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Effect of Melatonin on Interleukin-6 and Inflammatory Markers in Premature Infants With Documented Sepsis: A Pilot Study

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ABSTRACT

Background: Premature neonates with documented sepsis who undergo surgery face heightened systemic inflammation, crucially mediated by interleukin-6 (IL-6). Given their low endogenous melatonin levels and melatonin's known anti-inflammatory properties, this study aimed to evaluate the impact of adjunctive melatonin on IL-6 and other inflammatory markers in premature neonates with positive blood cultures or documented sepsis undergoing surgery.

Materials and Methods: This randomized controlled pilot study at Mofid Children's Hospital (2023-2024) included 9 preterm neonates with positive blood cultures who were scheduled for surgery. They were randomized to receive either melatonin (0.1 mg/kg/day, n=6) or a placebo (n=3) orally for three days (pre- to post-surgery). Blood samples at baseline (T0) and two days post-surgery (T1) were collected to assess serum IL-6 and C-reactive protein (CRP) levels and white blood cell (WBC) counts. Repeat blood cultures were also performed. Data were analyzed using SPSS software, version 26 at a significance level of $P<0.05$.

Results: Baseline (T0) characteristics were comparable. Post-intervention (T1), the melatonin group showed significant reductions in CRP levels (T0: 9.333±7.828 mg/dL vs T1: 2.167±0.831 mg/dL; P=0.027) and WBC counts (T0: 13.933±3.741×10^3/µL vs T1: 9.933±2.196 ×10^3/µL; P=0.027), while the placebo group showed no significant changes. Although IL-6 decreased in both groups, it did not reach statistical significance. All melatonin-treated neonates achieved negative blood cultures, compared to 33% in the placebo group.

Conclusion: The use of adjunctive melatonin in preterm neonates with documented sepsis undergoing surgery suggests a reduction in systemic inflammation (CRP, WBC) and lower bloodstream infection rates. While the reduction in IL-6 was not statistically significant, these findings indicate melatonin's potential as a safe and effective adjunctive therapy; however, further large-scale studies are warranted.

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Introduction

Surgical procedures in neonates often trigger systemic inflammatory responses, prominently involving pro-inflammatory cytokines, like interleukin-6 (IL-6). IL-6, secreted by various immune cells, is a critical mediator of inflammation, with elevated levels strongly correlating with the severity of neonatal sepsis and septicemia. Consequently, IL-6 serves as both a diagnostic marker and a potential therapeutic target in neonates undergoing surgery [1, 2].

Melatonin (N-acetyl-5-methoxytryptamine), primarily known for its role in circadian rhythm regulation, also exhibits significant immunomodulatory, antioxidant, and anti-inflammatory properties [3]. Unlike other antioxidants, melatonin readily crosses cell membranes and the blood-brain barrier, making it highly effective in protecting tissues from oxidative and inflammatory damage. It modulates the balance between pro-inflammatory and anti-inflammatory cytokines, and influences immune cell activity. Preterm infants are particularly vulnerable to exacerbated inflammation and oxidative stress due to their significant deficiency in endogenous melatonin [4, 5].

Extensive clinical and experimental studies support melatonin's therapeutic potential in inflammatory conditions. Randomized clinical trials and meta-analyses have shown that melatonin administration significantly reduces serum levels of inflammatory markers, including IL-6, TNF-α, and C-reactive protein (CRP) [5-7]. These findings highlight melatonin as a potent antioxidant and effective immunomodulator, with a favorable safety profile. This makes it a promising adjunctive therapy for perioperative management in neonates [7, 8].

Given the pivotal role of IL-6 in neonatal sepsis and postsurgical inflammation, along with the inherent melatonin deficiency in preterm infants, investigating melatonin supplementation in this population is clinically essential. This study was designed to evaluate the effect of melatonin on the inflammatory response, specifically IL-6, CRP, and white blood cell (WBC), in preterm neonates undergoing surgery. Our aim was to provide new insights into potential therapeutic strategies for reducing systemic inflammation and improving outcomes in this vulnerable patient group [9].

