism and acne is high (80%, 13.3%) respectively when compared to the same group of patients after Combination treatment (3.3%, 0%) respectively that means there was a highly significant reduction and improvement in these symptoms (P=.0001, .0073) respectively **(Tab. 2.)**.

DISCUSSION

The present study evaluated the metabolic outcomes changes of LH, LH/FSH ratio and Pr that were affected by two drugs in reproductive-aged women suffering from PCOS with a BMI mean of 30.7 kg/m^2. The subjects employed MET and SPR treatment and adjusted their lifestyle as the principal interventions.

The PCOS pathophysiology remains intricate. It is also unclear what causes PCOS. A combination of factors, including obesity, insulin resistance, and excess androgens, can lead to PCOS. The impact of oxidative stress (OS) is crucial to releasing these metabolic chemicals. Impaired energy metabolism in obese persons with PCOS may primarily manifest in mitochondria. Obesity can be caused by an increase in OS, which enlarges mature adipocytes and promotes the proliferation of preadipocytes [19] When women with PCOS have elevated amounts of Reactive

Tab. 1. Parameters comparison in reproductive-aged women between pre- and post-combination treatment.

	Mean ± SD		
	Pre-Treatment (n=30)	**Post-Treatment (n=30)**	***P*-Value**
Menstrual Cycle/Year	5.9 (3.07)	11.9 (0.8)	.0001 *
BMI (kg/m²)	30.7 (6.1)	29.2 (5.4)	.297 NS
FSH (IU/L)	6.0 (1.6)	5.7 (0.6)	.296 NS
LH (IU/L)	11.7 (3.7)	3.7 (0.4)	.0001 *
LH/FSH ratio	1.9 (0.4)	0.6 (0.08)	.0001 *
Pr (μg/L)	21.0 (10.6)	13.6 (2.2)	.0004 *
	* ($P \leq .01$)		
BMI = Body Mass Index * Highly Significant NS Not Significant			

Tab. 2. Distribution of sample study according to hirsutism and acne.

Factor		**Pre-Treatment (n=30)**	**Post-Treatment (n= 30)**	***P*-value**
Hirsutism	Yes	24 (80.0%)	1 (3.3%)	.0001 *
	No	6 (20.0%)	29 (96.6%)	
Acne	Yes	4 (13.3%)	0 (0.0%)	.0073 *
	No	26 (66.6%)	30 (100.0%)	
*($P \leq .01$)				
* Highly Significant				

Oxygen Species (ROS) in their ovarian Granulosa Cells (GCs), it can hinder mitochondrial oxidative metabolism, cause GCs to have aberrant shapes, lead to insufficient energy supply and increased OS, and significantly induce GC cell death. The quality of the oocyte and embryo is negatively correlated with elevated ROS levels in follicular fluid. Consequently, it is believed that infertility or poor pregnancy outcomes in PCOS women are caused mainly by changed oocyte quality or capacity [20].

MET is a hypoglycaemic medication that helps with PCOS by lowering androgen levels, improving insulin resistance, and increasing ovulation [21].

Research conducted by Sohrevardi, et al. in which fifty women were given 500 mg of MET q.i.d/day for three months corroborated the results shown in the current study (**Tab. 1.**), which indicated a highly significant decrease in blood LH levels after three months of therapy [22], on the other hand, this finding is consistent with a previous study by Fattah, et al. in which hundred women were given MET 500mg t.i.d/day for six months and had the same outcome [23,24].

