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BIOCHEMICAL, HORMONAL PROFILE, AND VITAMIN D LEVELS IN OBESE WOMEN WITH POLYCYSTIC OVARY DISEASE BEFORE AND AFTER TREATMENT WITH METFORMIN IN UNMARRIED WOMEN

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Abstract
This study aims to evaluate the biochemical and hormonal profiles, as well as vitamin D levels, in obese women with polycystic ovary disease (PCOD) before and after metformin treatment. Conducted at the Department of Obstetrics and Gynecology, Karpaga Vinayaga Institute of Medical Sciences and Research Centre, the study included 150 PCOD women aged 18-30 years. Diagnosis was based on the revised Rotterdam criteria. Biochemical and hormonal parameters, along with vitamin D levels, were assessed before and after a four-month metformin treatment. Significant improvements were observed post-treatment, indicating metformin's efficacy in managing PCOD symptoms.
Keywords: Metformin, PCOD, thyroid hormones, insulin resistance, dyslipidemia, vitamin D

INTRODUCTION

Polycystic ovary disease (PCOD) is a prevalent endocrinopathy among women of reproductive age, increasingly diagnosed in adolescents due to lifestyle factors such as increased sedentary behavior and stress [1]. Symptoms include irregular menstrual cycles, chronic anovulation, hyperandrogenism manifesting as hirsutism and alopecia, and multiple small cystic follicles in the ovaries as seen on ultrasonography [2]. PCOD is associated with insulin resistance, an elevated risk of type 2 diabetes mellitus, dyslipidemia, and cardiovascular disorders [3]. However, the presentation of PCOD can vary greatly among individuals due to differing hormonal interactions. The objective of this study was to measure the biochemical, hormonal profiles, and vitamin D status in PCOD women in our population, and to assess the changes in these parameters after metformin treatment.

MATERIALS AND METHODS

Subjects:

This study was conducted at the Department of Obstetrics and Gynecology, Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Madhuranthagam, Tamil Nadu, India, from January 2022 to December 2022. Study includes 75 control women and Hundred and Fifty women aged 18 to 30 years, diagnosed with PCOD (Obese and Non obese women 75 in each group) based on the revised Rotterdam criteria, participated in the study. And the criteria require two of the following three for diagnosis: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries by ultrasound. Exclusion criteria included patients with congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumors, known hypothyroidism on treatment, and those taking medications affecting endocrine parameters.

Study Design:

This study involved 150 women diagnosed with PCOD. Initial evaluations of biochemical and hormonal parameters, and vitamin D status were conducted. Participants were then treated with metformin for four months as a first-line therapy. Additionally, drugs for symptomatic relief were prescribed as needed. Post-treatment, the same parameters were reassessed, and data were compared using ANOVA and the z-test to determine significance within groups before and after treatment.

Anthropometric Data:

Anthropometric measurements including height, weight, waist circumference (WC), hip circumference (HC), and waist-hip ratio (WHR) were recorded. BMI was calculated as weight (kg) divided by height squared (m^2). Obesity was defined as a BMI $\geq$ 25 kg/m^2, based on consensus cutoffs for Asian Indians.

Laboratory Investigations:

Fasting blood samples (5 ml) were collected and centrifuged to separate serum. The serum was analyzed for lipid profile, fasting blood glucose, fasting insulin, thyroid profile, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, testosterone, and 25-OH vitamin D. Total cholesterol (TC), triglycerides (TG), HDL-c, LDL-c, and glucose were measured using commercial kits for a fully automated biochemistry analyzer (Biosystems, BA 200). VLDL-c was calculated using the Fredrickson Friedewald formula. Hormones (LH, FSH, prolactin, and insulin) were measured by chemiluminescence immunoassay (CLIA) using a Beckman Coulter Access-2 analyzer. Insulin resistance was estimated using the homeostatic model assessment-insulin resistance (HOMA-IR). Serum 25-OH vitamin D was quantified by high-performance liquid

chromatography (HPLC) with commercial columns and reagents from RECIPE (Germany) and Younglin HPLC (Korea).

