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Effectiveness of Metformin and its Combination with Probiotic in Polycystic Ovarian Disease with Hyperprolactinemia: A Randomized Clinical Trial

Urooj Zafar[1*], Syeda Amber Zaidi[2], Hafiz Syed Muhammad Osama Jafri[3] Sana Imran[4], Afreen Bhatty[3] and Fizza Abidi[5]

[1]Department of Pharmacology, Baqai Medical University, Karachi, Pakistan.
[2]Department of Pharmacology, Hamdard College of Medicine & Dentistry, Hamdard University, Karachi, Pakistan.
[3]Department of Biochemistry, Ziauddin Medical College, Karachi, Pakistan.
[4]Department of Pharmacology, Jinnah Sindh Medical University, Karachi, Pakistan.
[5]Department of Oral Pathology, Ziauddin College of Dentistry, Karachi, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. Author UZ conceptualized the study, as well as the protocol and first draught of the manuscript, as well as the sampling and statistical analysis. The authors SAZ and HSMOJ were in charge of the literature searches and drafting of the manuscript. SI assisted with the manuscript. The authors AB and FA finished all of the final settings and assisted with the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: Polycystic ovarian syndrome (PCOS) is an endocrine disorder that predominantly affects women of the reproductive age. Anovulation and abnormal uterine bleeding are caused by hyperprolactinemia, which affects the hypothalamic-pituitary-ovarian axis.
Aim: In this study, the efficacy of combined Probiotic and Metformin therapy on hyperprolactinemia levels in PCOS patients was compared to Metformin therapy alone.
Methodology: 102 participants having hyperprolactinemia were enrolled via convenient sampling technique between January 2019 to August 2019**.** Out of them women having Polycystic Ovarian Syndrome (PCOS) and high serum prolactin levels were randomly assigned to one of two groups:

**Corresponding author: E-mail: urooj.aamir87@gmail.com;*

group one received oral Metformin tablet 500 mg T.D. for three months, and group two received oral Metformin tablet 500 mg T.D and Probiotic capsule 180 mg O.D for three months. Serum prolactin levels in both groups were compared before and after treatment.
Results: 54 (53%) of the 102 hyperprolactinemia women had PCOS. The Combination group showed improvement in reduction in hyperprolactinemia levels after 12 weeks of intervention.
Conclusion: The addition of Probiotic to Metformin improved prolactin levels in women with polycystic ovarian syndrome with hyperprolactinemia more than Metformin alone.

Keywords: Polycystic ovarian disease; prolactin; neurotransmitter dysregulation; metabolic disorders.

1. INTRODUCTION

PCOS is not merely a reproductive disorder but an endocrinological disorder affecting women in their reproductive years. It is defined by the Rotterdam criteria as a combination of oligo / amenorrhea, clinical or endocrine signs of hyperandrogenism, and polycystic ovaries on ultrasonography [1-3].

PCOS comprise of 5-10% women of childbearing age, affecting not just the fertility but also women's health [4]. The most likely reasons of female infertility are hyperprolactinemia and Polycystic Ovary Syndrome (PCOS) [5].

Prolactin is a hormone produced by the lactotrop cells of anterior pituitary gland. Excess of the prolactin levels impairs the release of LH and FSH by decreasing the pulsatile release of gonadotropin-releasing hormone resulting in impair ovulation and decreased fertility. Furthermore, increase in the prolactin levels also significantly affect the ovary's steroidogenetic activity, resulting in menstrual irregularities and androgen excess [6].

Metformin is the most commonly used agent in PCOS patients to regulate the steroid-related disorder [7]. Moreover a study by Krysiak R et al. reported favorable effect of Metformin in reducing the increase plasma prolactin levels [8]. However, the use of this drug has been restricted due to the growing likelihood of adverse effects [9,10]. Due to of the adverse effect associated with Metformin and different medication prescribed in PCOS women, dietary strategies to disease management have become much more popular.

Given this, Probiotics arise as efficacious nutraceuticals due to their potential future benefits to human health or preemptive medication [11]. Probiotics are defined as "live microorganisms that, when consumed in sufficient quantities, have a health-beneficial effect on the host"[12].