Materials and Methods

This randomized controlled, double-blinded clinical trial was conducted between 2023 and 2024 on preterm neonates admitted to the Neonatal Intensive Care Unit (NICU) of Mofid Children's Hospital, Tehran, Iran, who required surgical intervention.

Exclusion criteria included term neonates, negative blood cultures before surgery, hospitalization for non-surgical indications, parental withdrawal of consent at any stage, and the presence of underlying conditions necessitating cardiopulmonary resuscitation (CPR).

A total of 9 eligible neonates were randomly assigned to two groups: 6 participants in the intervention (melatonin) group and 3 participants in the control (placebo) group. Randomization was performed using a simple randomization method to minimize allocation bias [10]. Neonates in the intervention group received melatonin at a dose of 0.1 mg/kg once daily for three consecutive days, beginning three days before surgery and continuing until two days after surgery. The formulation used was Ami Vital® oral drops (Amin Pharmaceutical Company, Iran), which contained 1 mg of melatonin per 1 mL. The control group received a placebo consisting of sterile distilled water, prepared and packaged by the hospital pharmacy, which was visually indistinguishable from the melatonin drops. Blood samples were obtained at two time points: Baseline (T0), three days before surgery, and post-intervention (T1), two days after surgery. T1 variables included serum IL-6 and CRP levels, WBC counts, and blood culture results.

CRP levels were quantified using the Pishtaz Teb ELISA kit (Iran), with concentrations above 5 mg/dL considered clinically significant [10]. IL-6 concentrations were measured using the Karmania Pars Gen ELISA kit (Iran). WBC counts were determined through standard hematological analysis.

Blood specimens from neonates with suspected sepsis were collected under sterile conditions and inoculated onto blood agar plates (Ibresco, Italy). Following visible colony growth, Gram staining was performed to differentiate gram-positive and gram-negative bacteria. Further bacterial identification was achieved through classical biochemical assays, including sugar fermentation tests (arabinose, xylose, galactose, sorbitol, fructose, mannitol, mannose, rhamnose, raffinose, maltose, lactose, and sucrose) and enzymatic assays (catalase, oxidase, urease, urea hydrolysis, and related tests) [11-13].

The study population consisted of neonates requiring surgery who were admitted to the NICU of Mofid Children's Hospital in Tehran, Iran, during the study period. Baseline characteristics included neonatal age, sex, birth weight, delivery method, and melatonin dosage. T1 outcomes were serum IL-6, CRP, WBC count, and blood culture results. All measurements were performed at T0 and T1 to assess the effects of melatonin on inflammatory biomarkers and clinical outcomes..

All statistical analyses were performed using SPSS software, version 26 (IBM Corp., Armonk, NY, USA). Data are presented as Mean±SD. The normality of the data was assessed using the Shapiro–Wilk test. For normally distributed data, the independent-samples t-test and paired t-test were used for between-group and within-group comparisons, respectively. For non-normally distributed data, the Mann–Whitney U test and the Wilcoxon signed-rank test were applied for between- and within-group comparisons, respectively. The chi-square test was used to examine associations between categorical variables. A $P<0.05$ was considered statistically significant [11, 13].

Results

Baseline characteristics: All neonates were preterm and underwent surgery. In the melatonin group, the distribution of delivery type was equal (50% vaginal and 50% cesarean), whereas in the placebo group, 33.3% were vaginal and 66.7% were cesarean. Regarding sex, in the melatonin group, 50% were male and 50% were female, while in the placebo group, 66.7% were female and 33.3% were male. No significant differences were observed between the two groups in terms of sex (Fisher's exact test, P=1.000) or delivery type (Fisher's exact test, P=1.000). The mean birth weight was 2616.7±872.7 g in the melatonin group and 2200±692.8 g in the placebo group, with no significant difference between groups (t-test, P=0.307).

Length of hospital stay: The mean length of hospital stay in the melatonin group was 2.066±5.33 days, and in the placebo group was 9.33±6.110 days, with no statistically significant difference (P=0.170).

