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The effect of a combination therapy of spironolactone and metformin on women with polycystic ovary syndrome

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ABSTRACT

Many women of childbearing age are affected by polycystic ovary syndrome (PCOS), which is a complex endocrine disorder that has no one ideal treatment. This research aimed to assess the effect of the combination therapy of metformin and spironolactone on the levels of hormones and some PCOS symptoms. This study is a prospective study that conducted on 60 females with PCOS recruited from an Obstetrics and Gynecology private clinic in Baghdad, Iraq, between December 2022 and May 2023 who received a combination therapy of metformin and spironolactone for at least 2 months. Blood samples were taken for the assessment of LH, FSH, and prolactin levels, as well as other PCOS symptoms before and after receiving the treatment for ≥ 2 months. Results showed that LH and prolactin levels were significantly reduced with a significant decrease in duration of the menstrual cycle absence to approximately 30 days, and a significant reduction in the number of cases that suffer from hirsutism after receiving treatment in comparison with the prevalence of hirsutism before treatment. In conclusion, spironolactone in combination with metformin showed to improve hirsutism and the menstrual cycle frequency as it caused an improvement in the levels of LH, FSH, and prolactin in PCOS patients.

Keywords: metformin, polycystic ovary syndrome, spironolactone

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a complex endocrine disorder that affects a sizable fraction of reproductive-aged women around the world[1]. Ovarian enlargement and dysfunction, increased androgen levels, insulin resistance, and other factors are often seen in patients with this syndrome[2]. About 10% of women have polycystic ovary syndrome (PCOS) before menopause[3] and many of these women have to deal with the difficulties associated with this condition. Although it is known that an increased ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH) and increased frequency of gonadotropin-releasing hormone (GnRH) are contributing factors in polycystic ovary syndrome (PCOS)[4,5], the etiology and pathophysiology of PCOS remain poorly understood. Insulin resistance (IR), hyperandrogenism (HA), environmental factors, genetic factors, and epigenetic mechanisms are just a few examples of what we know to play a role in determining outcomes. Importantly, polycystic ovary syndrome (PCOS) has been linked to an increased risk of other health problems, including metabolic syndrome, type 2 diabetes mellitus, cardiovascular disease[5, 6] as well as melancholy and anxiety[7].

Considering the increasing prevalence of polycystic ovary syndrome (PCOS) and its associated problems, as well as the limited efficacy of current treatments and drugs, it is imperative to thoroughly explore the underlying mechanisms of PCOS and identify novel pharmacological targets[3]. The primary pharmacological treatments utilized in the management of polycystic ovary syndrome (PCOS) encompass various drug classes. These include oral contraceptive pills, insulin sensitizers such as metformin and pioglitazone, anti-androgens like spironolactone, cyproterone acetate, finasteride, and flutamide, as well as ovulation inductors such as gonadotropin-releasing hormone analogues and clomiphene. Those drugs have been used for different expectations of the patients with PCOS[8,9]. Since insulin resistance and hyperandrogenemia are among the main interacting causes, as well as overproduction of luteinizing hormone (LH) and androgenic hormones, in PCOS[10], a combined approach utilizing insulin sensitizers and anti-androgens may offer synergistic benefits in the management of PCOS.

Researches have demonstrated that metformin has the ability to reduce insulin resistance, augment the number of cycles in individuals diagnosed with polycystic ovary syndrome (PCOS), and maybe ameliorate hirsutism to a limited extent in certain people[11-13]. The administration of spironolactone has been shown to improve hyperandrogenism through its dual mechanism of action, which involves the inhibition of both androgen production and receptor-bind-

ing[14,15]. Therefore, in theory, the combination of metformin and spironolactone therapy has promise as a potentially successful and safe treatment option for polycystic ovary syndrome (PCOS). One could postulate that the concurrent administration of metformin and spironolactone may yield superior efficacy compared to individual medication usage, owing to their mutually reinforcing modes of action.

A recent study conducted in India demonstrated that the combination of metformin and spironolactone yielded superior results compared to either drug used individually. Specifically, this combination exhibited improvements in various factors including the number of menstrual cycles, Ferriman-Gallwey score (FGS), serum total testosterone (T) levels, and area under curve (AUC) measurements for glucose and insulin. However, it did not show significant effects on body mass index (BMI), homeostasis model assessment of insulin resistance (HOMA-IR) index, and blood pressure[10]. The objective of our study was to examine the effects of this combination therapy on some hormonal levels and several features of PCOS.

