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Effect of Metformin and Its Combination with Probiotic on Menstrual Irregularity and TSH Levels in Patients with Polycystic Ovarian Syndrome: A Randomized Controlled Trial

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Authors' contributions

All of the authors worked together to complete this project. The study was designed by author UZ, who also wrote the protocol and the first draft of the manuscript, as well as the sampling and statistical analysis. The authors NI and FM were in charge of the literature searches and final drafting. Author FQ assisted with the manuscript writing. Authors ANB and MM completed all of the final settings and facilitated with the statistical analysis. The final manuscript was read and approved by all of the authors.

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ABSTRACT

Background: Polycystic ovarian syndrome (PCOS), the far most common endocrine disorder among sexually active women. The disease is typically characterized by irregular menstrual cycles and appears to be influenced by associated thyroid dysfunction.
Aim: The goal of this research was to assess and compare the effects of Metformin and its combination with Probiotic on monthly cycle irregularity and TSH levels in PCOS women.

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Methodology: This was a single-center study conducted at Karachi's Gynecological Outpatient department from January 2019 to September 2019. A total of 52 PCOS patients aged 18 to 40 years were included in this trial, which followed Rotterdam criteria. After providing written and consent form, individuals were randomized into one of the two groups and received Metformin 500 mg TD (n = 26) or Metformin Combination (n = 26).
Results: After three months of treatment, both groups improved in terms of menstrual cycle irregularity and TSH levels, but the combination treatment improved the most.
Conclusion: Probiotics may be considered in conjunction with Metformin for improving TSH levels to achieve better results.

Keywords: Menstrual irregularity; TSH levels; polycystic ovarian syndrome.

Keywords

PCOS : Polycystic Ovarian Syndrome
TSH : Thyroid Stimulating Hormone
OD : Once Daily
BD : Twice Daily
TD : Thrice Daily
GI : Gastrointestinal

1. INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a commonly diagnosed reproductive disorder and one of the leading causes of ovulatory infertility. PCOS is well-defined by the Rotterdam criteria as a combination of oligo / amenorrhea, clinical or biochemical signs of hyperandrogenism, and polycystic ovary on ultrasonography [1]. Being a heterogeneous disorder, it affects many body functions, leading to a variety of health complications such as infertility, menstrual dysfunction, androgenic symptoms, metabolic syndrome, and autoimmune disease.

Thyroid autoimmunity is associated with an increased risk of infertility, spontaneous miscarriage, and metabolic dysfunctions, all of which are common in PCOS [2,3]. In women with PCOS Subclinical hypothyroidism (SCH) and thyroid autoimmunity are more common than in women of the general population [4,5]. Recent studies have explored the relationship between thyroid autoimmunity and metabolic parameters in PCOS, particularly dyslipidemia and insulin resistance[6,7].

Thyroid hormones can act as insulin agonists in muscle and antagonists in the liver, so thyroid deficiency may result in a decline in glucose production and utilization [8,9]. Moreover, there are studies that have identified insulin resistance(IR),as a primary factor in the pathogenesis of PCOS, which might also play a keyroleindevelopinghypothyroidism [10,11].

The most frequently recommended drug for the treatment of insulin resistance is Metformin. It is considered a safer drug, with no clinically relevant pharmacologic interaction that has been described yet [12]. In the recent years, there has been a growing body of evidence supporting Metformin's beneficial effects in PCOS [13]. Multiple studies resulted in consensus statements and recommendations for Metformin use in PCOS patients[14,15].

Spite of the fact, that Metformin was instituted in clinical practice approximately five decades ago for the management of diabetes mellitus [16] however, its effect on clinically significant menstrual cycle irregularity was first reported in 1994 due to improvements in insulin resistance [17]. This was only lately, that this drug was observed to modify the thyroid hormone profile resulting in a substantial reduction of serum TSH levels [18,19].

There are number of adverse effects associated with Metformin which are commonly GI in nature: diarrhea, nausea, and/or abdominal discomfort. These effects are usually mild, transitory, and dose-related, but then they can arise in up to 50% of patients taking the drug [20,21]. And, even at low dosages, about 5% of individuals can't endure the medication [20].

Because of the increasing likelihood of side effects, the use of this drug has been limited. As a result of the challenges, dietary approaches are becoming more popular for managing different diseases [22].