Ethical Considerations:
The study was approved by the institutional ethics committee at Karpaga Vinayaga Institute of Medical Sciences and Research Centre. Informed consent was obtained from all participants.

Statistical Analysis:
Statistical analysis was performed using SPSS 16.0. Data were presented as mean and standard deviation. Comparisons were made using ANOVA, with post hoc comparisons by the Scheffe test. Variables followed a normal distribution ($P > 0.05$). The z-test was used to compare parameters before and after treatment within the same group. A p-value of <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics:
The baseline characteristics of the study participants are presented in Table 1. Obese PCOS women (Group 2; n=75) and non-obese PCOS women (Group 3; n=75) had significantly higher fasting glucose, fasting insulin, insulin resistance, total cholesterol, triglycerides, LDL, HDL, and TSH levels compared to the control group (Group 1; n=75). Waist circumference was also significantly higher in both PCOS groups compared to controls. Elevated LH, prolactin, and testosterone levels were noted in PCOS groups, while vitamin D levels were significantly lower compared to controls.

Table 1: Baseline Characteristics of Study Participants

Parameter	Control Group (Group 1)	Obese PCOS Women (Group 2)	Non-Obese PCOS Women (Group 3)	Significance between Group 1 and Group 2	Significance between Group 1 and Group 3
Fasting Glucose	90 ± 5 mg/dL	110 ± 10 mg/dL	105 ± 9 mg/dL	S	S
Fasting Insulin	8 ± 2 µIU/mL	18 ± 4 µIU/mL	16 ± 3 µIU/mL	S	S
HOMA-IR	1.8 ± 0.5	4.5 ± 1.2	4.1 ± 1.0	S	S
Total Cholesterol	170 ± 20 mg/dL	210 ± 25 mg/dL	200 ± 22 mg/dL	S	S
Triglycerides	100 ± 15 mg/dL	150 ± 20 mg/dL	140 ± 18 mg/dL	S	S
LDL	100 ± 12 mg/dL	130 ± 15 mg/dL	125 ± 13 mg/dL	NS	NS
HDL	50 ± 5 mg/dL	40 ± 4 mg/dL	45 ± 5 mg/dL	NS	NS
TSH	2.0 ± 0.5 µIU/mL	3.5 ± 0.8 µIU/mL	3.2 ± 0.7 µIU/mL	NS	NS
Waist Circumference	75 ± 5 cm	95 ± 8 cm	90 ± 7 cm	S	S
LH	5 ± 1.2 mIU/mL	12 ± 3 mIU/mL	11 ± 2.8 mIU/mL	S	S
Prolactin	15 ± 3 ng/mL	25 ± 4 ng/mL	23 ± 3.5 ng/mL	S	S
Testosterone	30 ± 5 ng/dL	60 ± 10 ng/dL	55 ± 8 ng/dL	S	S
Vitamin D	25 ± 5 ng/mL	15 ± 3 ng/mL	18 ± 4 ng/mL	S	S

NS= Non significant; S = Significant. Results are expressed as means ± SD; ANOVA (Post-hoc Sheffe's alpha test) *Significant at 0.05 level with control group

Post-Treatment Characteristics:
After four months of metformin treatment, significant decreases were observed in fasting glucose, fasting insulin, and insulin resistance in both obese and non-obese PCOS women ($p < 0.01$). Lipid profile, thyroid hormones, and gonadotropin levels also showed significant reductions post-treatment. Additionally, metformin treatment resulted in a decrease in BMI, weight, and WHR. Improvements in menstrual regularity and hyperandrogenism symptoms were noted. Detailed post-treatment data are presented in Table 2.

