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Research Article

A COMPARISON OF THE CLINICAL AND METABOLIC OUTCOMES OF METFORMIN ALONE VERSUS METFORMIN COMBINED WITH CALCIUM AND VITAMIN D IN WOMEN WITH POLYCYSTIC OVARY SYNDROME: A QUASI-EXPERIMENTAL STUDY

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ABSTRACT

Objective: This study evaluates the clinical and endocrine effects of metformin alone versus metformin combined with calcium and Vitamin D in women with Polycystic Ovary Syndrome (PCOS). PCOS is associated with insulin resistance, hormonal imbalances, and metabolic dysfunction.

Methods: A quasi-experimental interventional study was conducted on newly diagnosed PCOS women (18–45 years). Participants were divided into two groups: Group I received metformin (500 mg/day), calcium (1000 mg/day), and vitamin D (60,000 IU weekly) for 8 weeks. Group II received only metformin (500 mg/day) for 8 weeks. The study compared changes in homeostatic model assessment of insulin resistance, lipid profile, and clinical parameters from baseline to the end of treatment.

Results: Vitamin D supplementation along with calcium and metformin significantly improved body mass index (BMI) (24.93±3.78–23.84±1.11, $p<0.001$), waist circumference (WC) (87.83±7.83 cm–81.50±3.04 cm, $p=0.007$), and fasting glucose (95.48±10.64 mg/dL–92.18±6.43 mg/dL, $p<0.001$). Total cholesterol decreased ($p=0.030$), and high-density lipoprotein increased ($p=0.029$). Menstrual regularity (51.7–65%), acne (50–5%), and hirsutism (38.3–0%) improved significantly.

Conclusion: Vitamin D supplementation with calcium and metformin resulted in significant improvements in BMI, WC, glucose levels, lipid profile, menstrual regularity, acne, and hirsutism in women with PCOS. In contrast, metformin alone did not lead to notable metabolic benefits.

Keywords: Vitamin D, Metformin, Calcium, Metabolic status, Clinical outcome polycystic ovary syndrome, Polycystic ovary syndrome.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age, characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology. The prevalence of PCOS varies worldwide, but it is estimated to affect approximately 6–20% of women in this age group, depending on the diagnostic criteria used [1]. PCOS is associated with a range of metabolic abnormalities, including insulin resistance, obesity, dyslipidemia, and an increased risk of type 2 diabetes mellitus and cardiovascular disease (CVD) [2]. These metabolic disturbances are of particular concern as they not only impact reproductive health but also have long-term implications for overall health.

Metformin, an oral antihyperglycemic agent, is widely used in the management of PCOS due to its ability to improve insulin sensitivity and reduce hyperinsulinemia, which is a key pathophysiological factor in PCOS. Numerous studies have demonstrated that metformin can improve menstrual regularity, ovulation rates, and metabolic parameters in women with PCOS [3]. However, metformin alone may not fully address all aspects of the metabolic and reproductive abnormalities in PCOS, and there is growing interest in adjunct therapies that may enhance its efficacy.

Vitamin D deficiency has been suggested as a potential link between insulin resistance and PCOS [4]. Vitamin D, a fat-soluble vitamin, is synthesized in the skin through sunlight-induced conversion of cholesterol to 7-dehydrocholesterol, or it can be obtained from dietary sources. After synthesis, Vitamin D undergoes two hydroxylation processes: first in the liver, where it is converted to 25-hydroxyvitamin D (25(OH)D), and then in the kidney, where it is converted to the active form, 1,25-dihydroxyvitamin D (1,25(OH)2D) [5]. The active form binds to vitamin D receptors (VDR) in various tissues, including pancreatic beta-cells, immune cells, and reproductive organs, suggesting a role beyond calcium and bone homeostasis [6]. VDR has been identified in reproductive organs such as the ovaries, uterus, and placenta, indicating vitamin D's involvement in reproductive physiology [7,8]. Moreover, the active vitamin D-VDR complex regulates over 300 genes, including those related to glucose and lipid metabolism and gonadal function, highlighting its broader biological significance [9].

Calcium and Vitamin D are essential nutrients that play a significant role in various physiological processes, including bone health, immune function, and glucose metabolism. Emerging evidence suggests that Vitamin D deficiency and calcium imbalance may contribute to the pathogenesis of insulin resistance and metabolic disturbances in PCOS [10]. Moreover, Vitamin D and calcium supplementation have been proposed as potential therapeutic strategies to improve metabolic and reproductive outcomes in women with PCOS. Some studies have reported that supplementation with these nutrients can improve insulin sensitivity, reduce androgen levels, and enhance follicular maturation and ovulation in women with PCOS [11,12].

The combination of metformin with calcium and Vitamin D supplementation represents a novel therapeutic approach aimed at addressing both insulin resistance and the potential micronutrient deficiencies that may exacerbate the metabolic and reproductive disturbances in PCOS. This study aims to evaluate the efficacy of metformin combined with calcium and Vitamin D supplementation compared to metformin alone in improving clinical and metabolic outcomes in women with PCOS. The findings from this study could have significant implications for the management of PCOS, potentially supporting the use of a combined therapeutic approach to address the multifaceted nature of the syndrome. Understanding the impact of this combination therapy on insulin resistance, menstrual regularity, ovulation, and other metabolic parameters is crucial for optimizing treatment strategies in women with PCOS.

METHODS

This quasi-experimental interventional study was conducted on newly diagnosed women with PCOS at the Biochemistry and Obstetrics and Gynaecology Departments of Malabar Medical College Hospital and Research Centre, Kozhikode, from September 2021 to March 2023. The study included women aged 18–45 years, newly diagnosed with PCOS according to the Rotterdam criteria [13]. Who consented to participate and follow up. Exclusion criteria included a history of chronic liver or kidney disease, diabetes mellitus, hypothyroidism, hyperprolactinemia, recent Vitamin D intake (within 3 months), use of medications affecting endocrine parameters, and pregnancy.