Considering that, Probiotics emerge as effective nutritional supplements because of their possible future benefits to human health or preventative medicine [22]. Probiotics are characterized as live microorganisms that, when ingested in adequate amounts, present a

health beneficial effect on the host [23]. They are not delegated drugs under current worldwide law. Yet, they are still found to reduce pathogenic bacterial colonization in the intestinal tract, improve physiological gut barrier function, and impact the production of pro-inflammatory and anti-inflammatory cytokines [23-25].

Moreover, there seems to be currently little scientific proof of Probiotics interacting with concurrent drug ingestion, attributed to their impact on bacterial enzymatic activity. Given the popularity of probiotic-containing products, it is critical to identify any potential interactions with pharmacological therapies [26,27].

In view of above circumstances, prescriptions for insulin-sensitizing drugs, most notably Metformin, are not common among hypothyroid patients however, combination of Metformin and Probiotic have also not been used previously. Therefore, the purpose of this study is to directly investigate the effect of Metformin, alone or in combination with Probiotic, on the management of menstrual irregularities and TSH levels during PCOStreatment.

2. MATERIALS AND METHODS

2.1 Trial Design and Participants

It's an open label, randomly allocated, parallel-arm, and non-inferiority research in which 52 new participants with established PCOS, aged 18 to 40 years, were registered. This single-center cross-sectional study was conducted on women attending a gynecological clinic at a hospital in Karachi.

The participants were listed via convenient sampling technique between January 2019 to September 2019. It was the non-probability sampling technique so we cannot rule out the chancesofbiasness

The sample size was calculated by Sealed Envelope calculator version 201: (Significance level (alpha) 1%, 99% confidence interval Power (1-beta) 90, Percentage success in control group: 12%.

2.2 Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

2.3 Inclusion Criteria

Newly diagnosed PCOS patients were enrolled according to the Rotterdam criteria. According to the criteria, two of the following three characteristics are required to detect PCOS cases:

- Oligo-ovulation or anovulation: (Oligomenorrhea i.e. more than 45 days or less than 8 cycles per year and Amenorrhea i.e. more than 3 months in women with previous periodic menses) for a period of 6 months.
- Hyperandrogenism: clinical (including signs such as hirsutism) or biochemical (including a raised free androgen index or free testosterone) hyperandrogenism.
- Polycystic ovaries on ultrasonography: >12 follicles in one or both ovaries, 2-9 mm in diameter and/or increased ovarian volume >10m^3).

2.4 Exclusion Criteria

- Consent is missing or has been withdrawn
- Other than PCOS-related hyperandrogenism (Cushing's syndrome, hyperprolactinemia, adrenal tumors, congenital adrenal hyperplasia, rare genetic disorders)
- Pregnancy or breastfeeding period (first 6 months after giving birth)
- Allergies in relation to research procedures
- Any cancers that required treatment within the previous three years prior to the study procedures
- Any other chronic disease that necessitates medical examinations or hospitalization at least once every three months (exception: diabetes mellitus type 2)
- Type 1 diabetes mellitus
- Anti-diabetic and other drug therapy within the six months preceding the study procedures.

2.5 Protocol of the Study

Before obtaining informed consent, the study protocol was carefully described to all participants. Following the agreement, patients were randomized into two treatment groups and prescribed accordingly: group A: tablet Metformin 500mg T.D (n = 26) and group B (Met/Pro group): combination of Metformin

500mg B.D and Probiotic 180mg O.D (n = 26) for a 12-week period.

Lactobacillus acidophilus (1 X 10^9 CFU/g), *Lactobacillus delbruekii* (1 X 10^9 CFU/g), *Bifidobacterium bifidum* (1 X 10^9 CFU/g), *Lactobacillus bulgaricus* (1 X 10^9 CFU/g), and *Streptococcus Thermophilus* (1 X 10^9 CFU/g) were included in the Probiotic capsule.

Menstrual cycle and serum TSH levels were evaluated before and 3 months after starting Metformin and its combination with Probiotic treatment. Blood samples for assaying TSH were drawn between 08:00 and 09:00 a.m., after an overnight fast.

2.6 Statistical Analysis

SPSS 20 was used to analyze the data. The numeric factor was represented as mean standard deviation, while the categorical variable was represented as frequency and percentage. Normal distribution was verified by using the Shapiro–Wilk test. The paired-t test was used to compare the pre and post result of all numerical parameter. The Independent Samples t Test was used to test the differences between the means of two groups. A *p*-value of 0.01 was deemed statistically significant.

