The Effect of Erythropoietin on Cerebral Palsy Prevention in Hypoxic Ischemic Encephalopathy (HIE): A Systemic Review and Meta-Analysis

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ABSTRACT

Introduction: HIE plays a significant role in global disability, with 60% of newborns with severe HIE passing away or becoming severely disabled. Since there is still debate over the impact of EPO on HIE, we conducted a thorough analysis of the literature to determine whether erythropoietin may prevent cerebral palsy in HIE patients.

Methods: Database search included Cochrane Library, ProQuest, PubMed, ScienceDirect, and Scopus. RCTs reporting cerebral palsy, neurodevelopmental impairments, seizures, brain injury on MRI, or death were included. Qualitative studies were excluded. Critical appraisal was assessed using JBI while the I2, forest plot, and sensitivity analysis were performed on meta-analysis. The funnel plot and Egger’s test were used to assess bias. The GRADE method was used to assess the LOE independently.

Results: A total of 812 infants were included in 6 eligible articles. For the infant with HIE, EPO alone was statistically significant to prevent cerebral palsy in HIE patients (OR=0.90, p = 0.038, 95% confidence interval [95% CI] was 0.57-1.44). Erythropoietin administration also significantly reduced the risk of neurodevelopmental impairments, seizures, brain injury on MRI, or death were included. Qualitative studies were excluded. Critical appraisal was assessed using JBI while the I2, forest plot, and sensitivity analysis were performed on meta-analysis. The funnel plot and Egger’s test were used to assess bias. The GRADE method was used to assess the LOE independently.

Conclusion: With only 6 trials included, it is essential to do clinical trials with sufficient power to evaluate erythropoietin further. Researchers and clinicians must collaborate with specialists and form interest groups to define primary and secondary outcomes and acceptable guidelines for using erythropoietin in future trials.

Keywords: erythropoietin, cerebral palsy, hypoxic-ischemic encephalopathy, EPO.


INTRODUCTION

A brain condition known as hypoxic-ischemic encephalopathy is brought on by insufficient blood and oxygen delivery for a variety of reasons.1 1.5 cases of HIE are thought to occur for every 1,000 live births.2 All things considered, HIE is among the top causes of newborn deaths and plays a significant role in global handicaps. Severe HIE can cause up to 60% of baby deaths or serious disabilities.3 Conditions that are inflammatory and hypoxic-ischaemic are the main causes of cell death or loss of function. Excessive production of pro-inflammatory cytokines, oxidative stress, extracellular matrix alteration, growth factor deficiency, and excessive glutamate release set off excitatory cascade events. These processes lead to the mal-development of the thalamus in preterm neonates degeneration of the thalamus including secondary cortical and myelination gliosis abnormalities.4 Hypoxia-ischemia can result in lasting brain damage if it lasts long enough. This brain damage can then lead to neurodevelopmental diseases such as cerebral palsy (CP) and developmental delay.5 The main cause of CP is a neuromotor condition that impairs posture, muscle tone, and movement development.6 Many comorbidities, including visual impairment, epilepsy, cognitive impairment, sensory, communication, perception, and behavior disorders, are linked to CP. Associated comorbidities are the primary determinants of outcome and quality of life in many children with CP.7 Since CP is a permanent condition with little chance of full recovery, the main goals of treatment are to minimize or avoid abnormalities and improve the patient's ability to engage in activities at home and in the community.8 Erythropoietin (EPO) has been...
identified as a potential neuroprotective treatment for neonatal HIE recently. Brain cells produce more EPO under hypoxia, crucial for the central nervous system’s neuroprotection and neuroregeneration. The initial human trial performed by Zhu et al. demonstrated better long-term results. There were no adverse effects. According to a study protocol conducted by Juul et al., EPO has strong neuroprotective effects in preclinical research, and phase I/II trials indicate that giving several large doses of EPO protects term infants’ brain damage. Inversely, Wu et al., who performed multicenter research, there was no difference in the risk of death or neurodevelopmental impairment between erythropoietin-administered babies undergoing therapeutic hypothermia for HIE and placebo. Since there is still debate over the impact of EPO on HIE, we conducted a thorough analysis of the literature to determine whether erythropoietin may prevent cerebral palsy in HIE patients.

MATERIALS AND METHODS

This systematic review and meta-analysis were conducted and reported per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The protocol for the systematic review was not prepared and registered yet.

Eligibility criteria

We used the Population, Intervention, Comparator/s, Outcomes (PICO) framework for effectiveness review. The population were all newborns who were ≥34 weeks of gestation and had evidence of moderate or severe HIE. At least one of the following criteria was met: (i) Apgar score ≤5 at 5 min, (ii) cord or arterial blood pH ≤7.0, (iii) base deficit >12 mmol/L within the first hour after birth, or (iv) ongoing resuscitation or mechanical ventilation at 5 min of life. The intervention was EPO and its analogs, while the comparator was placebo. The outcome was effectiveness in preventing cerebral palsy (determined by neurological examination and severity classified based on the Gross Motor Functional Classification Scale or a comparable validated tool). Other outcomes included (i) moderate to severe neurodevelopmental impairment (determined by Alberta Infant Motor Scale (AIMS)), a standardized and validated exam that objectively rates motor function, or the Warner Initial Developmental Evaluation (WIDEA), a 43-item parental questionnaire that assesses 4 domains of infant development: self-care, mobility, communication, and social cognition or other validated tools; (ii) seizure; (iii) brain injury based on neonatal brain MRI; (iv) death. Studies that did not measure neurodevelopmental impairment or cerebral palsy, followed by other encephalopathy (other than hypoxic and ischemic), and consisted of children with special needs were excluded.