There are proposed theories that could explain the role of gut microbiota in the pathogenesis of polycystic ovary syndrome (PCOS). The first proposed theory is dysbiosis of the gut microbiota, which activates the host's immune response. The immunologic system's activation interferes with insulin receptor function, resulting in hyperinsulinemia. They have also been shown to decrease pathogenic bacterial colonization in the intestine, enhance biological gut barrier function, and influence the production of pro- and anti-inflammatory cytokines [12, 13] The second possibility is that the gut bacteria causes PCOS by increasing the secretion of gut-brain peptides. The third possible mechanism is that androgens influence the composition of the gut microbiota, which leads to the development of PCOS [14].

In light of the foregoing, prescriptions for insulin-sensitizing drugs, most notably Metformin, are most commonly given to women with PCOS but are uncommon among hyperprolactinemia women; and Probiotic, on the other hand, has shown effects in improving hormonal imbalances [15, 16]; thus, by giving a previously untested combination of Metformin and Probiotic on prolactin levels, we could find good results.

1.1 Primary Objective

- To evaluate the effect of Metformin, either alone or in combination with Probiotic, in improving hyperprolactinemia levels in women with PCOS.

1.2 Secondary Objective

- Prevalence of PCOS among hyperprolactinemia women

2. MATERIALS AND METHODS

2.1 Trial Design and Participants

It was an open label, randomized, parallel-arm study in which 102 women with hyperprolactinemia aged 18 to 45 were recruited. Women enrolled in this single-center cross-sectional study came from a gynecological clinic at a hospital in Karachi via a convenient sampling technique between January 2019 and August 2019. Then, women having PCOS with hyperprolactinemia were randomly assigned to one of the two groups for the trial from among the 102 women with hyperprolactinemia.

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%.

2.1.1 Inclusion criteria

- Women with Hyperprolactinemia without PCOS
- Women without Hyperprolactinemia with PCOS
- Patients with diagnosed PCOS were recruited using the Rotterdam criteria. Based on these two of the mentioned three features are necessary to detect PCOS instances, according to the criteria:

  - Oligo-ovulation or anovulation: (Oligomenorrhea, defined as more than 45 days or less than 8 cycles per year, and Amenorrhea, defined as more than 3 months in women with previous periodic menstruation) lasting 6 months.
  - Clinical hyperandrogenism (including hirsutism) or biochemical hyperandrogenism (with a higher free androgen index or free testosterone).
  - Ultrasonographic presentation of polycystic ovaries: >12 follicles in one or both ovaries, 2-9 mm in diameter, and/or enlarged ovarian volume >10 m3).

2.1.2 Exclusion criteria

- Other than PCOS-related hyperandrogenism (Cushing's syndrome, hyperprolactinemia adrenal tumours, congenital adrenal hyperplasia, uncommon genetic diseases)
- Consent is absent or has been revoked
- Period of pregnancy or nursing (first 6 months after giving birth)
- Allergies
- Any malignancies that required treatment in the three years before the study procedures
- Any other chronic condition requiring medical checks or hospitalization
- Type 1 diabetes mellitus
- Anti-diabetic therapy and other medication therapy within six months of the research procedures

2.1.3 Protocol of the study

The study methodology was clearly communicated to all participants prior to gaining informed consent and before the allocation of groups by the principal investigator. Agreements were signed and then patients were randomly assigned to one of two treatment groups and were given the following medications: For a 12-week period, group A received tablet Metformin 500mg T.D (n = 28) and group B (Met/Pro group) received a combination of Metformin 500mg B.D and Probiotic 180mg O.D (n = 28).

The probiotic supplement included *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).

Hyperprolactinemia in patients with and without PCOS was evaluated before and 3 months after starting Metformin and its combination with Probiotic treatment. Blood samples for assaying were drawn between 08:00 and 09:00 a.m., after an overnight fast.

2.2 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 the intervention. 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

Initially, 120 patients were eligible to participate in this study; however, 18 participants were removed, including those who refused to sign the

consent form (n= 14) and those who did not return for follow-up (n= 4). As previously indicated, all eligible individuals were randomized at random to one of two interventional groups: Metformin or Metformin and probiotics (Met/Pro).

At the start of the study, the women were evaluated for hyperprolactinemia levels, there was no significant difference between the two treatment groups (p = 0.07). The Combination group showed improvement in reduction in hyperprolactinemia levels after 12 weeks of intervention (p = 0.01) whereas Metformin alone showed insignificant improvement and, when the two groups were compared significant change exist between the two groups after treatment (p = 0.000).