3. RESULTS

At first, 60 patients were able to enroll in this trial; however, 8 participants were excluded, including those who refused to sign the consent form (n= 5) and those who did not return for follow-up (n= 3). As previously stated, all eligible participants were randomly assigned to one of two interventional groups: Metformin and Combination (Met/Pro) (Fig. 1).

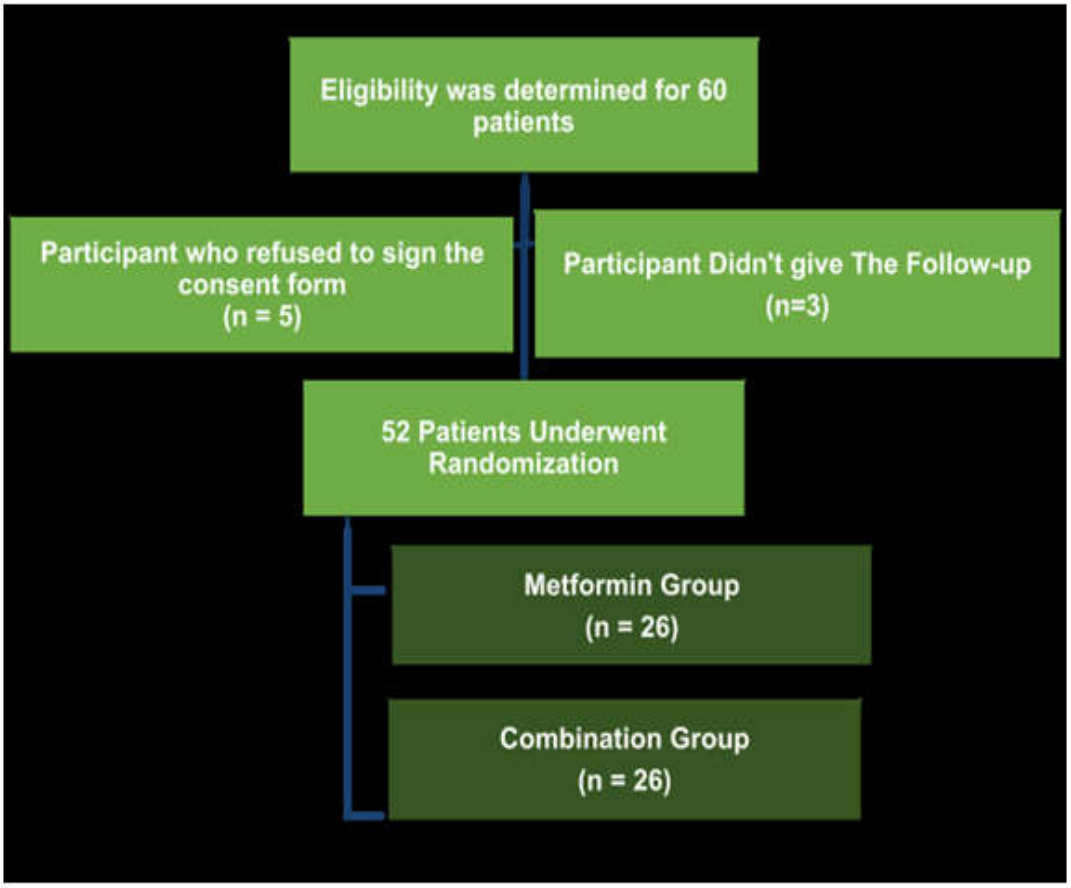

Fig. 1. Schematically depicted flow of patients

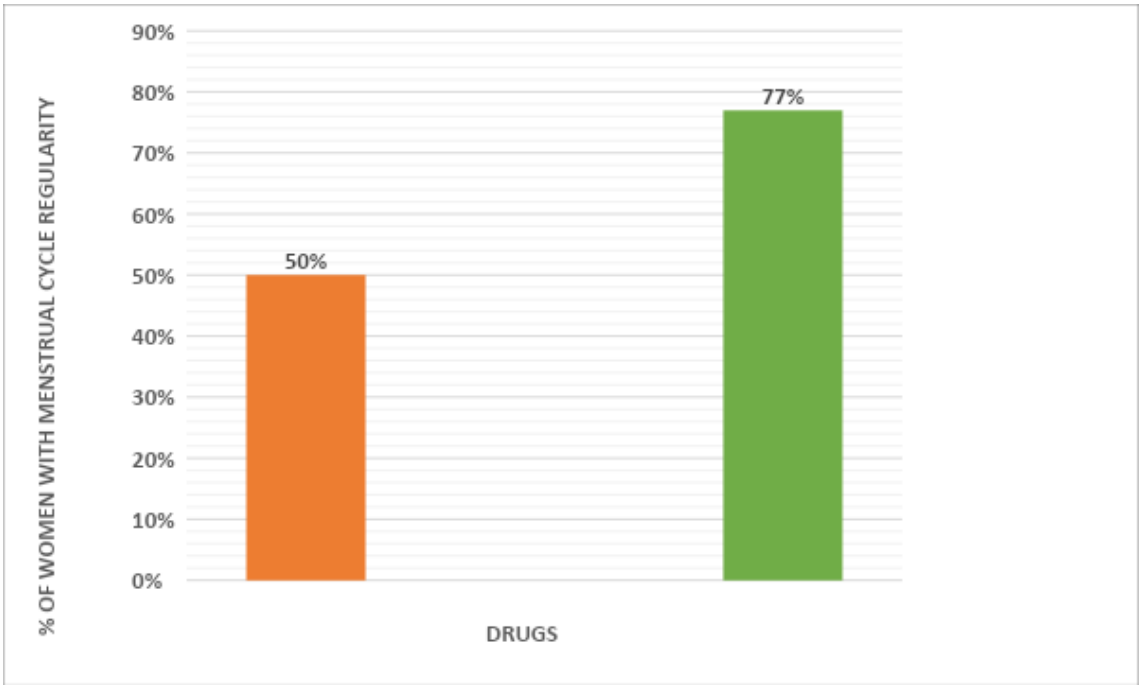

Fig. 2. Effect of Metformin and its Combination with Probiotic on Improving Menstrual cycle

Table 1. Effect of Metformin and its combination with Probiotics on TSH levels

Thyroid-Stimulating Hormone (TSH)			
Drug Treatment	**Metformin**	**Combination (Metformin + Probiotic)**	***p*-value**
Mean age	27.2 ± 4.6	25.1 ± 5.3	0.134
Before Treatment	2.23 ± 1.13	2.02 ± 0.88	0.736
After 12 weeks Treatment	1.85 ± 0.89	1.45 ± 0.55	0.163
p-value	0.005	0.000	

Menstrual Cycles: The 26 patients in both groups had irregular menstrual cycles at the start of the study. The current study found that symptoms improved in 13 women in the Metformin group (50%) and 20 women in the Met/Pro group (77%) after 12 weeks of treatment (Fig. 2).

The mean age of the patient in the Metformin group and Combination group is 27.2 ± 4.6 and 25.1 ± 5.3 respectively**.** Both the groups showed no significant change in the age.