METHODOLOGY

Study protocol

A prospective study was done on 60 female patients with polycystic ovarian syndrome (PCOS) who were recruited from Obstetrics and Gynecology private clinic, Baghdad, Iraq, between December 2022 and May 2022. Ages of the patients ranged between 17 and 35 years (mean ± SD 24.83 ± 4.27 years). The practical part of the study was conducted at private laboratory in Baghdad, Iraq. The diagnosis of PCOS was based on Rotterdam Revised criteria (2003)[3,16]. Those individuals who meet at least two of the following criteria were included in this study: oligo- or anovulation, such as amenorrhea (absence of menstruation >180 days) or oligomenorrhea (menstrual periods occur at intervals of >35 days), and clinical and/or biochemical signs of hyperandrogenism. Polycystic ovaries on ultrasound are defined as an ovary containing 12 or more follicles measuring 2–9 mm in diameter or an ovary that has a volume of greater than 10 ml. Patients received metformin (1700 mg/day), spironolactone (100 mg/day) and a hypocaloric diet and the levels of studied markers were measured before and after receiving the treatment.

Exclusion criteria

Pregnancy was ruled out by human-chorionic gonadotropin measurement[17]. Normal thyroid function was established by hormonal evaluation because hypothyroidism cause an increase in the prolactin levels which in turn inhibits

gonadotrophins that lead to a reduction in the levels of serum FSH and serum LH[18]. Late-onset nonclassical congenital hyperplasia was excluded by values of basal 17-hydroxy progesterone less than 2 ng/ml. Cushing syndrome was also ruled out by hormonal evaluation. Patients who administered drugs included oral contraceptives, antihypertensive agents, anti-diabetic drugs, and agents for weight loss were also ruled out from this study as they interfere with the treatment used.

Anthropometric tests

The only anthropometric parameter specified in the study was BMI calculated as:

BMI = weight (kg) / height square (m^2)

All subjects were weighed on the same scale, barefoot. Height was measured using the same measuring tape.

Methods

Patients who had fasted for 12 hours had five milliliters of blood drawn for the initial estimation of the studied markers with a clinical examination that included the presence of acne and hirsutism were recorded in addition to the age, BMI, and marital status that were obtained from all patients subjected to the current study. The collection of blood samples was repeated after 2-5 months of receiving the combination therapy, in addition to examining the patients for acne and hirsutism in addition to their BMI. After letting the samples clot for 30 minutes at room temperature, they were centrifuged at 4000rpm (1252 x g) for 10 minutes to separate the serum. The sera were then aliquoted and stored at -20°C until they could be evaluated for LH, FSH, and Prolactin using the enzyme-linked immunosorbent assay (ELISA).

Limitation

We faced a problem in convincing the patients to subject to the Ferriman-Gallwey scoring system for hirsutism, therefore, we replaced it by asking patients about their hirsutism condition.

Statistical analysis

The research data were entered into a Microsoft Excel spreadsheet, and then analyzed using SPSS 20 and Excel (2016). All numerical variables were presented as means and standard deviations. Statistical significance was set at a P value of ≤0.05 for all t-test and ANOVA comparisons. Cross tabulation was used to determine the frequency and percentage of each categorical variable

across the different groups. All relationships between parameters were analyzed using either the Pearson correlation test for numerical parameters[19] or the Chi-square test for categorical variables.

RESULTS and DISCUSSION

Some demographic characteristics of the studied groups were summarized in Table 1 and Figures 1 and 2 which showed non-significant differences in the age and the body mass index (BMI) between single and married patients. Results also revealed that the majority of cases were overweight to obese.

Table 1. Demographic characteristics of PCOS patients: a comparison between single and married patients

	Total patients	Single	Married	p-value
n	60	32	28	-
Age (year) (mean ± standard deviation)	26.3 ± 5.27	25.75 ± 5.11	27.21 ± 3.83	0.3
BMI (kg/m^2) (mean ± standard deviation)	30.79 ± 6.18	30.49 ± 7.44	31.13 ± 4.6	0.78

Table 2 and Figure 3 revealed that the level of LH and Prolactin were reduced significantly after treatment with metformin and spironolactone for at least 2 months. It was also noticed that the duration of the absent of menstrual cycle reduced dramatically from about 80 days to approximately 30 days. On the other hand, a non-significant reduction in BMI and the levels of FSH were obtained while the duration of menstrual bleeding where a non-significantly increased from average of 5.5 days to about 6 days after receiving the treatment.