At the baseline visit, a comprehensive clinical history was recorded, including menstrual and obstetric history, anthropometric measurements, and any prior illnesses such as diabetes, thyroid disorders, CVDs, or chronic kidney or liver diseases. Menstrual status, marital status, and ultrasound findings of the ovaries were also documented. Clinical assessments included noting the presence of hirsutism, acne, and blood pressure. Anthropometric measurements, such as height, weight, and waist circumference (WC), were taken, and body mass index (BMI) was calculated.

Twelve-hour fasting blood samples were collected at baseline and after the 8th week of the intervention. The blood samples were immediately centrifuged at 4000 rpm for 10 min to separate serum, and the serum was stored at −20°C until assayed. Fasting blood sugar was measured using Hexokinase/G-6-PDH method on an automated analyzer (Abbott Architect 4100 integrated autoanalyzer). The intra-assay and inter-assay coefficients of variation (CV%) were <5%. Serum Vitamin D was measured using Chemiluminescent Microparticle Immunoassay (CMIA) with a CV of 2.8% for high control and 4.8% for low control. Serum calcium was measured using Arsenazo III dye method with expect CV of within assay 1% and between assay <2%. Serum Total cholesterol was measured using the enzymatic method (cholesterol oxidase-peroxidase method) with <2% CV. Triglycerides were measured using Glycerol Phosphate Oxidase method with CV % between 0.8% and 1.7%. High-density lipoprotein (HDL) measured using Enzymatic-Accelerator Selective Detergent with <2% CV. Low-density lipoprotein (LDL) measured by the direct LDL assay method, and the precision of the method is <4% CV and very LDL (VLDL) by calculation (Triglycerides/5). Serum insulin was measured by using CMIA with a CV of <5%. All biochemical parameters were assayed by Abbott Architect 4100 integrated autoanalyzer. Haematological parameters such as hemoglobin (Hb) and mean corpuscular volume (MCV) were also evaluated using Mind ray BC 780. To estimate insulin resistance, homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as fasting plasma insulin (μU/mL) × fasting plasma glucose (mg/dL)/405.

Sample size

The sample size was calculated based on a previous randomized controlled trial by Maktabi *et al*. [14]. Using the reported mean difference in HOMA-IR (−0.3±0.8 for the intervention group vs. +0.6±1.6 for the placebo group), and assuming a significance level of 0.05 and power of 80%, Based on the formula,

$$N=[(Z\alpha/2+Z\beta\times\sigma)^2/\delta^2]$$

The minimum required sample size was estimated to be 32 participants per group. However, to enhance the statistical power, account for potential dropouts, and meet academic and ethical standards for doctoral-level research, the sample size was increased to 60 participants in the intervention group (vitamin D + calcium + metformin) and 40 participants in the control group (metformin alone). This increased sample size also improves the generalizability and robustness of the findings.

Study design

This was a quasi-experimental interventional study conducted to compare the clinical and metabolic outcomes of metformin alone versus metformin combined with calcium and Vitamin D supplementation in newly diagnosed women with PCOS, aged 18–45 years. Participants were nonrandomly assigned to two parallel groups based on their order of recruitment and availability, due to logistical and ethical considerations. Group I received metformin (500 mg/day), calcium (1000 mg/day), and vitamin D (60,000 IU weekly), whereas Group II received metformin (500 mg/day) alone. Both interventions were continued for 8 weeks.

The weekly dose of 60,000 IU of Vitamin D3 was selected based on established clinical guidelines and prior interventional studies demonstrating its safety and efficacy in correcting Vitamin D deficiency, particularly in women with PCOS. According to the Endocrine Society Clinical Practice Guidelines (2011), Vitamin D-deficient adults can be treated with 50,000 IU of vitamin D2 or D3 weekly for 8 weeks, followed by maintenance dosing [15]. In the Indian context, where deficiency is highly prevalent, 60,000 IU/week is commonly prescribed and readily available as a standard formulation. Previous studies, including that by Maktabi *et al*., have successfully used similar dosages in PCOS patients, reporting significant improvements in metabolic parameters without adverse effects [14]. This dosing strategy was therefore considered appropriate to ensure effective repletion of Vitamin D stores and assess its impact on metabolic and endocrine profiles in Vitamin D-deficient women with PCOS.

Given the quasi-experimental nature of the study, random allocation and allocation concealment were not employed. However, to minimize selection bias and baseline imbalances, statistical adjustments (such as ANCOVA) were applied during data analysis.

Statistical analysis

Continuous data with a normal distribution were presented as means with standard deviations (SD), whereas continuous data with a skewed distribution were shown as medians with interquartile ranges. Categorical data were expressed as percentages. The distribution of the data was analyzed using descriptive statistics and the Kolmogorov–Smirnov test. Baseline comparisons between the experiment and control groups were conducted using the unpaired Student's independent t-test, Mann–Whitney *U*-test, Wilcoxon signed-rank test, and Chi-square (χ^2) test depending on the type of variable and data distribution.

The data entry and the final analysis were done with the use of the Statistical Package for the Social Science software version 23.0.

Ethical clearance was obtained from the institutional ethics committee MMCH&RC/IEC/2021/02.

RESULTS

The present study was conducted on 100 women diagnosed with PCOS and Vitamin D deficiency. The results and observations of both groups (Group I [n=60] received metformin 500 mg plus calcium 1000 mg daily for 8 weeks along with 60,000 IU of Vitamin D weekly [Experimental group] and Group II [n=40] received only metformin 500 mg daily for 8 weeks) are as follows (Control group).

The study population group age ranged between 18 and 45 years with a mean age of 25.5±5.05 in the experimental group and 28.63±4.70 in the control group (Fig. 1). Both the experimental and control groups experienced significant weight reduction over the study period, though the intergroup difference remained non-significant. BMI decreased significantly in the experimental group (p<0.001), whereas no significant change was observed in the control group, suggesting a potential role of Vitamin D in BMI reduction (Table 1).