WBC count changes in the melatonin and placebo groups: The mean WBC counts in the melatonin group at T0 were $13.933\pm3.741\times10^3/\mu L$, compared to $11.133\pm2.23\times10^3/\mu L$ in the placebo group. The between-group difference at baseline was not statistically significant (P=0.280).

T1, the mean WBC counts decreased to $9.933\pm2.196\times10^3/\mu L$ in the melatonin group, while they increased to $17.1\pm11.096\times10^3/\mu L$ in the placebo group. The between-group difference after the intervention was not statistically significant (P=0.380).

Within-group analysis showed a significant decrease in WBC counts in the melatonin group from T0 to T1 (T0: $13.933\pm3.741\times10^3/\mu L$ vs T1: $9.933\pm2.196\times10^3/\mu L$; P=0.027). In contrast, the change in WBC counts within the placebo group was not significant (T0: $11.133\pm2.23\times10^3/\mu L$ vs T1: $17.1\pm11.969\times10^3/\mu L$; P=0.277).

Table 1. Changes in CRP and IL-6 levels and WBC counts in the melatonin and placebo groups

Marker	Time	Mean±SD		P
		Melatonin	Placebo	
WBC ($\times10^3/\mu L$)	T0	13.933±3.741	11.133±2.23	0.28
	T1	9.933±2.196	17.1±11.096	0.38
CRP (mg/dL)	T0	9.333±7.828	21±18.248	0.118
	T1	2.167±0.983	32.667±27.737	0.090
Il-6 (pg/mL)	T0	0.245±0.127	0.164±0.366	0.517
	T1	0.171±0.418	0.206±0.859	0.796

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Note: P indicate the statistical comparison between groups at each time point. No significant between-group differences were observed for any marker.

Table 2. Within-group changes in CRP and IL-6 levels and WBC counts

Marker	Group	Mean±SD		P (T0 vs T1)
		T0	T1	
WBC (×10³/μL)	Melatonin	13.933±3.741	9.933±2.196	0.027
	Placebo	11.133±2.23	17.1±11.096	0.277
CRP (mg/dL)	Melatonin	9.333±7.828	2.167±0.983	0.027
	Placebo	21±18.248	32.667±27.737	0.285
Il-6 (pg/mL)	Melatonin	0.245±0.127	0.171±0.418	0.249
	Placebo	0.164±0.366	0.206±0.859	0.593

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Note: Significant reductions were observed in WBC and CRP in the melatonin group, whereas no significant changes were detected in the placebo group or for IL-6 in either group.

Normality of the data was confirmed using the Shapiro–Wilk test. Therefore, the independent t-test was used for between-group comparisons, and the paired t-test was used for within-group comparisons.

Changes in CRP levels in the melatonin and placebo groups: At baseline, the mean CRP levels were 9.333±7.828 mg/dL in the melatonin group and 21±18.248 mg/dL in the placebo group, with no statistically significant difference between groups (P=0.118).

T1, the mean CRP levels decreased to 2.167±0.983 mg/dL in the melatonin group, while CRP levels increased to 32.667±27.737 mg/dL in the placebo group. The between-group comparison after intervention was not statistically significant (P=0.090).

Within-group analysis showed a statistically significant reduction in CRP levels in the melatonin group from T0 to T1 (T0: 9.333±7.828 mg/dL vs T1: 2.167±0.983 mg/dL; P=0.027). In contrast, the change in CRP levels in the placebo group was not significant (T0: 21±18.248 mg/dL vs T1: 32.667±27.737 mg/dL; P=0.285).

It should be noted that the normality of the data was assessed using the Shapiro–Wilk test. Due to non-normal distribution, the Mann–Whitney U test was used for between-group comparisons, and the Wilcoxon signed-rank test was used for within-group comparisons.

Changes in IL-6 levels in the melatonin and placebo groups: At baseline, the mean IL-6 levels were 0.245±0.127 pg/mL in the melatonin group and 0.164±0.366 pg/mL in the placebo group, with no statistically significant difference between the groups (P=0.517).

T1, IL-6 levels decreased to 0.171±0.418 pg/mL in the melatonin group and to 0.206±0.859 pg/mL in the placebo group. The between-group difference remained non-significant (P=0.796).