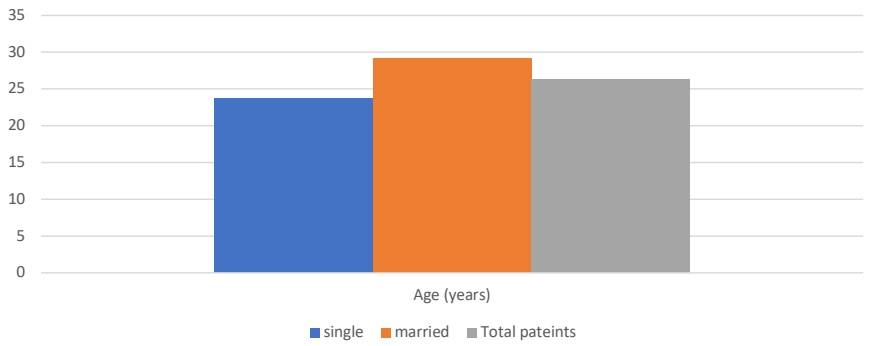

Figure 1. Averages of age in all studied groups

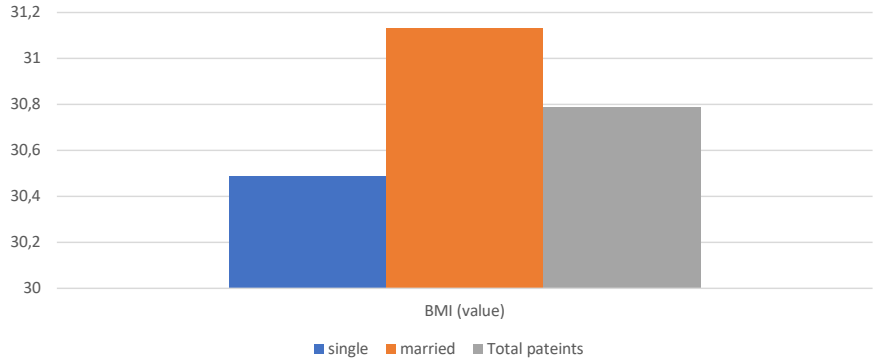

Figure 2. Averages of BMI in all studied groups

Table 2. Levels of LH, FSH, Prolactin and BMI with the duration (in days) since the last menstrual period and the duration of menstrual bleeding in patients before and after receiving the treatment

	Before treatment	After treatment	P value
LH (mIU/mL) (Mean ± SD)	11.7 ± 3.79	3.8 ± 0.46	<0.001
FSH (mIU/mL) (Mean ± SD)	6.06 ± 1.67	5.72 ± 0.68	0.3
Prolactin (ng/mL) (Mean ± SD)	21.07 ± 10.61	13.62 ± 2.28	<0.001
Duration since the last menstrual period (days) (Mean ± SD)	80.13 ± 44.01	30.6 ± 2.34	<0.001
Duration of bleeding in the last period (days) (Mean ± SD)	5.5 ± 1.91	6.07 ± 0.789	0.14
BMI (kg/m2) (Mean ± SD)	30.79 ± 6.18	29.2 ± 5.48	0.3

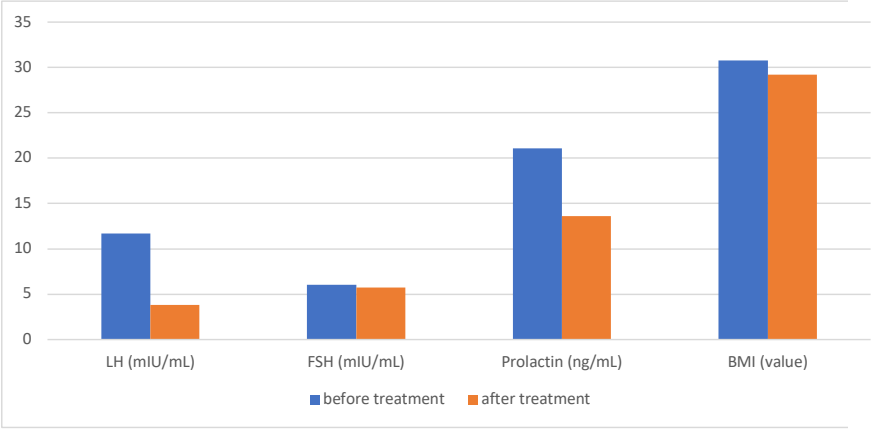

Figure 3. Averages of LH, FSH, Prolactin and BMI levels in all studied groups

Results illustrated in Table 3 and Figures 4 and 5 showed that the prevalence of hirsutism was significantly reduced in patients who received the combination therapy in comparison with the prevalence of hirsutism before treatment in that about 80% of patients were suffered from hirsutism before treatment which reduced to only 3.3% of patients after receiving the treatment for at least two months. On the other hand, the prevalence of acne was reduced non-significantly which may be owned to that the number of cases which suffer from acne were only four cases who recovered after receiving treatments.