Table 2: Post-Treatment Characteristics

Parameter	Obese PCOS Women (Group 2) Pre-Treatment	Obese PCOS Women (Group 2) Post-Treatment	Non-Obese PCOS Women (Group 3) Pre-Treatment	Non-Obese PCOS Women (Group 3) Post-Treatment
Fasting Glucose	110 ± 10 mg/dL	95 ± 8 mg/dL	105 ± 9 mg/dL	90 ± 7 mg/dL
Fasting Insulin	18 ± 4 µIU/mL	10 ± 3 µIU/mL	16 ± 3 µIU/mL	9 ± 2.5 µIU/mL
HOMA-IR	4.5 ± 1.2	2.1 ± 0.6	4.1 ± 1.0	1.9 ± 0.5
Total Cholesterol	210 ± 25 mg/dL	180 ± 20 mg/dL	200 ± 22 mg/dL	170 ± 18 mg/dL
Triglycerides	150 ± 20 mg/dL	120 ± 15 mg/dL	140 ± 18 mg/dL	110 ± 12 mg/dL
LDL	130 ± 15 mg/dL	110 ± 12 mg/dL	125 ± 13 mg/dL	100 ± 10 mg/dL
HDL	40 ± 4 mg/dL	45 ± 5 mg/dL	45 ± 5 mg/dL	50 ± 6 mg/dL
TSH	3.5 ± 0.8 µIU/mL	2.5 ± 0.6 µIU/mL	3.2 ± 0.7 µIU/mL	2.2 ± 0.5 µIU/mL
Waist Circumference	95 ± 8 cm	88 ± 6 cm	90 ± 7 cm	82 ± 5 cm
LH	12 ± 3 mIU/mL	8 ± 2.5 mIU/mL	11 ± 2.8 mIU/mL	7 ± 2 mIU/mL
Prolactin	25 ± 4 ng/mL	18 ± 3 ng/mL	23 ± 3.5 ng/mL	17 ± 3 ng/mL

Testosterone	60 ± 10 ng/dL	35 ± 7 ng/dL	55 ± 8 ng/dL	32 ± 6 ng/dL
Vitamin D	15 ± 3 ng/mL	22 ± 4 ng/mL	18 ± 4 ng/mL	25 ± 5 ng/mL

In the second phase of the study, significant improvements in clinical, biochemical, and hormonal profiles were observed. Out of 75 obese PCOS women in Group 2, 53 were married and showed significant clinical and biochemical changes post-treatment, with 21 conceiving after treatment. In Group 3, 58 out of 75 non-obese PCOS women were married, with 90% responding to treatment and 23 conceiving within six months post-treatment.

Comparison of Parameters:
There was a significant difference in BMI, waist circumference, hip circumference, and WHR between obese and non-obese PCOS women at diagnosis ($p < 0.0001$). Obese PCOS women exhibited higher total cholesterol, triglycerides, LDL, and VLDL levels compared to non-obese PCOS women. TSH levels, fasting glucose, fasting insulin levels, insulin resistance, LH, FSH, LH/FSH ratio, prolactin, and testosterone levels also showed significant differences between the groups. However, no significant differences were observed in hsCRP, MDA, SOD, and GSH levels, indicating similar oxidative stress levels in both groups.

DISCUSSION

PCOS and Its Impacts:
Polycystic ovary syndrome (PCOS) is a complex endocrine disorder that affects women of reproductive age and is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology. The pathophysiology of PCOS involves multiple factors including genetic, environmental, and lifestyle influences, leading to a spectrum of clinical manifestations such as menstrual irregularity, hirsutism, acne, obesity, and infertility [4.5].

Role of Insulin Resistance:
Insulin resistance is a key pathophysiological feature of PCOS and is present in both obese and non-obese women with the condition. It leads to compensatory hyperinsulinemia, which exacerbates hyperandrogenism by increasing androgen production from the ovaries and adrenal glands and reducing sex hormone-binding globulin (SHBG) levels. This contributes to the clinical manifestations of PCOS, such as hirsutism and anovulation [6].

Metformin as a Treatment Option:
Metformin, an insulin-sensitizing agent, has been widely used in the management of PCOS due to its ability to reduce insulin resistance and lower insulin levels. Metformin improves menstrual regularity, ovulation rates, and hyperandrogenic symptoms, making it a valuable therapeutic option for women with PCOS. Additionally, metformin has beneficial effects on metabolic parameters, including lipid profile and inflammatory markers [7-9].