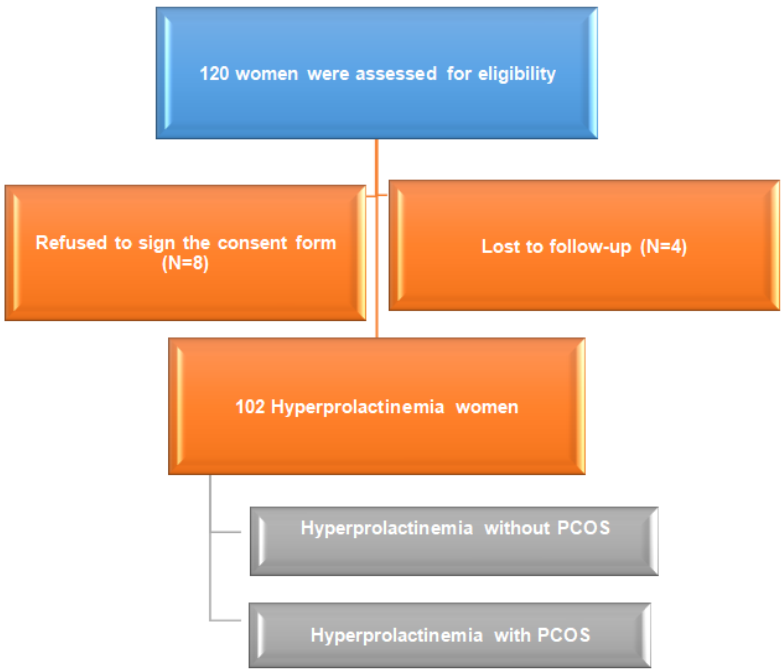

Fig 1. Flowchart of sample analysis

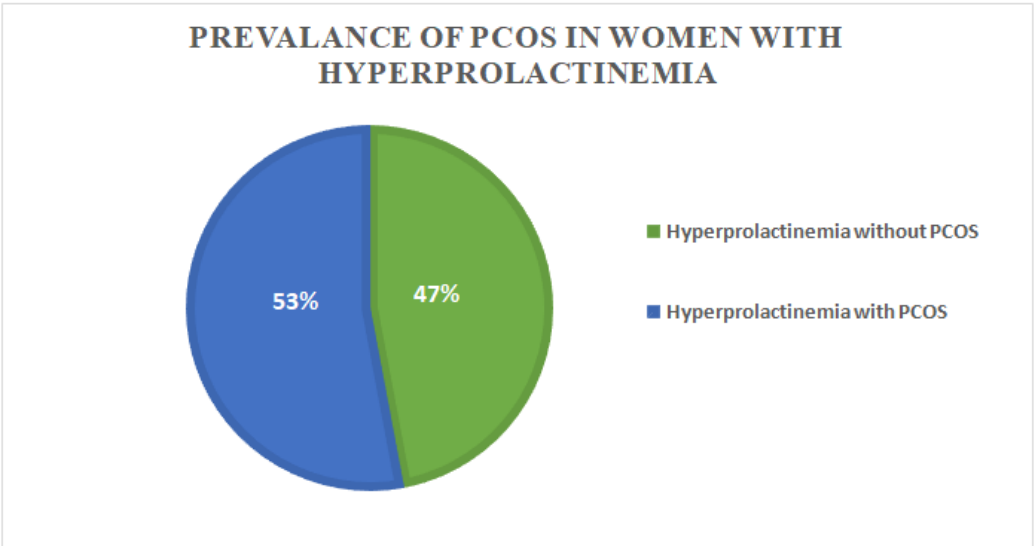

Fig. 2. Prevalence of PCOS in women with Hyperprolactinemia

Hyperprolactinemia: Among the 102 hyperprolactinemia women 54 (53%) had PCOS and without 48 (47%) had only hyperprolactinemia

Table 1. Hyperprolactinemia in women with PCOS

Hyperprolactinemia in Women with PCOS			
Drug Treatment	**Metformin**	**Combination (Metformin + Probiotic)**	***p*-value**
Mean age	28.2 ± 5.6	26.1 ± 6.3	0.13
BMI	25.2 ± 4.01	26.5 ± 5.8	0.34
Before Treatment	35.1 ± 8.9	36.8 ± 9.0	0.51
After 12 weeks Treatment	33.4 ± 9.1	27.2 ± 7.6	0.01
p-value	0.072	0.000	

The mean age of the patient in the Metformin group and Combination group is 28.2 ± 5.6 and 26.1 ± 6.3 respectively. Both the groups showed no significant change in the age. BMI of women was 25.2 ± 4.01 in Metformin group whereas in the Combination the BMI was found to be 26.5 ± 5.8

4. DISCUSSION

The purpose of this study was to see how adding Probiotic to Metformin affected PCOS women with hyperprolactinemia.