Table 3. Prevalence of hirsutism and acne in PCOS patients before and after receiving treatments

Yes No			Hirsutism		Acne	
			Yes	No	Yes	No
Groups	Before treatment	Count	24	6	4	26
		% within Groups	80.0%	20.0%	13.3%	86.7%
	After treatment	Count	1	29	0	30
		% within Groups	3.3%	96.7%	0.0%	100.0%
Chi^2 value			<0.001		0.056	

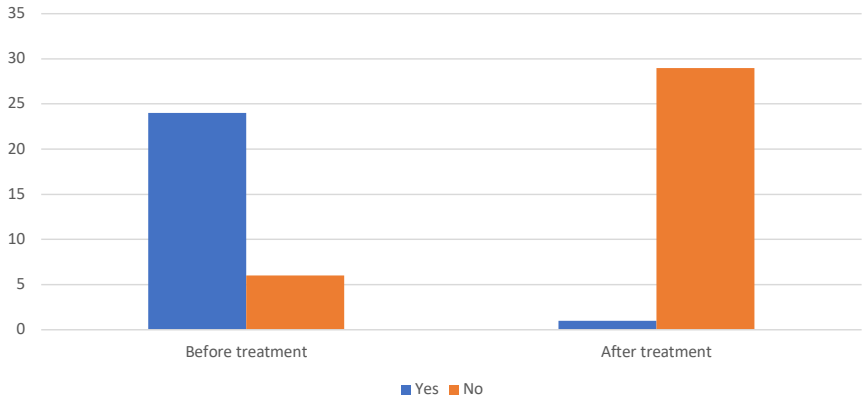

Figure 4. Prevalence of hirsutism in PCOS patients before and after receiving treatments

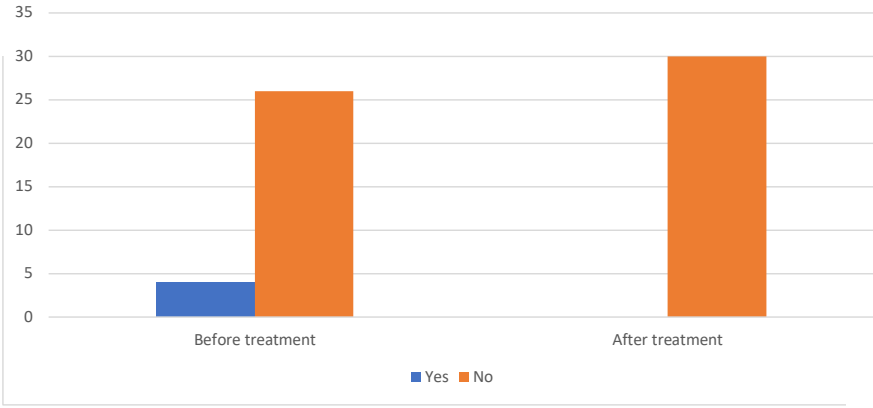

Figure 5. Prevalence of acne in PCOS patients before and after receiving treatments

Table 4 revealed that there were significant positive (direct) correlations between FSH and the levels of both LH (r=0.669, p<0.001) and prolactin (r=0.405, p=0.026) in PCOS patients before receiving the treatment. Moreover, levels of LH also showed a positive significant correlation with the duration of time since the last menstrual period (r=0.381, p=0.038). In contrast, all other parameters showed non-significant correlations with each other in these patients before receiving the treatment.