An ANCOVA was conducted to assess the effect of Vitamin D and calcium supplementation on WC while adjusting for baseline differences. The corrected model was statistically significant (F(2,77)=2589.173, p<0.001), with a partial eta squared of 0.982, indicating that 98.2% of the variance in post-intervention WC was explained by the model. WC at baseline emerged as a highly significant covariate (F=4038.774, p<0.001). To account for potential deviations from normality, a bootstrap ANCOVA was performed, further reinforcing the robustness of the findings (Table 2). The experimental group demonstrated a mean post-intervention WC of 88.93 cm (bias=0.0014, SE=1.1214; 95% CI: 86.68–91.29 cm), while the control group had a mean of 81.50 cm (bias=0.0037, SE=0.4709; 95% CI: 80.57–82.43 cm).

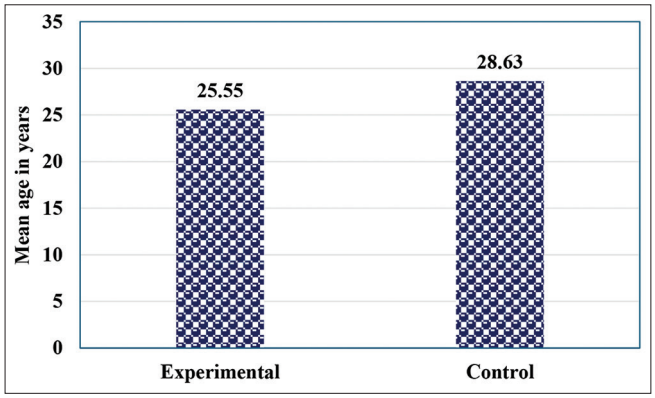

Fig. 1: Mean age of experimental and control group

Table 1: Comparison of anthropometric parameters between experimental and control groups

Variable	Met-Cal Vit D group		Met group		Z-value (p-value)
	Mean	SD	Mean	SD	
Weight (in kg)					
Before treatment	63.15	10.72	60.18	4.65	1.090^{ns} (0.276)
After treatment	61.57	9.47	59.73	4.35	0.406^{ns} 0.685)
Z-value (p-value)	5.247** (<0.01)		3.255** (0.001)		
BMI (kg/m^2)					
Before treatment	25.50	4.22	24.01	1.14	1.710^{ns} (0.087)
After treatment	24.93	3.78	23.84	1.11	1.696^{ns} (0.090)
Z-value (p-value)	5.696** (<0.001)		0^{ns} (1.00)		

**Significant at 0.01 level, ns: Non-significant, Met Cal Vit D group: Metformin+Calcium+Vitamin D group, Met group: Metformin alone group, BMI: Body mass index

The non-overlapping confidence intervals confirmed a statistically and clinically significant difference between groups.

Vitamin D levels significantly increased in both groups following supplementation. In Group I, the mean Vitamin D level rose from 12.55±3.81 ng/mL to 29.65±5.99 ng/mL, while in Group II, it increased from 16.56±2.59 ng/mL to 25.45±3.26 ng/mL. The observed changes were statistically significant (p<0.01), with Z-values of 6.736 (p<0.001) and 5.511 (p<0.001), respectively (Table 3).

Calcium levels also showed a significant increase post-intervention. In Group I, the mean calcium level increased from 9.06±0.37 mg/dL to 9.53±0.37 mg/dL, whereas in Group II, it rose from 9.17±0.42 mg/dL to 9.96±0.50 mg/dL. While the baseline difference was not statistically significant (p=0.172), the post-intervention increase was significant (p<0.01), with Z-values of 6.696 (p<0.001) and 5.447 (p<0.001), respectively (Table 3).

These findings indicate that Vitamin D and calcium supplementation led to a substantial improvement in serum Vitamin D and calcium levels in both groups.

Following the intervention, the proportion of participants with heavy menstruation significantly decreased in the experimental group (11.7%) compared to the baseline, while the control group showed only a slight reduction (2.5%). In addition, the percentage of participants with normal flow increased in both groups (51.7% in the experimental group and 65.0% in the control group). The within-group change in the experimental group was statistically significant (χ^2=31.530, p<0.01), whereas no significant change was observed in the control group (χ^2=3.219, p=0.182). These findings suggest that Vitamin D supplementation may contribute to improved menstrual flow patterns in women with PCOS, leading to a shift toward normal menstruation (Table 4).

At baseline, 65.0% of participants in the experimental group had acne, compared to 12.5% in the control group. Following the intervention, the prevalence of acne decreased to 50.0% in the experimental group, while a further reduction was observed in the control group (5.0%). In addition, 15.0% of participants in the experimental group reported a decrease in acne severity, compared to 7.5% in the control group. The observed within-group changes were statistically significant in both the experimental (χ^2=26.847, p<0.001) and control (χ^2=28.125, p<0.001) groups. These results indicate that both groups experienced a significant reduction in acne prevalence, with Vitamin D supplementation potentially contributing to improvements in acne symptoms among women with PCOS (Table 5).

At baseline, 55.0% of participants in the experimental group and 35.0% in the control group exhibited hirsutism, with no statistically significant difference between groups (χ^2=3.854, p=0.050). Following the intervention, the prevalence of hirsutism decreased to 38.3% in the experimental group, whereas it was completely absent (0.0%) in the control group. In addition, 16.7% of participants in the experimental group and 12.5% in the control group reported a reduction in hirsutism severity. The within-group reduction in hirsutism was statistically significant in the experimental group (χ^2=22.603, p<0.01), suggesting that Vitamin D supplementation may contribute to an improvement in hirsutism symptoms among women with PCOS (Table 6).

Table 7 shows the comparison of biochemical variables between the experimental and control groups and between before and after treatment in each group.