Potential reasons for the pituitary gland's restoration of normal LH secretion encompass MET's ability to traverse the blood-brain barrier and access the hypothalamus and other cerebral regions, where it normalizes Adenosine Monophosphate activated Protein Kinase (AMPK)-dependent signalling in neurons secreting GnRH. The neurons that produce neurohormones such as kisspeptin, melanocortins, agouti-related peptide, neuropeptide Y, □-aminobutyric acid, and other biogenic amines are likely to be pharmacological targets for MET because these neuropeptides regulate the production of GnRH. To normalize the functioning of feedback loops in the HPG axis and prevent hyperandrogenism associated with PCOS, Restoring functional interactions between GnRH-expressing neurons and other components of the neural network responsible for hypothalamic axis regulation is essential.(24)

Additionally, as shown in **Tab. 1.**, after three months of treatment, the combined treatment response resulted in a highly significant reduction in the LH/FSH ratio. These findings align with a recent study conducted by Tao Long, et al. in which fifty-one women were treated with 500mg of MET daily and 40mg of SPR daily for the same duration of treatment [16].

There was no statistically significant improvement in BMI or FSH levels throughout therapy in this trial. Consistent with earlier work of three months by Sohrevardi SM, et al. [22] and by recent work of six months period by Ganie, et al. [25]. Consistent with our findings, the meta-analysis by Zeng, et al. found that the combination of MET with SPR had no more significant impact on FSH and LH than MET alone [26].

The current investigation revealed that around (23%) of women diagnosed with PCOS experience hyperprolactinemia. This finding aligns with the classification of PCOS as a causative factor for hyperprolactinemia, as indicated by the Endocrine Society Clinical Practice Guideline and supported by recent research studies [27]. Additionally, Pr inhibits the activity of the aromatase enzyme in granulosa cells and negatively affects folliculogenesis in the ovary; it is in line with the results of a previous study that found that these behaviours cause hypoestrogenism and anovulation, which are linked to oligomenorrhea and amenorrhea in women [11]. The results show that out of the 23 women whose serum Pr levels were normal, 8 had an abnormally high LH/FSH ratio. The production of Pr may be stimulated by oestradiol, as women with hyperprolactinemia have elevated levels of this hormone, according to a recent research by Davoudi, et al. [28]. In a research conducted by Ali D-e-S et al., fifty-three women were given 500mg of MET b.i.d/day for three months. The results revealed a substantial drop in Pr levels, consistent with our findings [29].

Like our findings, Ganie et al. found that hirsutism and menstrual cycle frequency were both improved in sixty-

two women with combination therapy of MET 500mg b.i.d/day and SPR 50mg/day, and these improvements were highly significant and achieved in twice the time as long as our trial [25].

The administration of SPR 50mg/day effectively inhibits the biosynthesis of steroids. Irregular menstruation, acne, and hirsutism are some of the high androgen-induced symptoms that have led to its use in the treatment of PCOS. According to the results in **Tab. 1.**, women who had SPR therapy saw a significant improvement in menstrual irregularity. The results of the SPR therapy also showed a considerable reduction in acne and hirsutism in women, as shown in **Tab. 2.** These findings agree with the conclusion of Rajashekar, et al., who conducted a systematic review [30]. By simultaneously blocking androgen production and receptor binding, SPR improves hyperandrogenism [26].

Since it did not produce any significant menstrual abnormalities to impact patient compliance, the current study shows that the medicine is more acceptable in women with PCOS when used as a combination of low-dose SPR 50mg/day and MET 500mg q.i.d/day [25].

Some limitations of the present study include the recommendation for a larger sample size to improve the BMI approach. However, the study's sample size was sufficient to show significant results in other variables. In addition, this trial was done in two clinics, and the fact that MET's gastrointestinal side effects make long-term treatment inappropriate for some people necessitates supervision.

CONCLUSION

In conclusion, the current study demonstrated that the combination therapy had the same or better results on androgens after three months compared to a longer duration. A complementary effect may occur when MET and SPR are combined, leading to improved LH levels, LH/FSH ratio, Pr, menstrual irregularity, hirsutism, and acne in PCOS patients than each treatment alone. As a result, to reverse metabolic outcomes in women with PCOS, we propose a combination therapy of MET 500mg q.i.d/day with SPR 50mg/day for future studies.

LIMITATIONS

We recommend a large sample size for the body mass index approach and MET gastrointestinal side effects limitation use.

CONFLICT OF INTEREST

None.

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