Table 4. Correlations between all studied parameters in PCOS patients before treatment

		FSH	LH	Prolactin	Duration since the last menstrual period	Duration of bleeding in the last period	BMI
Age	r	- 0.082	0.081	0.092	- 0.012	- 0.070	- 0.228
	p	0.667	0.669	0.629	0.948	0.712	0.226
FSH	r		0.650**	0.405*	0.119	- 0.285	0.033
	p		<0.001	0.026	0.530	0.126	0.862
LH	r			0.312	0.381*	- 0.069	- 0.214
	p			0.093	0.038	0.719	0.257
Prolactin	r				- 0.018	- 0.154	0.189
	p				0.925	0.415	0.318
Duration since the last menstrual period	r					0.279	0.079
	p					0.135	0.677
Duration of bleeding in the last period	r						- 0.110
	p						0.563

As demonstrated in Table 5, the levels of LH were positively and significantly correlated with duration of receiving treatment (r=0.513, p=0.004) whereas all other parameters showed non-significant correlations with the duration of receiving combined therapy. Moreover, FSH showed a positive and significant correlation with LH (r=0.388, p=0.034) in PCOS patients receiving treatment whereas Prolactin levels were negatively (inversely) significantly correlated with the duration of menstrual bleeding in the last menstrual period (r=-0.463, p=0.01). It was also demonstrated that the duration of treatment non-significantly correlated with levels of FSH and prolactin and the results also showed that in spite of the improvement of menstrual frequency it didn't correlate to the levels of FSH, LH and Prolactin. The bleeding duration in each menstrual period showed a non-significant correlation with the levels of LH and FSH that assessed in this study and also showed a non-significant correlation with the duration of receiving the combined treatment. All other parameters were non-significantly correlated with each other.

Table 5. Correlations between all studied parameters in PCOS patients after treatment

		Duration of treatment (days)	FSH	LH	Prolactin	Duration since the last menstrual period	Duration of bleeding in the last period	BMI
Age	**r**	- 0.110	- 0.003	0.115	0.209	0.111	- 0.113	- 0.234
	p	0.564	0.988	0.544	0.268	0.561	0.551	0.213
Duration of treatment (days)	**r**		0.029	0.513	- 0.192	- 0.213	- 0.101	0.025
	p		0.878	0.004	0.310	0.258	0.597	0.896
FSH	**r**			0.388	0.091	0.118	- 0.314	0.169
	p			0.034	0.632	0.534	0.091	0.373
LH	**r**				- 0.155	- 0.006	- 0.037	0.211
	p				0.413	0.975	0.845	0.264
Prolactin	**r**					0.199	- 0.463	0.191
	p					0.292	0.010	0.313
Duration since the last menstrual period	**r**						- 0.154	0.187
	p						0.417	0.322
Duration of bleeding in the last period	**r**							- 0.140
	p							0.460

Polycystic ovary syndrome (PCOS) represents the prevailing manifestation of World Health Organization (WHO) type II anovulatory infertility, commonly accompanied by hyperandrogenemia. Additionally, it is worth noting that this particular endocrine anomaly is prevalent among women of reproductive age. The use of the new Rotterdam diagnostic criteria[3], has revealed a greater prevalence of polycystic ovary syndrome (PCOS) (11.9 ± 2.4%) compared to the prior estimates provided by the National Institute of Health (10.2 ± 2.2%) and the Androgen Excess Society (8.7 ± 2.0%). Insulin resistance and hyperandrogenemia are recognized as prominent contributing factors along with excessive production of luteinizing hormone (LH) and androgenic hormones in the development of polycystic ovary syndrome (PCOS), Consequently, a therapeutic approach combining insulin sensitizers and antiandrogens may offer synergistic benefits in the management of PCOS[17].

Research has demonstrated that metformin has the capacity to enhance insulin resistance, augment the frequency of menstrual periods in individuals diagnosed with polycystic ovary syndrome (PCOS). Previous literatures also proposed that metformin maybe ameliorate hirsutism in certain people. A multitude of studies have been conducted to investigate the clinical effectiveness and safety of various medication combinations for the treatment of polycystic ovary syndrome (PCOS). Nevertheless, the number of studies evaluating the clinical and laboratory effectiveness of the combined use of spironolactone and metformin is somewhat limited[17]. Our hypothesis posits that the combination of metformin and spironolactone would yield improved results compared to using either drug alone. This is based on the understanding that both medications are commonly employed to address certain components of the condition in question[20].

In the current study, the levels of LH and prolactin were reduced significantly ($p<0.001$ for both) in patients receiving the combined therapy of metformin and spironolactone, as illustrated in Table 2. Given that patients subjected to the current study received the treatment for a variable duration ranging from 2–10 months with an average of 101 days, this explains the heterogeneity of the results obtained. Regarding LH levels, results demonstrated in the present study were consistent with previous studies conducted by Abd Elaal et al. in 2020[17], who investigated the effect of combined therapy on 20 PCOS patients for 6 months, whereas Ganie et al. (2013)[10] and Diri et al. (2016)[20] demonstrated a non-significant change in the levels of LH in patients subjected to their studies.