Prolactin (lactogenic) is a polypeptide hormone that is primarily produced by the anterior pituitary gland's lactotroph cells. Moreover, it is also generated by a large number of extra-pituitary cells. It is well-known that PRL plays an essential role in nursing during pregnancy, but it is also involved in angiogenesis, immune-regulation, and osmoregulation. Hyperprolactinemia impairs both genders' reproductive function, resulting in hypogonadism, sterility, and galactorrhea [17].

The principal finding of the present study was PCOS women has higher incidence prolactin serum levels (53%) than non-PCOS healthy hyperprolactinemia women (47%) that was in line with a study done in Iraq by Muhjah Hassan which showed prevalence of hyperprolactinemia was 69.4% [18]. Another researcher also agreed with this finding, demonstrating that serum prolactin levels in PCOS individuals are greater than healthy women[19].

Prolactin is often generated in adipose tissue and functions as a cytokine in the regulatory oversight of the body's metabolism. The level of prolactin derived from adipose tissue seems to to be proportional to body fat [20]. The above phenomena are consistent with our findings, as most of the patients enrolled had a high BMI.

One of most prevalent explanation for the connection between HPRL and PCOS is a potentially shared hypothalamic-pituitary problem that can describe both PCOS and hyperprolactinemia.

Interestingly, investigation by Delcour have demonstrated that the excessive level of prolactin and LH production in women with PCOS are synchronized. Furthermore, certain investigations have indicated that dopamine may slow the production of LH. It has therefore been proposed that the increased levels of LH seen in PCOS women are a result of a reduction in dopaminergic tone, which is also responsible for the rise in prolactin levels [21, 22].

It is also suggested that plasma PRL levels have a wide range of impacts on gluconeogenesis. Previous research found that elevated PRL disturbed glucose homeostasis and caused metabolic disorders. Individuals with hyperprolactinemia have higher insulin-resistance and glucose intolerance than healthy people [23, 24].

The present study evaluated the effect of Probiotic with Metformin on rolactin levels in women with PCOS. The mean prolactin in the group treated with Metformin and Probiotics decreased after intervention, as anticipated, and the difference whereas no substantial change was observed in Metformin treated group after the intervention. Remarkably, a study showed amazing results in which they mentioned Metformin only reduced prolactin levels when given at high doses. Besides that, Metformin at the same daily doses (2.5–3 g) was found to be effective in lowering prolactin levels in hyperprolactinemia patients who received chronic Bromocriptine intervention. [25]. Given such findings, it could be suggested that a particular quantity of the drug in the pituitary could be required to affect lactotroph function. If this interpretation is true, a decrease in prolactin levels would be much more prominent in long-term treatment with maximum doses of this agent.

5. CONCLUSION

Finally, this study discovered for the first time that Metformin, when combined with a probiotic,

significantly reduced prolactin levels in women with PCOS. It is expected that high-dose metformin therapy will benefit women with PCOS. However, more research is needed to back up our findings.

6. LIMITATIONS

The study has certain limitations:

- Single-centered research
- Small sample size

7. FUTURE RECOMMENDATIONS

Multi-center and long-term clinical research is necessary to determine the findings, and different amounts of Metformin must be analyzed for unrivalled results.

STRENGTH OF THE STUDY

The study's strengths include:

- Strict criteria for interventional procedures' inclusion and exclusion
- Careful monitoring of treatment fidelity

CONSENT

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

ETHICAL APPROVAL

The study was accepted by the Ziauddin University Ethics Review Committee. It was carried out in line with the Helsinki Declaration, and all participants gave their informed permission. The present clinical study has been submitted with the United States National Library of Medicine at clinicaltrials.gov (identifier: NCT04009603, Unique Protocol ID: 651118UZPHA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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