Melatonin, an adjuvant for neonatal sepsis: a systematic review and meta-analysis

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INTRODUCTION

Neonatal sepsis is a clinical syndrome of systemic disease occurring in the first month of life resulting from bacteremia. Neonatal sepsis is a major cause of morbidity and mortality. Sepsis is responsible for 2.9 million people deaths worldwide every year and around 25% of them occur in neonates. Neonatal sepsis causes great pain and mortality in infants, accounting for 12-20% of newborn deaths. Some of the difficulties in solving sepsis problems include determining the diagnosis and difficulties in treatment, although it is now possible to provide follow-up care for newborns in some hospitals. Antibiotics are the standard treatment for sepsis.

Melatonin, a hormone produced by the pineal gland, has beneficial effects against free radicals, antioxidants, anti-inflammatory, and anti-apoptotic effects. However, in newborns, melatonin is minimally detectable. Adjuvant therapy is required for the deterrence and neonatal sepsis. This study aims to systematically review and perform a meta-analysis of melatonin as an adjunctive therapy in treating neonatal sepsis with the addition of the most recent articles.

METHODS: Databases of electronics used such as PubMed, Embase, and the Cochrane Central Controlled Trials Register were searched systematically in October 2022 for clinical trials reporting the efficacy of melatonin as adjunctive therapy for neonatal sepsis. We compared treatment responses between groups using serum C-reactive protein (CRP) levels as an endpoint biomarker. Data analysis was performed to appraise the quality of enrolled studies using a comprehensive meta-analysis Revman 5.4 and bias tool risk of the Cochrane Collaboration.

RESULTS: Four trials with a total of 175 subjects were listed in the systematic review and meta-analysis. Pooled analysis showed a statistically significant mean difference in serum CRP level (mg/L) between groups 24 hours after adjuvant melatonin therapy (-1.63 mg/L; 95% CI: -3.20 to -0.054; P=0.043).

Conclusion: Melatonin as an adjunctive therapy significantly lowers CRP levels. Larger, sample size studies are necessary to establish clear clinical benefits of this treatment.

Keywords: melatonin, meta-analysis, neonatal sepsis, systematic review.

Reviews and Meta-Analyses. We searched databases PubMed, Embase, and the Cochrane Central Register of Controlled Trials to identify all clinical trial articles published between July 12, 2001, and July 12, 2021. In addition, additional hand searches were performed to identify relevant studies by review bibliographies of searched articles. The following predefined search terms were used in combination: “melatonin” AND “sepsis” or “septicemia” AND “neonatal” or “neonates” or “newborn”. Only articles published in English were considered in the final selection.

**Eligibility criteria (inclusion and exclusion criteria)**
The inclusion criteria are clinically controlled trials on melatonin with the subjects of neonates and published in English in the last 20 years (2001-2021). The exclusion criteria are studies without relevant melatonin study outcomes. Eligible studies were independently selected by screening titles, abstracts, and language. Two researchers (DW and HS) conducted the article searching, and two other researchers (TW and BW) conducted bias analysis. Extracting the data was performed by VW, HH, and SS.

**Data extraction and quality assessment**
The following descriptive information was obtained from all eligible studies in a standard format: country; year of publication; study design; the number of participants; demographic information (pregnancy age and sex); intervention (dose of melatonin, duration, method of administration, and simultaneous antibiotic treatment); controls (type of antibiotics); and main outcomes (CRP levels). The second (HS) and third author (TW) confirmed the extracted data, while any discrepancies were resolved by the fourth author (BW). The Cochrane risk assessment tool was used to assess the risk of bias in the included studies for non-RCTs and RCTs. The Cochrane tool consists of six areas: (1) random sequence generation, (2) allocation concealment, (3) blinding of participants and personnel, (4) blinding of outcome assessment, (5) incomplete outcome data, and (6) selective outcome reporting.

**Data analysis**
Relative risks (RR) with 95% confidence interval (CI) were calculated for the dichotomous variable to measure improvement in clinical outcome. For a continuous variable such as serum CRP level, standardized mean differences (SMDs) with 95% confidence intervals were calculated by comparing the intervention and control groups after 24 hours of treatment. Pooled estimates and heterogeneity of included studies were calculated using Revman 5.4. Whether the model parameters are heterogeneous or homogeneous was assessed by heterogeneity (I² or Tau). The fixed effect and the random effect methods were applied for small heterogeneity and large heterogeneity, respectively. The calculation obtained heterogeneity, reflected in the I² value of 93%. The results demonstrated I² > 70%, so the Random Effects Model (REM) was used as a model.

**RESULTS**
**Study selection**
A sum of 133 researches were preliminary detected and 100 articles were yielded after removing duplicates. After examining the titles and abstracts, 23 of these studies and 73 of the persisting 77 studies were ruled out for the following reasons: review articles (n=65), publications in languages other than English (n=2), and insufficiency of related results (n=6). Eventually, four studies comprising three non-randomized clinical trials from Egypt and one randomized controlled trial (RCT) from Italy were included in the final analysis (Figure 1).