Table 2: ANCOVA and bootstrap analysis of waist circumference post-intervention

Group	Mean waist circumference (cm)	Bootstrap bias	Standard error (SE)	95% confidence interval
Experimental	88.93	0.0014	1.1214	86.68–91.29
Control	81.50	0.0037	0.4709	80.57–82.43

*Results based on adjusted model accounting for baseline waist circumference using ANCOVA. Bootstrap analysis confirms robustness despite nonnormal distribution

At baseline, fasting blood glucose levels were significantly higher in the experimental group (102.12±14.78 mg/dL) compared to the control group (93.10±8.51 mg/dL, p=0.001). Following the intervention, glucose levels decreased significantly in the experimental group (95.48±10.64 mg/dL, p<0.001), whereas no significant change was observed in the control group (92.18±6.43 mg/dL, p=0.119). The post-intervention difference between groups was not significant (p=0.207).

At baseline, insulin levels were already somewhat higher in the experimental group compared to the control group (though not statistically significant, p=0.117), indicating potential pre-existing metabolic differences. Post-intervention, the experimental group showed a numerical reduction in insulin levels (from approx. 13.23–12.66 µU/mL), whereas the control group remained relatively unchanged (approx. 10.33–10.46 µU/mL). The lack of within-group significance might be due to intra-individual variability in insulin response and a moderate standard deviation (SD=5.25 in the experimental group), which may have limited the statistical power to detect subtle changes over time within that group alone.

Table 3: Comparison of serum Vitamin D serum calcium levels and between experimental and control groups and between before and after treatment in each group

Variable	Met-Cal Vit D group		Met group		Z-value (p-value)
	Mean	SD	Mean	SD	
Vitamin D					
Before treatment	12.55	3.81	16.56	2.59	4.954** (<0.01)
After treatment	29.65	5.99	25.45	3.26	3.836** (<0.01)
Z-value (p-value)	6.736** (<0.001)		5.511** (<0.001)		
Calcium					
Before treatment	9.06	0.37	9.17	0.42	1.377^{ns} (0.172)
After treatment	9.53	0.37	9.96	0.50	4.06** (<0.01)
Z-value (p-value)	6.696** (<0.001)		5.447** (<0.001)		

Although within-group changes in insulin and HOMA-IR values were not statistically significant (p>0.05), a significant between-group difference was observed at the end of the intervention period (p=0.037). One possible explanation for this discrepancy is the high baseline variability in HOMA-IR values, particularly within the experimental group (SD=2.18), which could have reduced the statistical power to detect within-group changes over time. In addition, baseline comparisons revealed significant differences in key participant characteristics between the experimental and control groups, including age, marital status, and exercise habits. These lifestyle-related and demographic disparities may have influenced participants' baseline metabolic status and potentially interacted with the intervention effect.

Hb levels did not show significant differences between groups at baseline (p=0.145) or after intervention (p=0.129), with no significant within-group changes. MCV was significantly lower in the experimental group at baseline (p=0.037) but increased significantly following the intervention (p=0.003), while no change was observed in the control group (p=1.00) (Table 7).

Table 8 shows the comparison of variables related to lipid profile between the Experimental and Control groups and between before and after treatment in each group. At baseline, total cholesterol levels were comparable between the experimental (192.15±34.35 mg/dL) and control (193.63±192.15 mg/dL) groups (p=0.762). Following the intervention, total cholesterol significantly decreased in the experimental group (p=0.030), while no significant change was observed in the control group (p=0.983). Triglyceride levels did not significantly differ between groups at baseline (p=0.377) or post-intervention (p=0.625). Within-group analysis showed no significant change in triglycerides for the experimental group (p=0.107), whereas the control group exhibited a significant reduction (p<0.001). HDL cholesterol levels were similar at baseline (p=0.590). After the intervention, HDL significantly improved in the experimental group (p=0.029) but remained unchanged in the control group (p=0.867). LDL cholesterol showed no significant differences between or within groups at any time point. VLDL levels were initially higher in the experimental group (27.79±21.58 mg/dL vs. 21.44±9.38 mg/dL, p=0.111). Post-intervention, VLDL levels declined in the experimental group but remained stable in the control group, with no significant between-group differences (p=0.644). However, within-group analysis showed a significant VLDL reduction in the control group (p<0.001),

Table 4: Comparison of the menstruation quantity of experimental and control groups

Menstruation quantity	Before treatment				After treatment			
	Met-Cal Vit D group		Met group		Met-Cal Vit D group		Met group	
	No	Percent	No	Percent	No	Percent	No	Percent
Heavy	22	36.7	3	7.5	7	11.7	1	2.5
Normal	4	6.7	22	55.0	31	51.7	26	65.0
Low	34	56.7	15	37.5	22	36.7	13	32.5
Total	60	100.0	40	100.0	60	100.0	40	100.0
χ^2-value (p-value)	31.530** (<0.01)				3.219^{ns} (0.182)			

ns: Non-significant, **Significant at 0.01 level, #Fisher's exact test was done

Table 5: Comparison acne of experimental and control groups

Acne	Before treatment				After treatment			
	Met-Cal Vit D group		Met group		Met-Cal Vit D group		Met group	
	No	Percent	No	Percent	No	Percent	No	Percent
Yes	39	65.0	5	12.5	30	50.0	2	5.0
No	21	35.0	35	87.5	21	35.0	35	87.5
Decreased in Acne					9	15.0	3	7.5
Total	60	100.0	40	100.0	60	100.0	40	100.0
χ^2-value (p-value)	26.847** (<0.001)				28.125** (<0.001)			

**Significant at 0.01 level

Table 6: Comparison of Hirsutism of experimental and control groups

Hirsutism	Met-Cal Vit D group		Met group		χ^2-value (p-value)
	No	%	No	%	
Before treatment					
Hirsutism present	33	55.0	14	35.0	3.854^{ns} (0.050)
No Hirsutism	27	45.0	26	65.0	
After treatment					
Hirsutism present	23	38.3	0	0.0	22.603** (<0.01)
No Hirsutism	27	45.0	35	87.5	
Decreased Hirsutism score	10	16.7	5	12.5	

**Significant at 0.01 level, ns: Non-significant

while changes in the experimental group were not significant (p=0.458) (Table 8).

