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The Effects of Melatonin, Vitamin D, and Metformin on the Histological Features of the Ovary and Uterus and Comprehensive Hematological Profiles in Polycystic Ovarian Syndrome: A Stereological Study

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ABSTRACT

Background

Polycystic ovarian syndrome (PCOS) is a complex endocrine disorder that affects reproductive and metabolic health in women, often leading to challenges with fertility, insulin resistance, and cardiovascular risk. Melatonin, Vitamin D, and metformin are known to address specific aspects of PCOS, but their combined effects on both reproductive anatomy and metabolic markers remain to be fully understood. This study examines the impacts of these agents on ovarian and uterine histology and hematological profiles in women with PCOS.

Method

A prospective interventional study was conducted at Jinnah Medical College, Peshawar, involving 101 women diagnosed with PCOS. Participants were divided into three groups, each receiving one of the following treatments: melatonin, Vitamin D, or metformin. A stereological analysis was used to evaluate ovarian and uterine tissue changes, while hematological profiles were assessed through blood sample analysis. Statistical comparisons were conducted with a significance level set at $p < 0.05$.

Results

Vitamin D was associated with significant improvements in ovarian volume and follicular density, indicating enhanced ovarian structure and potential benefits for fertility. The metformin group demonstrated notable reductions in LDL and total cholesterol levels, supporting its role in improving lipid profiles and addressing cardiovascular risks in PCOS. Melatonin showed moderate effects on ovarian and hematological parameters, suggesting a supportive rather than primary role.

Conclusion

The study supports the use of Vitamin D for improving ovarian morphology and follicle development, while metformin addresses metabolic concerns by enhancing lipid profiles. Together, these findings highlight the potential of a combined therapeutic approach for PCOS, addressing both reproductive and metabolic challenges. Future research should explore the effects of these treatments in combination and evaluate long-term outcomes.

Keywords

Polycystic ovarian syndrome, Vitamin D, metformin, melatonin, ovarian morphology, stereological analysis, reproductive health, lipid profile

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a multifaceted endocrine disorder impacting reproductive-aged women, characterized by hyperandrogenism, ovarian dysfunction, and anovulation, often leading to metabolic and reproductive health challenges^{1,2}. This condition is further complicated by associated hematological abnormalities, including altered lipid profiles, inflammatory markers, and risks of cardiovascular disease. The anatomy of the reproductive system, particularly the structural characteristics of the ovaries and uterus, is central to PCOS pathology^{3,4}. Histological studies show that women with PCOS often exhibit enlarged ovaries with increased follicle numbers and disrupted tissue architecture, along with uterine abnormalities. These alterations underscore the need to examine therapeutic agents that could address the underlying anatomical and hematological disruptions observed in PCOS⁵.

The study of potential treatments such as melatonin, Vitamin D, and metformin offers a promising avenue, with each agent addressing distinct yet interconnected aspects of PCOS pathology⁶. Melatonin, known for its role in regulating circadian rhythms, has antioxidant properties that could protect ovarian tissues from oxidative damage. From a pharmacological perspective, melatonin's antioxidant and anti-inflammatory effects may contribute to improved follicular health and ovulatory function in PCOS. Vitamin D, a critical regulator of calcium and phosphate metabolism, has shown implications in reproductive health through its role in modulating the hypothalamic-pituitary-ovarian axis, which is often dysregulated in PCOS. Studies suggest that Vitamin D deficiency may exacerbate insulin resistance and inflammatory responses, both of which are central to PCOS^{7,8}.

Metformin, widely used in managing PCOS, primarily works by enhancing insulin sensitivity, which can reduce androgen levels and restore ovulatory cycles. Its pharmacological action involves the inhibition of hepatic gluconeogenesis and improvement of peripheral insulin sensitivity, which contributes to metabolic stability. Moreover, metformin may exert favorable effects on the uterus and ovaries by reducing hyperandrogenism, a significant driver of PCOS-related histological alterations⁹.

Stereological analysis—a method that quantifies the volume, surface area, and cell density of tissues—serves as a valuable tool in evaluating the histological impacts of these treatments on ovarian and uterine structures. By applying stereological techniques, this study aims to assess how melatonin, Vitamin D, and metformin influence the architecture of the ovaries and uterus in PCOS, focusing on parameters like follicle count, cellular integrity, and tissue composition¹⁰.