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**Figure 1.** Flowchart of the published works application and study selection process.
Table 1. The characteristics of the included trials

<table>
<thead>
<tr>
<th>Study (yr)</th>
<th>Design</th>
<th>Participants</th>
<th>GA (MT)</th>
<th>GA (CT)</th>
<th>Sex (MT)</th>
<th>Sex (CT)</th>
<th>Melatonin/Control</th>
<th>Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>El Gendy et al (2017)</td>
<td>Non-RCT</td>
<td>40 septic neonates</td>
<td>35.5 (2.55)</td>
<td>35.7 (2.48)</td>
<td>10/10</td>
<td>10/10</td>
<td>20 mg melatonin x1 dose</td>
<td>via NG tube plus antibiotics</td>
</tr>
<tr>
<td>El Frargy et al (2015)</td>
<td>Non-RCT</td>
<td>50 septic neonates</td>
<td>36.3 (1.98)</td>
<td>36.5 (2.2)</td>
<td>14/11</td>
<td>14/11</td>
<td>20 mg melatonin x1 dose</td>
<td>via NG tube and ampicillin + gentamycin</td>
</tr>
<tr>
<td>Gitto et al (2001)</td>
<td>RCT</td>
<td>20 septic neonates and 10 healthy newborns</td>
<td>39.1 (1.29)</td>
<td>38.60 (1.17)</td>
<td>N/A</td>
<td>N/A</td>
<td>20 mg melatonin x2 doses 1 hour apart</td>
<td>orally plus standard antibiotics</td>
</tr>
<tr>
<td>El Kabbany et al (2020)</td>
<td>RCT</td>
<td>40 premature infants diagnosed with NS and 15 healthy newborns</td>
<td>32.40 (2.60)</td>
<td>32.87 (2.07)</td>
<td>26/14</td>
<td>10/5</td>
<td>20 mg melatonin x2 doses 1 hour apart</td>
<td>orally plus standard antibiotics</td>
</tr>
</tbody>
</table>

Risk of bias

Bias analysis tools were applied to evaluate the presence of bias. The Cochrane algorithm of two Egyptian non-RCTs showed low potential bias in all but one category. Nevertheless, the design of the trials did not consider masking the staff or outcome assessors to minimize the risk of cumulative bias. Both significant and non-significant effects of the intervention were calculated, reducing the risk of selective consequence. Sex was a confounding variable, as we did not calculate the ratio of males to females in the intervention and control groups (Figure 2).

The characteristics of the included trials

The total study subjects of all four trials were 175 neonates with comparable gestational age (GA) as well as sex. The mean value of GA ranged from 32.40 to 39.1 weeks. Of these subjects, 75 newborns received a combination of melatonin and antibiotics, and 75 controls received only standard antibiotics. The remaining 25 subjects were healthy, sepsis-free neonates enlisted in an Italian and Egyptian RCT to compare laboratory measurements. In all four clinical trials, the intervention group was given a total of 20 mg of melatonin. The characteristics of the included studies are displayed in Table 1.
Melatonin, an endogenous indoleamine, provides several physiological functions, including circadian rhythm control and sleep induction, but in the case of neonatal sepsis therapy the most important role of melatonin is its potent antioxidant activity as well as pronounced anti-inflammatory effects. Melatonin as a free radical agent and antioxidant agent indirectly employs pleiotropic action in the body. Marseglia L et al. revealed the effectiveness of melatonin in counteracting oxidative stress. During septic conditions, pathogens invade the immune system activating pro-inflammatory cytokines along with some oxidative. Direct cell destruction promoted by ROS, inflammation, as well as oxidative stress is augmented by gene expression activation together with mitochondrial function.

Adding antioxidants therapy to conventional ones has been proposed, to reduce oxidative stress in neonatal sepsis. Melatonin is one of the antioxidants that can be used as an adjuvant. Melatonin is capable of providing direct and indirect protection against oxidative stress. Protection is directly linked to the suppression of free radical activity. Indirect protection is mainly due to inhibition of DNA damage results from metal induction, activation of antioxidant enzymes, inhibition of pro-oxidative enzymes, and increased DNA repair. Melatonin reduces inflammation by decreasing pro-inflammatory cytokines.

In this meta-analysis, serum CRP levels at baseline differed from those 24 hours after melatonin treatment in which the CRP levels decreased significantly. The literature suggested CRP as a clinical indicator. It should be noted that the Italian RCT achieved the greatest decrease in CRP levels compared to the other three studies. This can be explained by the heterogeneity of the three Egyptian studies and the Italian study. The newborns included in the Italian study had greater weight and they were older than the neonates in the three Egyptian studies.

Several limitations should be considered when interpreting the results of our study. First, our meta-analysis included only four studies with a total of 175 subjects from two countries. So that the results of our study cannot be generalized. Further study is required to support our findings. Due to, the methodological design of our study we could not assess the publication bias, as at least ten studies are needed to create a funnel plot. Second, using a random effects model, the Italian study showed high heterogeneity (ie, I² > 50%) in the assessment of CRP levels 24 hours after melatonin administration compared to the three Egyptian studies. Finally, the number of studies included in this meta-analysis was limited by the number of existing clinical trials that had to be relevant to the subject of this study.

Administering melatonin as an adjunct therapy for neonates with sepsis significantly reduces serum CRP levels.

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DH and HS contributed to the wider study concept and design. TW and BW contributed to the literature identification and determination of study inclusion and exclusion criteria. VM contributed to data extraction. HH and SS contributed to the draft of the manuscript. All authors contributed to interpreting the analyses critically revising the article and approving the final draft.

The author reports no conflicts of interest in this work.
DATA AVAILABILITY
The data that support the findings of this study are openly available in [figshare] at http://doi.org/10.6084/m9.figshare.23633643

ETHICAL STATEMENT
Due to its meta-analysis design, the data used were based on previously existing data, no further data were requested from the previous authors.

REFERENCES