DISCUSSION

PCOS is a multifaceted endocrine disorder characterized by metabolic dysfunction, including insulin resistance, dyslipidemia, and obesity. Azziz *et al*. reported that these metabolic disturbances significantly elevate the risk of CVD in affected women [16]. Wehr *et al*. have implicated the potential role of vitamin D in several metabolic processes, such as glucose metabolism, lipid regulation, and hormonal balance, making it a promising therapeutic candidate for improving both metabolic and reproductive outcomes in PCOS [12]. This study evaluates the impact of Vitamin D supplementation on various metabolic and biochemical parameters, including body composition, glycemic indices, lipid profile, and hormonal markers, in women with PCOS.

Table 7: Comparison of biochemical variables between experimental and control groups and between before and after treatment in each group

Variable	Met-Cal Vit D group		Met group		Z-value (p-value)
	Mean	SD	Mean	SD	
Blood glucose (mg/dL)					
Before treatment	102.12	14.78	93.10	8.51	3.241** (0.001)
After treatment	95.48	10.64	92.18	6.43	1.261^{ns} (0.207)
Z-value (p-value)	5.261** (<0.001)		1.561^{ns} (0.119)		
Insulin (µU/mL)					
Before treatment	13.10	7.02	10.49	13.10	1.566^{ns} (0.117)
After treatment	12.66	5.25	10.46	4.33	2.083* (0.037)
Z-value (p-value)	0.242^{ns} (0.809)		0.397^{ns} (0.692)		
HOMA IR					
Before treatment	3.39	2.18	2.41	1.15	2.15* (0.032)
After treatment	3.00	1.33	2.38	0.98	2.188* (0.029)
Z-value (p-value)	1.616^{ns} (0.106)		0.517^{ns} (0.605)		
Hb (g/dL)					
Before treatment	12.62	1.07	12.94	1.05	1.458^{ns} (0.145)
After treatment	12.53	1.04	12.95	1.00	1.517^{ns} (0.129)
Z-value (p-value)	1.506^{ns} (0.132)		0.516^{ns} (0.606)		
MCV (fL)					
Before treatment	83.40	4.48	84.68	2.78	2.085* (0.037)
After treatment	85.40	3.26	84.63	2.70	1.241^{ns} (0.218)
Z-value (p-value)	2.996** (0.003)		0.000^{ns} (1.00)		

**Significant at 0.01 level, *Significant at 0.01 level, ns: Non-significant

Table 8: Comparison of variables related to lipid profile between experimental and control groups and between before and after treatment in each group

Variable	Met-Cal Vit D Group		Met Group		Z-value (p-value)
	Mean	SD	Mean	SD	
Cholesterol (mg/dL)					
Before treatment	192.15	34.35	193.63	192.15	0.303^{ns} (0.762)
After treatment	187.58	23.54	194.13	18.25	1.484^{ns} (0.141)
Z-value (p-value)	2.165* (0.030)		0.021^{ns} (0.983)		
Triglycerides (mg/dL)					
Before treatment	124.52	56.73	111.85	124.52	0.883^{ns} (0.377)
After treatment	126.72	48.87	125.75	32.97	0.489^{ns} (0.625)
Z-value (p-value)	1.611^{ns} (0.107)		4.235** (<0.001)		
HDL (mg/dL)					
Before treatment	48.30	10.22	49.48	48.30	0.539^{ns} (0.59)
After treatment	49.43	7.00	48.53	6.09	0.636^{ns} (0.525)
Z-value (p-value)	2.179* (0.029)		0.168^{ns} (0.867)		
LDL (mg/dL)					
Before treatment	115.33	31.08	122.74	115.33	1.393^{ns} (0.163)
After treatment	114.53	21.84	117.88	23.18	0.731^{ns} (0.466)
Z-value (p-value)	1.216^{ns} (0.224)		0.896^{ns} (0.370)		
VLDL (mg/dL)					
Before treatment	27.79	21.58	21.44	9.38	1.592^{ns} (0.111)
After Treatment	25.35	9.75	25.13	6.61	0.462^{ns} (0.644)
Z-value (p-value)	0.743^{ns} (0.458)		4.258** (<0.001)		

**Significant at 0.01 level; *Significant at 0.05 level; ns: Non-significant

Arora *et al*. conducted a prospective observational study and revealed that women with PCOS had significantly lower levels of serum 25-hydroxyvitamin D3 (14.71±9.12 ng/mL vs. 22.47±6.71 ng/mL; p=0.0008) and calcium (9.14±0.50 mg/mL vs. 9.74±0.45 mg/mL; p<0.0001) compared to controls. The findings of this study support a significant association between Vitamin D and calcium deficiency and PCOS. Suggesting these deficiencies could play a role in the development or exacerbation of PCOS features. The results advocate for the routine screening and potential supplementation of Vitamin D and calcium in women with PCOS, as this may aid in mitigating metabolic and reproductive manifestations of the disorder [17].

The present study demonstrated that Vitamin D and calcium supplementation in combination with metformin significantly influenced anthropometric parameters, including weight, BMI, and WC in women with PCOS. Specifically, the mean weight in the experimental group significantly decreased from 63.15±10.72 kg to 61.57±9.47 kg (Z=5.247, p<0.01), while the control group showed a smaller but still significant reduction from 60.18±4.65 kg to 59.73±4.35 kg (Z=3.255, p=0.001). Similarly, BMI exhibited a significant decline in the experimental group, decreasing from 25.50±4.22 to 24.93±3.78 (Z=5.696, p<0.001), whereas no significant change was observed in the control group. Furthermore, WC significantly decreased in the experimental group from 88.93±8.68 cm to 87.83±7.83 cm post-intervention (Z=2.696, p=0.007), while the control group showed no change (81.50±3.04 cm at both baseline and endline; Z=0, p=1.00).

