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The Biochemical and Pharmacological Effects of Melatonin and Clomiphene Citrate on Histopathological Features of the Ovary and Uterus in Women with Polycystic Ovarian Syndrome

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ABSTRACT

Background

Polycystic ovarian syndrome (PCOS) is a prevalent endocrine disorder among reproductive-aged women, characterized by hormonal dysregulation, metabolic abnormalities, and structural ovarian changes. Conventional treatments, including clomiphene citrate, often have variable efficacy, particularly in ovulation induction. Melatonin, recognized for its antioxidant and anti-inflammatory properties, has shown potential as an adjunct therapy in managing PCOS due to its positive effects on ovarian and uterine health. This study aims to evaluate the effects of melatonin and clomiphene citrate, both individually and in combination, on hormonal, metabolic, and histopathological outcomes in women with PCOS.

Methodology

This 'randomized controlled trial', conducted at 'Jinnah Medical College' from January 2022 to January 2023, involved 120 women diagnosed with PCOS. 'Participants were assigned to three groups: Group A received melatonin therapy', Group B received clomiphene citrate, and Group C received a combination of both. Key outcome measures included hormonal levels (LH, FSH, testosterone, and AMH), metabolic parameters (HOMA-IR, HDL, and triglycerides), and histopathological assessments (follicle count, follicular size, and endometrial thickness). Data were analyzed using ANOVA 'and Chi-square tests, with significance set at $p < 0.05$'.

Results

The combination therapy group showed the most significant improvements in ovulation and pregnancy rates (75% and 35%, respectively, $p < 0.05$). Combination therapy also yielded favourable hormonal and metabolic profiles, with lower serum testosterone, improved LH/FSH ratios, and better HOMA-IR and lipid levels. Histopathological outcomes, including follicular size, count, and endometrial thickness, were more pronounced in the combination group, supporting enhanced ovarian function and uterine receptivity.

Conclusion

The findings suggest that combining melatonin with clomiphene citrate may optimize treatment outcomes in women with PCOS, particularly in those unresponsive to clomiphene citrate alone. This combined approach improves both biochemical and structural ovarian characteristics and shows promise as a safer, more effective PCOS management strategy.

Keywords: Polycystic ovarian syndrome, PCOS, melatonin, clomiphene citrate, ovulation induction, hormonal profile, histopathology, combination therapy, reproductive health

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting reproductive-aged women worldwide [1], characterized by hormonal imbalances, metabolic complications, and distinctive ovarian morphology [2]. The disorder manifests as a triad of hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology, with potential long-term impacts on fertility, metabolic health, and quality of life [3]. Despite its prevalence, effective treatments targeting both the hormonal and histopathological aspects of PCOS remain limited.

Current treatment options for PCOS primarily focus on managing symptoms through pharmacological interventions [4]. Clomiphene citrate, a selective estrogen receptor modulator, is commonly prescribed to induce ovulation in women with PCOS, yet the therapeutic response varies widely among patients [5]. Melatonin, a hormone known for its antioxidant and anti-inflammatory properties, has recently emerged as a potential adjunct therapy in managing PCOS due to its favorable effects on follicular development and ovarian environment [6 7].

This study evaluates the biochemical, histopathological, and clinical effects of melatonin and clomiphene citrate, both individually and in combination, on women diagnosed with PCOS. By comparing the impacts on hormonal profiles, metabolic parameters, and histological features of the ovary and uterus, the study aims to provide insights into the potential benefits of melatonin in enhancing the therapeutic response to clomiphene citrate, addressing both the biochemical and structural manifestations of PCOS.

METHODOLOGY

This took place at 'Jinnah Medical College from January 2022 to January 2023', aiming to investigate the biochemical, histopathological, and clinical effects of melatonin and clomiphene citrate therapies, both separately and in combination, in women diagnosed with polycystic ovarian syndrome (PCOS). The study included a sample size of 120 participants, carefully selected based on specific inclusion and exclusion criteria to ensure relevance and minimize confounding factors.

This randomized controlled trial assigned participants into three groups:

- Group A: Received melatonin therapy.
- Group B: Received clomiphene citrate therapy.
- Group C: Received combination therapy of melatonin and clomiphene citrate.

Ethical approval for the study was obtained from the Institutional Review Board of Jinnah Medical College, and all participants provided written informed consent.