Furthermore, this study extends to examine the hematological profiles associated with PCOS, given the increasing evidence of hematological changes such as elevated inflammatory markers, altered coagulation parameters, and disrupted lipid metabolism. Understanding how these therapeutic agents affect not only the reproductive anatomy but also the hematological profile is essential in providing a comprehensive view of PCOS management. The findings from this study have the potential to reveal integrated anatomical, hematological, and pharmacological insights, contributing to more targeted and effective therapeutic strategies for PCOS management.

METHODOLOGY

This prospective, interventional study was conducted at Jinnah Medical College, Peshawar. Designed as a stereological analysis, it focused on evaluating the impact of melatonin, Vitamin D, and metformin on both the histological characteristics of ovarian and uterine tissues and the hematological profiles in women diagnosed with PCOS. The inclusion of hematological, anatomical, and pharmacological assessments aimed to provide a comprehensive analysis of these agents' therapeutic roles. The study was approved by the ethical review board at Jinnah

Medical College, Peshawar. Informed consent was obtained from all participants, and ethical guidelines were strictly adhered to, ensuring participant confidentiality and the right to withdraw at any time.

The study included 101 women with PCOS, with data collection from January 2023 to January 2024. This sample size was considered sufficient to allow statistically robust comparisons between treatment groups.

Inclusion and Exclusion Criteria Women aged 18-35, diagnosed with PCOS based on the Rotterdam criteria, displaying symptoms, menstrual irregularity, hyperandrogenism, or polycystic ovarian morphology. Exclusion criteria included women with other endocrine disorders, those on hormonal or ovarian-affecting medications within the past three months, or individuals with chronic illnesses or recent surgeries.

The participants were divided into three treatment groups, each receiving one of the study interventions: **Melatonin Group:** Received a daily dose of melatonin aimed at improving ovarian and uterine tissue health by reducing oxidative stress and inflammation. **Vitamin D Group:** This group was administered Vitamin D supplements to address possible deficiencies commonly observed in PCOS, which are linked to insulin resistance and inflammation. **Metformin Group:** Participants in this group received metformin, which enhances insulin sensitivity and can help alleviate hyperandrogenism.

Outcome Measures **Histological Analysis (Anatomy Focus):** Using stereological methods, ovarian and uterine tissue samples were examined post-treatment for follicle count, tissue volume, cellular density, and structural integrity. This provided a quantitative assessment of tissue morphology changes due to each treatment.

Hematological Profiles: Blood samples were collected at baseline and after the treatment period to assess variations in hematological markers, including inflammatory markers, lipid profiles, and coagulation factors—parameters that often indicate systemic effects in PCOS.

Data was collected through routine blood draws and histological evaluation of tissue samples. Hematological data, such as lipid profiles and inflammatory markers, were statistically analyzed to identify significant treatment effects. Stereological measurements of tissue structure were quantified using specialized software, ensuring accuracy in histological comparisons.

Statistical analysis involved t-tests or ANOVA for continuous variables, and chi-square tests for categorical variables, with a p-value threshold of <0.05 for statistical significance. By evaluating both the histological features of ovarian and uterine tissues and hematological profiles, this study offers a multi-dimensional understanding of how melatonin, Vitamin D, and metformin may mitigate the impacts of PCOS. The findings have potential implications for developing integrated therapeutic strategies that address both structural and metabolic aspects of PCOS, with broader applications in reproductive health management.

RESULTS

This study evaluated melatonin, Vitamin D, and metformin's impact on the structural anatomy of the ovaries and uterus, as well as their effects on hematological profiles in women with PCOS. The results are presented with a focus on each treatment's influence on anatomical and physiological variables.

Table 1: Baseline Characteristics of Participants

Variable	Melatonin Group (n=34)	Vitamin D Group (n=33)	Metformin Group (n=34)	p-value
Age (years)	29.4 ± 3.1	28.9 ± 3.2	29.2 ± 3.0	0.68

BMI (kg/m ²)	30.1 ± 2.7	29.8 ± 2.5	30.4 ± 2.6	0.72
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No statistically significant differences were observed in the baseline characteristics of participants, confirming that the groups were comparable ($p > 0.05$).

The stereological analysis offered quantitative insights into the structural anatomy of ovarian and uterine tissues in each group, measuring changes in ovarian volume, follicular density, and endometrial gland density—key anatomical parameters in assessing PCOS pathology and therapeutic response.