At the start there was no significant difference between the two treatment groups. The TSH levels were decreased significantly after 12 weeks of intervention in both the treatment groups. The Combination group showed more prominent results than Metformin alone however, no significant change exist between the two groups after treatment.

4. DISCUSSION

PCOS is thought to be a multifactorial dysfunction with a heterogeneous etiology. It has also been related to hypothyroidism in 6.3% of women with PCOS [28]. Women who test positive for thyroid antibodies do have 2–3-fold increased risk of spontaneous miscarriage compared to all those who test negative.

PCOS together with a thyroid disorder causes increased risk of ovarian dysfunction and pregnancy related problems [29].

Menstrual Irregularity is one of the features of PCOS. It along with subclinical hypothyroidism could be a sign of underlying insulin resistance. Oligomenorrhea has been linked to hyperinsulinemia with increase future risk of type 2 diabetes mellitus [30-32].

Thyroid-stimulating hormone (TSH) is a pituitary hormone that stimulates the thyroid gland to produce thyroxine (T4), followed by triiodothyronine (T3), which stimulates the metabolic processes of nearly all other tissues of the body [33]. It is indeed a glycoprotein hormone that is produced by thyrotrope cells within the anterior pituitary gland that helps regulate the thyroid's endocrine function[34].

Thyroid autonomic dysfunction affects 8–14 % of sexually active women world - wide. In eastern Chinese women, the prevalence is 21.4 % [35].