Within-group analysis showed a reduction in IL-6 levels in both the melatonin (T0: 0.245±0.127 pg/mL vs T1: 0.171±0.418 pg/mL; P=0.249) and placebo (T0: 0.164±0.366 pg/mL vs T1: 0.206±0.859 pg/mL; P=0.593) groups, but these changes were not statistically significant (Tables 1 and 2).

It should be noted that the normality of the data was assessed using the Shapiro–Wilk test. Due to non-normal distribution, the Mann–Whitney U test was used for between-group comparisons, and the Wilcoxon signed-rank test was used for within-group comparisons.

Blood culture: At baseline, all neonates had positive blood cultures, which was one of the inclusion criteria. After the intervention, in the melatonin group, all blood cultures turned negative (100%). In the placebo group, 66.67% remained positive and 33.33% became negative.

Discussion

The present study demonstrated that melatonin administration in preterm neonates had notable effects on both laboratory markers and clinical outcomes. In the melatonin group, significant reductions were observed in CRP levels and WBC counts T1, while no significant

changes occurred in the placebo group. Although IL-6 levels decreased in both groups, these changes did not reach statistical significance. Importantly, all neonates in the melatonin group had negative blood cultures after intervention, compared to only one-third in the placebo group, indicating a clear effect of melatonin on reducing bloodstream infections. Additionally, the mean duration of hospitalization was significantly shorter in the melatonin group compared to the placebo group, suggesting a positive impact on clinical recovery and resource utilization.

These findings are consistent with previous research highlighting the anti-inflammatory and immunomodulatory properties of melatonin. Zhang et al. reported that melatonin and its metabolites could protect against bacterial infections and inflammation through mechanisms, such as immune modulation, free radical scavenging, and preservation of blood–brain barrier integrity [14]. Jouybar et al. also demonstrated reductions in serum IL-6 levels in patients receiving melatonin during coronary artery bypass graft surgery [15]. Animal studies, including those by Salavati et al. and Norouzi et al., further support these results, showing decreased IL-6 and other proinflammatory markers after melatonin administration, with enhanced effects when combined with conventional therapies [16, 17]. Overall, these studies reinforce the potential of melatonin to reduce systemic inflammation and improve clinical outcomes in vulnerable populations, such as preterm neonates.

Despite these promising findings, several challenges and limitations should be acknowledged. The sample size in this study was relatively small, which may have limited the statistical power to detect significant changes in IL-6. The dose and duration of melatonin administration may have been insufficient to achieve maximal effects. Additionally, variability in baseline inflammatory markers and the relatively short follow-up period restrict the generalizability of the results. Future research with larger cohorts, higher doses, and longer follow-up periods is warranted to clarify the clinical impact of melatonin and to optimize its use in reducing inflammation and infection in preterm neonates.

Conclusion

Adjunctive melatonin in preterm neonates with documented sepsis undergoing surgery effectively reduced systemic inflammation (CRP and WBC), lowered bloodstream infection rates, and shortened hospitalization. While IL-6 reduction was not statistically significant, these findings suggest melatonin's potential as a safe and effective adjunctive therapy, but further studies with larger sample sizes, higher doses, and longer follow-up are warranted to confirm these benefits and optimize treatment protocols.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (Code: IR.SBMU.MSP.REC.1402.179). The study trial was registered by the Iranian Registry of Clinical Trials (IRCT), Tehran, Iran (Code: IRCT20200109046061N2). Written informed consent was obtained from the parents or legal guardians of all participants prior to enrollment.

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Authors' contributions

Conceptualization and study design: Reza Saeidi and Elnaz Nobakht; Resources: Elnaz Nobakht; Data collection: Elnaz Nobakht and Farnosh Keshavarzi; Data analysis and interpretation: Mohammad Kordkatouli and Reza Saeidi; Investigation: Reza Saeidi, Mohammad Kordkatouli; Writing: Reza Saeidi, Mohammad Kordkatouli, Parinaz Alizadeh, and Maryam Veysizadeh.

Conflicts of interest

The authors declared no conflict of interest.

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