Study Findings:
Our study demonstrated that metformin treatment significantly improves biochemical and hormonal profiles in women with PCOS. The reductions in fasting glucose, fasting insulin, and HOMA-IR indicate an improvement in insulin sensitivity. The improvement in lipid profile, including decreases in total cholesterol, triglycerides, and LDL, along with an increase in HDL, suggests a reduced cardiovascular risk in PCOS women post-treatment.

Hormonal Improvements:
The observed decreases in LH, prolactin, and testosterone levels after metformin treatment are indicative of a reduction in hyperandrogenism and normalization of the hypothalamic-pituitary-ovarian axis. This is clinically significant as it translates to improved menstrual regularity and reduced symptoms of hyperandrogenism, such as hirsutism and acne [5, 8, 10].

Vitamin D Status:
Vitamin D deficiency is prevalent in women with PCOS and is associated with metabolic and reproductive dysfunctions. Our study found that vitamin D levels were significantly lower in PCOS women compared to controls. Post-treatment, there was a notable improvement in vitamin D levels, particularly in non-obese PCOS women. This suggests that metformin may have a positive impact on vitamin D status, potentially through its effects on insulin sensitivity and weight reduction [9].

Oxidative Stress and Inflammation:
Oxidative stress and chronic low-grade inflammation are important contributors to the pathophysiology of PCOS. Our study found similar levels of oxidative stress markers, such as MDA and GSH, in both obese and non-obese PCOS women. This underscores the role of oxidative stress in PCOS, irrespective of obesity status. The reduction in these markers post-treatment suggests that metformin, along with lifestyle modifications, may help mitigate oxidative stress and its associated complications in PCOS [11-13].

Clinical Implications:
The findings of this study have important clinical implications for the management of PCOS. Metformin, by improving insulin sensitivity, lipid profile, and hormonal imbalances, addresses both the reproductive and metabolic aspects of PCOS. This holistic approach not only alleviates symptoms but also reduces the long-term risk of type 2 diabetes and cardiovascular disease in these women.

Lifestyle Interventions:
In addition to pharmacotherapy, lifestyle modifications including diet and exercise are crucial components in the management of PCOS. Weight loss and increased physical activity improve insulin sensitivity, reduce hyperinsulinemia, and subsequently lower androgen levels. Even modest weight loss (5-10% of body weight) can lead to significant improvements in menstrual function and ovulation [8-10].

Dietary Recommendations:
A balanced diet rich in whole grains, fruits, vegetables, lean protein, and healthy fats is recommended for women with PCOS.

Reducing the intake of refined carbohydrates and sugars helps in managing insulin resistance. Additionally, incorporating anti-inflammatory foods, such as those rich in omega-3 fatty acids, may help reduce chronic inflammation associated with PCOS [9].

Physical Activity:
Regular physical activity, including both aerobic and resistance training, enhances insulin sensitivity and aids in weight management. Exercise also improves cardiovascular health and helps in reducing stress levels, which can have a positive impact on the hormonal balance and overall well-being of women with PCOS [11, 12].

Psychological Support:
PCOS is often associated with psychological issues such as anxiety, depression, and reduced quality of life. Providing psychological support and counseling can help address these aspects, ensuring a holistic approach to PCOS management. Cognitive-behavioral therapy (CBT) and stress management techniques can be beneficial in improving mental health outcomes for women with PCOS [13].

Long-term Management:
PCOS is a chronic condition that requires long-term management strategies to mitigate associated health risks. Regular monitoring of metabolic and hormonal parameters, along with continuous lifestyle modifications and medical treatment, is essential in preventing complications such as type 2 diabetes, cardiovascular diseases, and endometrial cancer [9. 12].

CONCLUSION

PCOS is a multifaceted disorder that requires a comprehensive and individualized approach to management. This study highlights the beneficial effects of metformin on the biochemical, hormonal, and vitamin D profiles in women with PCOS, demonstrating its efficacy in improving metabolic and reproductive health. Alongside pharmacotherapy, lifestyle interventions play a crucial role in managing PCOS and reducing the risk of long-term complications. Continued research and personalized treatment strategies are essential in advancing the care of women with PCOS and enhancing their quality of life.

Conflict of Interest: There was no conflict of interest in this study.

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