The significant reduction in prolactin levels was in agreement with several previous studies[21,22] and evidence from these studies suggests that metformin has a

significant impact on pituitary function in PCOS women, as evidenced by changes in LH and, in ovulatory responders, prolactin PRL secretion dynamics after intervention[21]. As a consequence, it increases the number of cycles in PCOS patients, as indicated by the dramatic reduction in the period ($p<0.001$) since the last cycle that is presented in Table 2, which is consistent with previous literature[17,20].

In accordance with previous literature, Table 3 revealed that patients subjected to the current study showed a significant ($p<0.001$) improvement in hirsutism as a consequence of restoring the levels of LH, FSH, and prolactin. In the current study, about all subjects experienced a reduction in hirsutism, given that in our study the patients were asked about hirsutism and not as in previous studies that conducted a hirsutism score. Ganie and his co-workers reported in 2013 reported that the combination of metformin (1000 mg/d) and spironolactone (50 mg/d) was better than either drug alone for improving the number of menstrual cycles and hirsutism score, which is completely compatible with the current research findings[10].

Correlation studies revealed that in patients with PCOS, the levels of FSH were directly and significantly correlated to the levels of LH ($p<0.001$) and prolactin ($p=0.026$) before receiving treatments, as illustrated in Tables 4, which is a classical finding that has been approved since 1978[23,24]. Before receiving treatments, patients showed a significant positive correlation between LH levels and the duration of the last menstrual period ($p=0.038$), as shown in Tables 4. Increasing the durations of the menstrual cycle indicates a decrease in the number of cycles, so, the high ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH) and the increased frequency of gonadotropin-releasing hormone (GnRH) which are known as the underlying causes of PCOS involved in the elongation of the menstrual cycle period that reduce its frequency[3].

It was demonstrated in Table 5 that after at least two months of treatment, the correlation studies showed that the LH-FSH positive significant ($p=0.034$) association persists, which may indicate that the reduction in the levels of both hormones run in parallel to each other. However, it was noticed that the correlation before receiving treatment was more significant ($r=0.65$; $p<0.001$) whereas the correlation after receiving treatment was less significant ($r=0.388$; $p=0.034$). The reduction in the significancy of the LH-FSH correlation might be caused by the fact that the reduction in LH levels were more pronounced than the reduction in FSH levels, which also led to a lower LH/FSH ratio, which is mentioned above as one of the underlying causes of PCOS, i.e., using this combination therapy affect the levels of LH to higher extent than FSH which may be contributed to that reduction in the significancy of their correlation[3].

Additionally, results illustrated in Table 5 showed that a significant positive correlation (p=0.004) was obtained between the levels of LH and the duration of treatment. It was also noticed that patients treated with this therapy for more than 3 months showed a slight increase in LH levels that may be caused by the poor compliance of patients to the therapeutic regime after a long term of treatment and the observed improvement while patients receiving treatment for less than three months showed a higher compliance rate. Furthermore, results presented in Tables 5 revealed that a significant negative (p=0.01) correlation was obtained between prolactin and the duration of menstrual bleeding, which is also compatible with the previous literature, which reported that high levels of prolactin cause menstrual abnormalities[25].

In conclusion, spironolactone in combination with metformin was effective on hirsutism and also caused an increase in the menstrual cycle frequency as it caused an improvement in the levels of LH, FSH, and prolactin in our patients with PCOS. The most significant result of the study was that when spironolactone was combined with metformin, both drugs were more effective and more likely to be taken as prescribed. One possible explanation for these oligoammenorrhoic women's improved adherence is the combination's enhanced efficacy in restoring regular menstrual cyclicity. The relatively small sample sizes of the treatment groups in the present study are a significant limitation that necessitates additional research using a larger patient population.

STATEMENT OF ETHICS

The study has approved by the Institutional Review Board (IRB) of the Department of Pharmacy, Al-Yarmouk University College, Diyala, Iraq (YUC3982-2022).

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

Design – Khadir FK, Ajeed AM; Acquisition of data – AlSahaf DM; Analysis of data – Ajeed AM; Drafting of the manuscript – Khadir FK, AlSahaf DM; Critical revision of the manuscript– Ajeed AM; Statistical analysis– AlSahaf DM; Technical or financial support– Khadir FK, Ajeed AM; supervision – Khadir FK.

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