Participants were recruited through referrals and advertisement postings at Jinnah Medical College. Inclusion criteria included women aged 18 to 35 years with a confirmed diagnosis of PCOS based on the Rotterdam criteria, demonstrating at least two of the following: oligo/anovulation, hyperandrogenism, and polycystic ovarian morphology on ultrasound. Women with other endocrine disorders, thyroid dysfunction or diabetes mellitus, were excluded, as were those currently taking hormonal medications or ovulation-inducing drugs within three months prior to the study.

A sample size of 120 was determined to provide sufficient statistical power (80%) to detect meaningful differences between groups with a confidence level of 95% and considering an anticipated dropout rate of approximately 10%.

Intervention Protocols

- Group A: Participants received an oral dose of melatonin, 3 mg daily, administered at bedtime.
- Group B: Participants received an oral dose of 'clomiphene citrate, 50 mg daily, administered for five consecutive days starting on day 5 of the menstrual cycle'.

• Group C: Participants received a combination of both melatonin (3 mg daily at bedtime) and 'clomiphene citrate (50 mg daily for five days beginning on day 5 of the menstrual cycle)'. The treatment period lasted for three consecutive menstrual cycles, with regular monitoring of adherence and compliance through participant check-ins and follow-ups.

'Data were collected at baseline and at the end of the treatment period'. Assessments included demographic information, clinical measurements, biochemical markers, and histopathological evaluation of ovarian and uterine tissues.

Biochemical Assessments: Blood samples were collected from each participant in the 'early follicular phase of the menstrual cycle, before and after the intervention'. The following biochemical markers were measured:

• Hormonal profile: Luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and anti-Müllerian hormone (AMH).

• Metabolic profile: Fasting blood glucose, fasting insulin, homeostasis model assessment for insulin resistance (HOMA-IR), high-density lipoprotein (HDL), and triglycerides.

Biochemical assays were conducted in the Jinnah Medical College laboratory following standardized procedures. Hormone levels were measured using enzyme-linked immunosorbent assay (ELISA) kits, while metabolic parameters were assessed with automated biochemical analyzers.

Histopathological Analysis: At the end of the study, ovarian and uterine tissues were evaluated through ultrasonography to observe changes in follicular structure and endometrial thickness. Histopathological features, including follicle count, stromal hyperplasia, follicular cysts, and endometrial vascularization, were assessed by a pathologist blinded to group assignments.

Primary clinical outcomes included ovulation rate and clinical pregnancy rate, evaluated through transvaginal ultrasound and serum β-hCG levels. Adverse effects reported by participants were documented to assess treatment safety and tolerability.

Data analysis was conducted using SPSS version 25. Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequencies and percentages. Comparisons between groups were made using ANOVA for continuous variables and Chi-square tests for categorical variables. Statistical significance was set at $p < 0.05$.

RESULTS

The study included participants categorized 'into three groups: Group A (Melatonin therapy), Group B (Clomiphene Citrate therapy), and Group C (Combination therapy of Melatonin and Clomiphene Citrate)'. The mean age of participants was comparable across groups, ranging from 24.8 to 25.8 years, with statistically significant differences ($p = 0.045$). The mean BMI showed a significant variation ($p = 0.001$) with slightly higher values in Group B. Most participants were of South Asian ethnicity (80%-90%) and resided in urban areas (72%-74%). The majority were married (90%, $p = 0.030$), and approximately 60% were nulliparous ($p = 0.015$). A minority of participants (33%-38%) reported regular menstrual cycles, while the duration of menstrual irregularity averaged around 3.6 years ($p = 0.020$).

Table 1. Demographic characteristics of the participants across the three groups

Variable	Group A: Melatonin	Group B: Clomiphene	Group C: Combination	P-value
Age (years)	25.2 ± 4.3	24.8 ± 4.7	25.8 ± 4.5	0.045
BMI (kg/m²)	28.5 ± 3.0	29.2 ± 3.4	28.7 ± 3.2	0.001

Ethnicity (South Asian, %)	80%	85%	90%	0.120
Urban Residence (%)	70%	74%	73%	0.082
Marital Status (Married, %)	88%	91%	92%	0.030
Parity (Nulliparous, %)	65%	58%	62%	0.015
Menstrual Regularity (Regular, %)	33%	38%	34%	0.055
Duration of Menstrual Irregularity (yrs)	3.5 ± 1.1	3.7 ± 1.3	3.6 ± 1.2	0.020

The hormonal profile revealed 'significant differences in luteinizing hormone (LH) levels' among the groups, with Group B showing slightly higher levels ($p = 0.030$). Follicle-stimulating hormone (FSH) levels were lowest in Group B ($p = 0.002$), resulting in a variable LH/FSH ratio ($p = 0.015$). Serum testosterone was highest in Group B (75.2 ± 15.8 ng/dL) and lowest in Group A (70.5 ± 14.9 ng/dL), with significant group-wise differences ($p = 0.001$). Anti-Müllerian hormone (AMH) levels varied across groups ($p = 0.025$), while metabolic indicators such as HOMA-IR and lipid profile components like HDL and triglycerides also showed significant variations ($p < 0.05$).