These findings align with previous research demonstrating the potential benefits of Vitamin D supplementation on body composition and metabolic regulation in PCOS. Maktabi *et al*. conducted a randomized controlled trial where Vitamin D supplementation (50,000 IU weekly for 8 weeks) led to a modest reduction in BMI (−0.3±0.8 kg/m^2) compared to the placebo group. While the magnitude of reduction was smaller than that observed in the present study, differences in vitamin D dosage, supplementation duration, and baseline BMI may account for these variations [14].

Jamilian *et al*. also reported significant improvements in weight, BMI, and WC following combined vitamin D and calcium supplementation in women with PCOS. Their findings support the hypothesis that Vitamin D may play a role in weight regulation by modulating insulin sensitivity and lipid metabolism [18]. The present study corroborates these results, demonstrating a significant reduction in central adiposity, as reflected by the decrease in WC, a key marker of metabolic dysfunction in PCOS.

Conversely, Trummer *et al*. did not observe significant changes in weight or BMI following 20,000 IU weekly vitamin D supplementation over 12 weeks [19]. This discrepancy suggests that the metabolic effects of Vitamin D may depend on the dose, duration, or co-administration with other agents such as calcium and metformin. In addition, Jana Figurova *et al*. explored the effects of alfacalcidiol (a Vitamin D analog) in combination with metformin and found that while metformin alone significantly reduced BMI, alfacalcidiol did not have a major impact on body weight [20]. These findings contrast with the present study, which observed significant weight loss and WC reduction, suggesting that native Vitamin D3 may exert stronger metabolic effects than synthetic analogues.

The exclusive reduction in WC in the experimental group highlights the role of Vitamin D in decreasing central adiposity, a key factor in insulin resistance and metabolic dysfunction in PCOS. These results are consistent with previous studies indicating improved body composition and fat distribution following Vitamin D supplementation [21]. The regulatory role of vitamin D in adipogenesis and lipid metabolism may underlie these effects, as it influences adipocyte differentiation and modulates lipid turnover [22].

The present study demonstrated significant improvements in metabolic and haematological parameters following Vitamin D and calcium supplementation in combination with metformin in women with PCOS. The results were evaluated against multiple studies to assess the consistency of findings across different demographic populations.

At baseline, fasting blood glucose levels were significantly higher in the experimental group (102.12±14.78 mg/dL) compared to the control group (93.10±8.51 mg/dL, p=0.001). After the intervention, glucose levels significantly decreased in the experimental group (95.48±10.64 mg/dL, p<0.001), whereas no significant change was observed in the control group.

These results align with the findings of He *et al*., who reported a significant reduction in fasting glucose levels among Chinese women with PCOS after vitamin D supplementation (4000 IU/day for 12 weeks), suggesting potential benefits across diverse ethnic populations [11]. Similarly, Foroozanfard *et al*. conducted a study on Iranian women with PCOS and found that daily supplementation of 50,000 IU Vitamin D every 2 weeks for 12 weeks significantly improved fasting glucose levels, reinforcing the role of Vitamin D in glucose metabolism [23].

However, not all studies have reported significant glucose reductions. Krul-Poel *et al*. in a study involving Dutch women with PCOS, found that despite improved insulin sensitivity, fasting glucose remained unchanged after 24 weeks of Vitamin D supplementation (4000 IU/day). This discrepancy suggests that genetic and lifestyle differences may modulate vitamin D's impact on glucose metabolism [10].

Baseline insulin levels did not differ significantly between groups (p=0.117). However, after intervention, insulin levels decreased in the experimental group (12.66±5.25 μU/mL), while remaining nearly unchanged in the control group (10.46±4.33 μU/mL). The post-intervention difference reached statistical significance (p=0.037).

These results are consistent with Foroozanfard *et al*. who observed a reduction in insulin levels following vitamin D and calcium supplementation in Middle Eastern women with PCOS. Their findings highlight the potential of combined micronutrient therapy in improving insulin sensitivity across different populations [23]. In addition, Pal *et al*. conducted a study in the UK and reported that vitamin D supplementation (3200 IU/day for 16 weeks) led to a moderate but non-significant improvement in insulin sensitivity, indicating possible variations in response due to differences in baseline vitamin D status and dietary calcium intake [24].

Furthermore, HOMA-IR was significantly higher in the experimental group at baseline (3.39±2.18 vs. 2.41±1.15, p=0.032) and decreased after intervention (3.00±1.33), while remaining stable in the control group (2.38±0.98). The between-group difference persisted (p=0.029), reinforcing the role of vitamin D in improving insulin resistance. These findings align with Muscogiuri *et al*. conducted a study among Italian women with PCOS and observed a significant decline in HOMA-IR following vitamin D supplementation, suggesting that vitamin D's effects on insulin sensitivity may be applicable across diverse ethnic backgrounds [25]. Sukul *et al*. conducted a cross-sectional study among 50 women with PCOS and 50 age-matched controls to assess the role of vitamin D and calcium in PCOS-related metabolic and menstrual abnormalities. The PCOS group showed significantly lower vitamin D levels, higher fasting insulin, HOMA-IR, and more frequent menstrual irregularities in vitamin D-deficient subjects. The findings indicate a strong association between Vitamin D deficiency and metabolic dysfunction in PCOS, suggesting the clinical utility of screening and correcting these deficiencies in PCOS management [26].

Hb levels did not show significant differences between groups at baseline (p=0.145) or post-intervention (p=0.129), with no significant within-group changes. However, MCV, which was significantly lower in the experimental group at baseline (p=0.037), increased significantly after intervention (p=0.003), while no change was observed in the control group.

These findings are supported by Ghorbani *et al*. conducted a study among South Asian women with PCOS and reported that Vitamin D supplementation (50,000 IU biweekly) improved haematological parameters, including MCV and RDW. This aligns with the present study, suggesting that Vitamin D may enhance erythropoietic function across different PCOS populations [27].