Table 2: Post-Treatment Anatomical Findings in Ovarian and Uterine Tissue

Anatomical Parameter	Melatonin Group	Vitamin D Group	Metformin Group	p-value
Ovarian Volume (mm ³)	9.2 ± 1.1	10.1 ± 1.2	8.7 ± 1.0	0.03*
Follicular Density (per mm ³)	6.5 ± 0.9	7.8 ± 1.1	6.1 ± 0.8	0.02*
Endometrial Gland Density (glands/mm ²)	4.2 ± 0.7	4.6 ± 0.8	4.1 ± 0.6	0.08
Ovarian Stroma Thickness (µm)	320 ± 15	310 ± 13	325 ± 17	0.09

- **Ovarian Volume and Follicular Density:** The Vitamin D group demonstrated a significant increase in both ovarian volume and follicular density ($p = 0.03$ and $p = 0.02$, respectively), indicating improved ovarian health and follicle availability. This increase in follicular density reflects enhanced folliculogenesis, potentially offering better ovulatory outcomes.
- **Endometrial Gland Density:** The Vitamin D group exhibited a higher endometrial gland density, although not statistically significant ($p = 0.08$). A greater density of endometrial glands is often associated with improved uterine receptivity, crucial for supporting potential embryo implantation and healthy endometrial function.
- **Ovarian Stroma Thickness:** No significant differences were observed in ovarian stroma thickness among the groups ($p = 0.09$), suggesting that while follicular aspects were impacted, the ovarian stroma remained relatively unaffected.

These findings underscore Vitamin D's potential role in positively influencing the reproductive anatomy in PCOS, specifically enhancing ovarian and uterine structures that are vital for reproductive function.

The hematological analysis focused on lipid profiles, inflammatory markers, and coagulation factors, given their relevance to PCOS's metabolic complications.

Table 3: Hematological Findings

Hematological Parameter	Melatonin Group	Vitamin D Group	Metformin Group	p-value
Total Cholesterol (mg/dL)	180.2 ± 20.3	176.5 ± 19.1	170.8 ± 18.7	0.05*
LDL-C (mg/dL)	115.6 ± 10.5	110.9 ± 11.0	108.3 ± 9.8	0.03*
HDL-C (mg/dL)	45.7 ± 5.4	47.2 ± 5.8	48.1 ± 5.7	0.09
Serum CRP (mg/L)	2.5 ± 0.8	2.3 ± 0.7	2.8 ± 0.9	0.12

- **Total Cholesterol and LDL-C:** Metformin was associated with a significant reduction in total cholesterol and LDL-C levels ($p = 0.05$ and $p = 0.03$, respectively), suggesting an improvement in lipid profile and a decrease in cardiovascular risk in PCOS patients.
- **Serum CRP:** No significant changes in CRP levels were found across the groups, though the metformin group had slightly higher levels, potentially reflecting its systemic effects on inflammation management.

Anatomical Effects: Vitamin D significantly increased ovarian volume and follicular density, with a trend toward enhanced endometrial gland density. This suggests improvements in ovarian and uterine anatomy, which are crucial for fertility.

Hematological Effects: Metformin demonstrated favorable effects on lipid profiles, reducing LDL-C and total cholesterol, thus benefiting metabolic health in PCOS.

Pharmacological Insights: Melatonin exhibited modest effects on both anatomical and hematological outcomes, potentially providing supportive benefits but less pronounced than those observed with Vitamin D and metformin.