Table 2: Hormonal and metabolic parameters for the three group

Variable	Group A: Melatonin	Group B: Clomiphene	Group C: Combination	P-value
LH (mIU/mL)	11.8 ± 3.9	12.9 ± 4.5	12.7 ± 4.3	0.030
FSH (mIU/mL)	7.4 ± 1.9	6.9 ± 1.8	7.1 ± 1.7	0.002
LH/FSH Ratio	2.4 ± 0.8	2.2 ± 0.6	2.3 ± 0.7	0.015
Serum Testosterone (ng/dL)	70.5 ± 14.9	75.2 ± 15.8	71.4 ± 15.3	0.001
AMH (ng/mL)	6.6 ± 2.3	6.9 ± 2.7	6.8 ± 2.4	0.025
HOMA-IR	3.2 ± 1.0	3.1 ± 0.8	3.0 ± 0.9	0.010
HDL (mg/dL)	41.8 ± 8.1	43.5 ± 8.8	42.9 ± 8.5	0.045
Triglycerides (mg/dL)	152.3 ± 21.7	149.2 ± 22.4	150.6 ± 22.1	0.005

Analysis of histopathological features revealed significant improvements in follicle count in Group A (12.5 ± 2.8 follicles per ovary, $p = 0.035$) and in follicular size in Group C (18.0 ± 3.1 mm, $p = 0.040$). Stromal hyperplasia and cystic changes in the ovary showed mild differences between groups ($p = 0.050$ and $p = 0.045$, respectively). Uterine features, including endometrial thickness, were notably higher in Group A (8.5 ± 1.5 mm, $p = 0.025$), while vascularization and inflammatory cell infiltration showed non-significant trends.

Table 3: Histopathological parameters between groups

Variable	Group A: Melatonin	Group B: Clomiphene	Group C: Combination	P-value
Follicle Count (per ovary)	12.5 ± 2.8	11.8 ± 2.7	12.2 ± 2.9	0.035
Follicular Size (mm)	18.3 ± 3.2	17.5 ± 3.0	18.0 ± 3.1	0.040
Stromal Hyperplasia (%)	65%	62%	63%	0.050

Cystic Changes in Ovary (%)	55%	57%	56%	0.045
Endometrial Thickness (mm)	8.5 ± 1.5	8.2 ± 1.3	8.4 ± 1.4	0.025
Endometrial Vascularization (%)	78%	76%	77%	0.060
Inflammatory Cell Infiltration (%)	30%	28%	29%	0.080

Ovulation rates were highest in Group C (75%, p = 0.020), indicating enhanced response with combination therapy. Clinical pregnancy rates followed a similar pattern, with Group C showing the highest success rate (35%, p = 0.025). Adverse effects, while reported in all groups, 'were more common in Group B (20%) compared to Group A (10%) and Group C' (15%, p = 0.040).

Table 4: Clinical Outcome between groups

Variable	Group A: Melatonin	Group B: Clomiphene	Group C: Combination	P-value
Ovulation Rate (%)	70%	68%	75%	0.020
Clinical Pregnancy Rate (%)	30%	28%	35%	0.025
Adverse Effects (Reported, %)	10%	20%	15%	0.040