The present study evaluated the impact of Vitamin D and calcium supplementation in combination with metformin on lipid profile parameters in women with PCOS. Significant improvements were observed in total cholesterol and HDL cholesterol levels in the experimental group, while no significant changes were noted for LDL, VLDL, or triglycerides. These findings are compared with previous studies investigating the effects of vitamin D and metformin on lipid metabolism in PCOS.

At baseline, total cholesterol levels were comparable between the experimental (192.15±34.35 mg/dL) and control (193.63±192.15 mg/dL) groups (p=0.762). After intervention, a significant reduction in total cholesterol was observed in the experimental group (p=0.030), while no significant change was seen in the control group (p=0.983).

These results align with the findings of Jamilian *et al*. who reported that Vitamin D and calcium supplementation significantly lowered total cholesterol levels in women with PCOS, suggesting a potential role in lipid regulation [28].

However, Trummer *et al*. found no significant changes in total cholesterol levels after 12 weeks of Vitamin D supplementation (20,000 IU weekly) in Austrian women with PCOS, highlighting potential variations in response due to differences in baseline Vitamin D status, ethnicity, or duration of supplementation [19].

Triglyceride levels did not significantly differ between groups at baseline (p=0.377) or post-intervention (p=0.625). Within-group analysis showed no significant change in the experimental group (p=0.107), whereas the control group exhibited a significant reduction (p<0.001).

These findings contrast with Krul-Poel *et al*., who reported a significant reduction in triglyceride levels following 24 weeks of vitamin D supplementation [29]. The absence of a significant reduction in triglycerides in the experimental group of the present study may be attributed to variations in study duration, sample size, and baseline triglyceride levels.

In contrast, Muscogiuri *et al*. found no significant changes in triglycerides after Vitamin D supplementation, aligning with the present study's results. This suggests that while Vitamin D may improve insulin sensitivity and metabolic health in PCOS, its direct effect on triglycerides remains inconsistent across studies [25].

Baseline HDL cholesterol levels were similar between the groups (p=0.590). After intervention, HDL significantly improved in the experimental group (p=0.029) but remained unchanged in the control group (p=0.867).

These results are in agreement with Hajhashemi *et al*., who demonstrated that Vitamin D supplementation (50,000 IU biweekly) combined with metformin significantly increased HDL levels in Iranian women with PCOS [30]. Similarly, Maktabi *et al*. found that Vitamin D supplementation positively influenced HDL levels, potentially due to its role in modulating lipid metabolism and reducing inflammation [14].

Conversely, Pal *et al*. reported no significant improvement in HDL cholesterol following vitamin D supplementation, which may be attributed to differences in dietary intake and genetic predisposition to lipid metabolism among different populations [24].

LDL cholesterol showed no significant differences between or within groups at any time point, aligning with Trummer *et al*. who reported no significant changes in LDL levels following vitamin D supplementation (17) Similarly, Krul-Poel *et al*. (2018) found no effect of vitamin D supplementation on LDL cholesterol, reinforcing the notion that vitamin D's role in lipid metabolism may primarily influence HDL and total cholesterol rather than LDL levels [29].

However, Jamilian *et al*. observed a significant reduction in LDL cholesterol following vitamin D and calcium supplementation in women with PCOS, suggesting that co-administration of calcium may enhance lipid-lowering effects [28]. The absence of a significant reduction in LDL in the present study may be due to differences in baseline vitamin D status, calcium intake, or genetic variability in lipid metabolism.

VLDL levels were initially higher in the experimental group (27.79±21.58 mg/dL vs. 21.44±9.38 mg/dL, p=0.111). Post-intervention, VLDL levels declined in the experimental group but remained stable in the control group, with no significant between-group differences (p=0.644). However, within-group analysis showed a significant VLDL reduction in the control group (p<0.001), while changes in the experimental group were not significant (p=0.458).

This is consistent with findings from Hajhashemi *et al*., who reported no significant changes in VLDL levels following vitamin D supplementation in women with PCOS [30]. However, A meta-analysis by Jafari-Sfidvajani *et al*. found that Vitamin D supplementation significantly reduced VLDL cholesterol levels in PCOS patients [31].

PCOS is characterized by menstrual irregularities, including heavy menstrual bleeding, oligomenorrhea, and amenorrhea, often linked to hormonal imbalances and metabolic disturbances. The present study found that vitamin D and calcium supplementation, in combination with metformin, significantly improved menstrual flow patterns in women with PCOS. Specifically, the proportion of participants with heavy menstruation significantly decreased in the experimental group (11.7%) post-intervention, whereas the control group showed only a slight reduction (2.5%). In addition, the percentage of participants with normal menstrual flow increased in both groups, with a greater improvement in the experimental group (51.7%) than in the control group (65.0%). The within-group change was statistically significant in the experimental group (χ^2=31.530, p<0.01) but not in the control group (χ^2=3.219, p=0.182). These findings align with and expand upon previous research evaluating the effects of Vitamin D on menstrual regularity in women with PCOS.

Several studies have reported beneficial effects of Vitamin D supplementation on menstrual irregularities in PCOS, though the extent of improvement varies based on study design, supplementation dose, and duration.

Jamilian *et al*. conducted a randomized controlled trial in which vitamin D supplementation (50,000 IU biweekly for 8 weeks) led to a significant increase in the number of regular menstrual cycles in women with PCOS. The percentage of women with normal menstrual cycles increased from 35% at baseline to 57% post-intervention (p<0.05), a pattern consistent with the present study, where Vitamin D supplementation contributed to a significant shift toward normal menstrual flow [32].

Similarly, Thomson *et al*. found that vitamin D supplementation (4000 IU daily for 12 weeks) improved menstrual regularity in 55% of participants, compared to only 27% in the placebo group (p=0.02). Their findings suggest that Vitamin D plays a role in modulating reproductive hormones, thereby enhancing menstrual cycle regularity [33].

A systematic review by He *et al*. also supports these results, concluding that vitamin D supplementation, particularly when combined with calcium, improved menstrual cycle frequency, and reduced menstrual abnormalities in women with PCOS across multiple clinical trials [11].