Regardless of the fact that the etiology of hypothyroidism and PCOS is exclusively different, both of these conditions share many characteristics [19]. Thyroid levels that are too high or too low can cause ovulatory dysfunction and menstrual irregularities [36].

The purpose of this study was to see how adding a Probiotic to Metformin and Metformin alone affected PCOS women with menstrual irregularities and TSH levels.

Metformin is used for not just the prevention and treatment of hyperglycemia-related disorders, but it is also used for the management of other metabolic, endocrine, and non-endocrine diseases [37]. Current medical research is revealing new aspects in the pharmacology of Metformin. As, it is known that hypothyroidism has been linked to polycystic ovarian syndrome (PCOS). As long as, Metformin is a key component in the treatment of this disorders, it is prudent to investigate its use in subclinical hypothyroidism [38].

Although Probiotics have been shown to be effective in a variety of physiopathological conditions, but little is known about the impact on thyroid function in patients [39].Probiotics have been shown to be beneficial in thyroid diseases. Furthermore, microbes have the ability to prevent thyroid hormone fluctuations. Probiotics may be used as an adjunctive treatment for thyroid diseases [40].

The outcome of the present study showed that approximately 50% of the women develop normal menstrual cycles after taking Metformin whereas the combined effect of Metformin and Probiotic showed improvement in the cycle length in nearly 77% after 3 months of the treatment.

The findings are in line with those of Eltbogen et al., who studied 211 individuals with menstrual disorders (Amenorrhea, Oligomenorrhea) [41]. The other study also mentioned decrease in the menstrual intervals following 3 months of Metformin treatment. The drug had significant effects on the treatment of Oligomenorrhea and menstrual cycle regulation, with Oligomenorrhea alleviated and menstrual cycles regulated [42]. Furthermore, there is a plethora of evidence indicating an improvement in menstrual cyclility following Metformin treatment [43-45].

There was a study that observed the outcome measure criterion of menstrual irregularities were improved greatly in the Metformin-treated women, based on Insulin Resistance, so they concluded that women without Insulin Resistance had no significant improvement in their menstrual irregularities[46].

There are no such data available regarding the Combination treatment on menstrual cycle irregularity. However, data is present that links the effects of gut dysbiosis to menstrual disorders [47]. As a result, this could be prophesied that Probiotic bacteria may have positive benefits in regulating the menses.

The current study considered the TSH levels in PCOS patients and found a significant reduction in the mean TSH levels after Metformin treatment. According to Garber et al, clearly observed hypothyroidism in PCOS patients [48]. Likewise, a study conducted by Metformin treatment reduces serum TSH concentrations significantly in thyroid dysfunction women with PCOs, independent of consequent l-thyroxine replacement therapy [16]. Another study also mentioned decreased TSH levels significantly after 6 months of Metformin treatment [49]. Conversely, according to the study findings of Velija-Ašimi 2013, Metformin treatment showed no effect on TSH levels [50].

To the best of knowledge, this is the very first step in determining the combined effect of

Metformin and Probiotic on TSH levels in PCOS women. Even so, researches have shown that combining Probiotics with Metformin tends to improve liver aminotransferase levels in non-alcoholic steatohepatitis [51], Colitis-associated Colorectal Cancer [52], and diabetes in mice, along with lipid levels in PCOS women [23]. Moreover, a study also found the synergistic effect of probiotic V and Metformin in improving cellular injury and liver histopathology in ethanol fed rats [53].

5. CONCLUSION

In patients with polycystic ovarian syndrome, the use of Probiotics in addition to Metformin had a more favorable effect on cycle regularity and TSH levels reduction. More research is needed to determine the mode of action of these supplements in relation to Thyroid modulation.

6. LIMITATIONS

It was an only centered study and because we used a non-probability survey method, there is indeed a possibility of selection bias.

7. FUTURE RECOMMENDATIONS

More far multi-center and long-term clinical studies are needed to validate the findings and different amounts of Metformin and Probiotics must be evaluated for unrivalled results.

CONSENT

The authors obtained written and informed consent from the patients and kept it.

ETHICAL APPROVAL

The Ziauddin University Ethics Review Committee approved the analysis. It was conducted in accordance with the Helsinki Declaration, and all participants provided the informed consent. The current clinical trial has been registered at clinicaltrial.gov in the United States National Library of Medicine (identifier: NCT04009603, Unique Protocol ID: 651118UZPHA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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