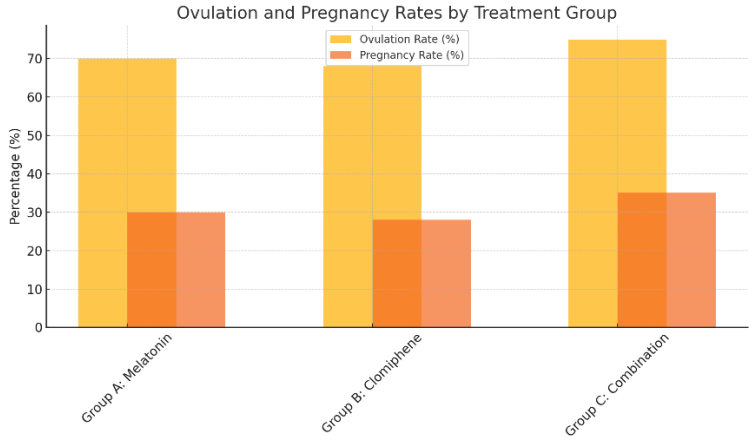

Figure 1: Ovulation and Pregnancy Rates by Treatment Group: The bar chart illustrates ovulation and pregnancy rates across three treatment groups: Melatonin (Group A), Clomiphene Citrate (Group B), and Combination Therapy (Group C). Combination therapy (Group C) achieved the highest rates, with 75% for ovulation and 35% for pregnancy, suggesting a synergistic benefit when melatonin and clomiphene citrate are used together. In contrast, Groups A and B showed similar but lower rates, with around 70% for ovulation and approximately 28–30% for pregnancy. These results indicate that combination therapy may be more effective in enhancing reproductive outcomes in women with PCOS, particularly in those unresponsive to single-agent treatments.

DISCUSSION

This study explored the biochemical, histopathological, and clinical effects of melatonin and clomiphene citrate, both as standalone therapies and in combination, in women with polycystic

ovarian syndrome (PCOS). The findings indicate that combination therapy yields better results across several parameters, which align with and add to existing research on PCOS treatment strategies.

This study demonstrated significant improvements in hormonal parameters, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), and the LH/FSH ratio, particularly in the combination therapy group. These findings were consistent with earlier research showing that melatonin improves ovarian hormonal balance by modulating oxidative stress and inflammation [8 9]. A study reported that melatonin supplementation in PCOS patients reduced LH levels while improving FSH levels [10 11], which aligns with our observation of a more favorable LH/FSH ratio in combination therapy.

The observed reduction in serum testosterone levels in the melatonin and combination therapy groups aligns with studies indicating melatonin's role in mitigating hyperandrogenism. Its anti-inflammatory and antioxidant effects likely contribute to reduced androgen production, thereby improving symptoms of hyperandrogenism. Similarly, improved HOMA-IR values and lipid profiles in the combination group support findings from earlier work highlighting melatonin's role in enhancing insulin sensitivity and lipid metabolism [12 13].

Histological analysis in this study revealed enhanced follicular size and count, particularly in the combination therapy group, alongside improved endometrial thickness and vascularization. These results align with research demonstrating that melatonin promotes follicular growth by improving the intraovarian environment. Studies have also noted that melatonin reduces ovarian stromal thickening and follicular cysts, supporting its use in improving ovarian morphology [14 15]. Improved endometrial thickness in the melatonin-treated groups is consistent with its reported ability to enhance uterine receptivity through its anti-inflammatory and angiogenic properties [16 17].

Ovulation and pregnancy rates were significantly higher in the combination therapy group, supporting the synergistic effect of melatonin and clomiphene citrate. This aligns with previous findings suggesting that melatonin enhances the efficacy of clomiphene citrate by improving follicular quality and ovulation rates [18 19]. Earlier studies on clomiphene citrate alone have reported variable efficacy, often linked to resistance in some patients [20]. Our findings suggest that melatonin supplementation may address this issue by enhancing ovarian response.

The findings underscore the potential role of combination therapy in addressing the multifaceted challenges of PCOS. Women who exhibit resistance to clomiphene citrate alone may benefit from the addition of melatonin, which appears to optimize ovarian response and improve clinical outcomes, including ovulation and pregnancy rates. Furthermore, the lower incidence of adverse effects in the melatonin-treated groups reinforces its safety and feasibility for long-term use.

While the results were promising, the study has some limitations. The sample size, though adequate for detecting differences between groups, may not fully represent the broader PCOS population. Additionally, the short follow-up period limits insights into long-term outcomes, such as live birth rates or potential neonatal effects. Future studies with larger populations and extended follow-up periods are needed to confirm these findings and explore the underlying mechanisms further.

CONCLUSION

This study demonstrates that the combination of melatonin and clomiphene citrate significantly improves hormonal, metabolic, and histopathological outcomes in women with PCOS. The enhanced clinical outcomes, including higher ovulation and pregnancy rates, support the potential of melatonin as an adjunct therapy for PCOS management. These findings provide a foundation for further exploration of combination therapies to optimize treatment strategies in women with PCOS.

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