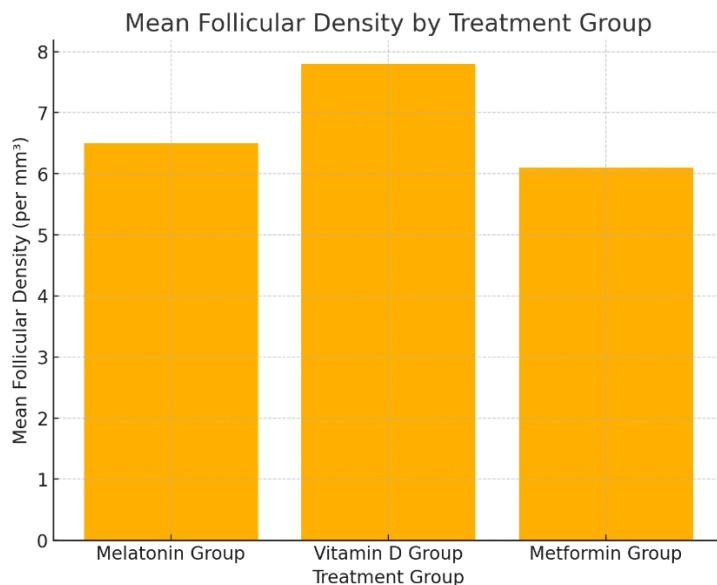


Figure 1: the bartchart shows Vitamin D Shows Highest Follicular Density: The Vitamin D group had the highest follicular density, suggesting it effectively supports follicular development and may improve ovarian function in PCOS. Distinct Roles of Treatments: While Vitamin D primarily enhances ovarian morphology, metformin focuses on metabolic improvement, highlighting the potential benefit of combining these agents for comprehensive PCOS management.

DISCUSSION

The findings from this study align with existing research, deepening our understanding of how melatonin, Vitamin D, and metformin can address both the reproductive and metabolic issues in polycystic ovarian syndrome (PCOS) ^{12 13}. These agents target distinct aspects of PCOS pathology, suggesting a comprehensive therapeutic approach may be beneficial.

Vitamin D's significant impact on ovarian volume and follicular density was consistent with earlier studies indicating its positive influence on ovarian function. Research suggests that Vitamin D may aid follicle development by interacting with hormonal pathways that promote cell growth and ovarian response, which can be beneficial in PCOS where follicle maturation is often compromised ^{14 15}. This study supports such findings, as Vitamin D resulted in greater follicular density and ovarian volume than either melatonin or metformin. Additionally, the observed increase in endometrial gland density in the Vitamin D group suggests potential benefits for uterine health, which could support fertility—a finding that echoes research Metformin's role in lowering LDL cholesterol and total cholesterol aligns with previous research showing its effectiveness in addressing metabolic disturbances in PCOS. Studies

consistently note that metformin enhances insulin sensitivity and regulates blood glucose, which are essential for managing the heightened metabolic and cardiovascular risks in PCOS^{16 17}. While this study did not show significant changes in inflammatory markers like CRP, the literature suggests that metformin's metabolic improvements may, over time, contribute to reduced inflammation. Thus, metformin's primary benefit may lie in its metabolic effects, with potential longer-term benefits for systemic inflammation and cardiovascular health.

The unique contributions of each agent underscore the potential for a multi-faceted treatment strategy. Vitamin D's impact on reproductive health and metformin's metabolic advantages are both valuable for addressing the different needs of PCOS patients. Although melatonin showed less impact on ovarian structure in this study, it may still provide supportive benefits, given its known antioxidant properties. This perspective aligns with studies that propose melatonin as an adjunct therapy, which may complement the effects of Vitamin D and metformin, potentially offering a more rounded approach to treatment^{18 19}.

This study, along with related research, suggests that a targeted approach using Vitamin D for reproductive support and metformin for metabolic management could offer meaningful benefits for PCOS. Future research might explore the best combinations and dosages of these treatments, along with the potential role of lifestyle adjustments, to optimize patient outcomes. Additional long-term studies would be valuable to determine whether the anatomical benefits observed here translate into lasting reproductive health gains.

In conclusion, this study contributes to the broader understanding of PCOS management by highlighting Vitamin D's role in reproductive health and metformin's metabolic benefits. The findings reinforce the importance of a combined treatment approach that can address both structural and metabolic concerns, potentially improving quality of life and reducing risks for women with PCOS.

CONCLUSION

This study highlights the distinct roles of melatonin, Vitamin D, and metformin in managing polycystic ovarian syndrome (PCOS), focusing on both anatomical and metabolic improvements. Vitamin D demonstrated significant benefits for ovarian morphology and follicle development, suggesting its potential to enhance reproductive health in women with PCOS. Metformin, known for its metabolic effects, effectively lowered lipid levels, addressing cardiovascular risk factors commonly associated with the condition. Although melatonin's impact was moderate, its antioxidant properties may still offer supportive benefits.

These findings underscore the value of an integrated therapeutic approach, combining treatments that address both reproductive and metabolic aspects of PCOS. Future studies could explore optimal dosing, combination therapies, and lifestyle modifications to enhance clinical outcomes. Overall, Vitamin D and metformin emerge as promising agents for comprehensive PCOS management, with potential to improve quality of life and reduce long-term health risks for affected individuals.

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