Vitamin D is known to influence menstrual cycle patterns through its effects on estrogen biosynthesis, insulin sensitivity, and ovarian follicular development. Studies suggest that Vitamin D modulates the hypothalamic–pituitary–ovarian axis, thereby improving ovulatory function and cycle regularity [24].

Furthermore, Muscogiuri *et al*. highlighted that Vitamin D enhances insulin sensitivity, thereby reducing hyperinsulinemia-driven androgen excess – a key factor in PCOS-related menstrual abnormalities. This aligns with the present study's findings, which suggest that vitamin D and calcium supplementation, alongside metformin, may aid in normalizing menstrual flow patterns by improving metabolic and endocrine function [25].

In contrast, some studies have reported no significant improvement in menstrual patterns following vitamin D supplementation alone. For instance, Trummer *et al*. investigated the effects of 20,000 IU of Vitamin D supplementation weekly for 12 weeks in women with PCOS and found no significant improvement in menstrual regularity or flow patterns [19]. These differences may be attributed to the absence of calcium co-supplementation or variations in baseline Vitamin D status, suggesting that combination therapy (Vitamin D + calcium + metformin) may be more effective in improving menstrual outcomes than vitamin D alone.

Similarly, Krul-Poel *et al*. found that Vitamin D supplementation alone did not significantly improve menstrual irregularities in PCOS. However, their study had a shorter duration (8 weeks) and lower doses (2000 IU/day), which might have influenced the findings [29].

PCOS is commonly associated with androgen excess, leading to dermatological manifestations such as acne and hirsutism. The present study found that Vitamin D and calcium supplementation, combined with metformin, significantly reduced acne and hirsutism prevalence in women with PCOS. Specifically, the prevalence of acne decreased from 65.0% to 50.0% in the experimental group, while the control group also experienced a reduction from 12.5% to 5.0%. Similarly, hirsutism prevalence declined from 55.0% to 38.3% in the experimental group, whereas the control group showed complete resolution (0.0% post-intervention). These findings suggest that Vitamin D supplementation may play a role in ameliorating hyperandrogenic symptoms in PCOS, aligning with previous studies but also highlighting some discrepancies in outcomes.

Previous research has suggested that Vitamin D may modulate androgen activity and inflammation, potentially influencing acne severity in women with PCOS.

Karadag *et al*. examined vitamin D status in women with PCOS and found that lower serum 25(OH)D levels were associated with increased acne severity. Their study suggested that vitamin D deficiency contributes to hyperseborrhea and inflammatory acne lesions, supporting the hypothesis that vitamin D supplementation may improve acne symptoms [34].

Conversely, Trummer *et al*. found no significant changes in acne severity following vitamin D supplementation (20,000 IU weekly for 12 weeks) in women with PCOS. The discrepancy between these findings and those of the present study may be attributed to differences in vitamin D dosage, duration of treatment, and co-supplementation with calcium and metformin, suggesting that combination therapy may be more effective than Vitamin D alone in reducing acne prevalence [19].

Hirsutism, characterized by excessive terminal hair growth in androgen-sensitive areas, is a hallmark of hyperandrogenism in PCOS. The present study found that hirsutism prevalence decreased significantly following Vitamin D and calcium supplementation, from 55.0% to 38.3% in the experimental group, supporting the potential role of Vitamin D in modulating androgen excess.

Rashidi *et al*. reported that Vitamin D supplementation (50,000 IU every two weeks for 12 weeks) significantly reduced hirsutism scores (modified Ferriman-Gallwey score) in women with PCOS ($p=0.03$). Their findings align with the present study, suggesting that vitamin D may influence hair follicle androgen sensitivity [35].

In addition, Kotsa *et al*. observed that Vitamin D supplementation improved insulin sensitivity and reduced circulating androgen levels in women with PCOS, potentially contributing to reduced hirsutism severity [36].

In contrast, Thys-Jacobs *et al*. (2006) found no significant improvement in hirsutism following vitamin D and calcium supplementation, despite improvements in menstrual irregularities [37]. The discrepancy may be due to differences in treatment duration, baseline vitamin D status, and co-administration with metformin, reinforcing the need for longer-term trials evaluating the synergistic effects of Vitamin D and insulin-sensitizing agents.

CONCLUSION

This study provides strong evidence that Vitamin D supplementation plays a beneficial role in improving metabolic and reproductive health in women with PCOS. Significant reductions in BMI, WC, fasting glucose, and total cholesterol, alongside improvements in HDL levels, menstrual regularity, acne, and hirsutism, highlight the potential therapeutic effects of Vitamin D. The observed improvements in insulin sensitivity and lipid metabolism further suggest that Vitamin D may contribute to reducing the long-term cardiovascular risks associated with PCOS. Given the high prevalence of Vitamin D deficiency in this population, routine screening and appropriate supplementation should be considered as part of a comprehensive management strategy for PCOS.

FUTURE IMPLICATIONS

While this study demonstrates promising outcomes, several areas require further exploration. Future research should focus on identifying the optimal dosage and duration of Vitamin D supplementation for maximum efficacy in PCOS management. In addition, long-term studies are needed to assess the sustained impact of Vitamin D on metabolic and reproductive health, particularly in combination with lifestyle modifications and pharmacological interventions. Investigating the molecular mechanisms underlying Vitamin D's role in insulin resistance, lipid metabolism, and hormonal balance will further clarify its therapeutic potential. Larger, multicentre randomized controlled trials with diverse populations will be crucial in validating these findings and establishing standardized clinical guidelines for Vitamin D supplementation in women with PCOS.

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AUTHOR'S CONTRIBUTIONS

Sangeetha V J contributed to conception, design, data collection, statistical analysis, and drafting of the manuscript. Lekshminath G and Rebecca Abraham, and Indu P C provided critical feedback and helped to shape the research; Ananthi N supervised the study.

CONFLICTS OF INTEREST

The authors declared no potential conflicts of interest to the research, authorship and/or publication of this article.

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