Studies were eligible for inclusion if they used an intervention (primary randomized controlled trial) design that reported a comparison of EPO to placebo for preventing cerebral palsy on HIE. Studies had to provide quantitative data on that comparison, so qualitative studies were excluded. Protocol studies were not attempted.

Information sources

Standard searching methods were used to administer this review and respond to guidance from the Cochrane Collaboration. Relevant studies were discovered by the following electronic databases of peer-reviewed journal articles, such as Cochrane Library, ProQuest, PubMed, ScienceDirect, and Scopus.

Search strategy

The initial search terms were ['erythropoietin' (Mesh)] OR ['erythropoietin AND 'hematopoietic' AND 'hemopoietin'AND 'EPO' AND 'Epoetin Alfa'] AND ['cerebral palsy'(Mesh)] OR ['spastic paralysis'] AND ['brain diseases'(Mesh)] OR 'encephalopathy'AND ['hypoxic ischemic OR 'hypoxic ischaemic' OR 'hypoxic-ischaemic' encephalopathy].

Selection process

The same literature may be found in many databases. In this case, the retrieved literature will be reserved in a Rayyan (RIS format), and corresponding entries will be removed. If more than one report type is retrieved for the same study, preference will be given to peer-reviewed material and the hierarchy of research study design; further information will, however, be removed from the distinct citations where appropriate. The inclusion criteria for the review will be the primary screening criteria for the titles and abstracts of papers found using the search methods. This will help identify all possibly relevant studies. Studies will be categorized as “included” or “not included.” The titles and abstracts that cause the reviewers to disagree will be considered.

Full texts of any material thought pertinent or likely to cause disagreement between reviewers will subsequently be obtained. After that, a uniform checklist of the requirements will be used to determine which gets included. Both the first and second reviewers will conduct the second screening. If there is a debate on inclusion, the entire research team will deliberate and come to a consensus. The review saved on Rayyan will contain a separate folder, including the sources for the eliminated studies and the rationale behind their non-acceptance.
Developing a novel theory about Assessing the degree to which this data is found in a specified at-risk population. Prevalence rate analysis was performed to identify the pertinent research that qualified for full-text publication. The two writers (first and second author) evaluated the screening studies’ suitability for inclusion.

**Data items**
Following the literature validation appropriate for the review, a custom data abstraction framework will be used as a model to document important research features. Details about the study's design, participant count, intervention dosage, age of the participants, time period, location, diagnosis of cerebral palsy, neurodevelopmental assessment, analysis, findings, and quality assessment will all be included here, as applicable. Study outcomes were divided into primary and secondary outcomes. The effectiveness of erythropoietin in preventing cerebral palsy in HIE patients was the primary outcome. Neurodevelopmental impairments, seizure, and brain injury, which were detected using brain MRI, and death were secondary outcomes. Data were obtained and analyzed using Stata 14.0 (StataCorp LLC, USA).

**Study risk of bias assessment**
Before being included in the review using PRISMA, the identified papers that satisfy the publishing criteria will be separately evaluated by two reviewers for methodological validity. The Joanna Briggs Institute's (JBI) critical appraisal tools of randomized controlled trial (RCT) were used by the two authors (first and second author) to independently assess the risk of bias. Discussion and agreement helped to settle the differences, which have not met its decision and would be discussed with the third author. The data about the following areas is displayed in the "Risk of bias tables": selection bias, intervention bias, outcome bias, participation retention bias, and statistical validity. Any bias section which had "Yes" >50% is considered as "Yes", as well as "No".

**Effect measures**
Prevalence rate analysis was performed to measure how common a disease process is found in a specified at-risk population at a specific time or during a specified time period. Odds ratios were analyzed for categorical outcomes. Standardized mean differences (for continuous data) will be calculated from the data generated. Confidence intervals will be included in any analysis.

**Synthesis methods**
The Economic and Social Research Council has produced a comprehensive framework and a set of criteria for creating an apparent (clear) reliable narrative synthesis report incorporating specialized instruments and procedures. There will be four primary steps in creating a narrative synthesis of the quantitative data using this framework:
- Developing a novel theory about the function of erythropoietin in preventing cerebral palsy in HIE patients and the potential effects of erythropoietin intervention on cerebral palsy in HIE patients.
- Writing a preliminary summary of the included literature's findings.
- Investigating connections and any resemblances found inside and within research.
- Assessing the degree to which this data synthesis may be considered reliable.

The first and second authors assessed the features of the included studies and the results. If the authors (first and second author) believed that a certain outcome was not comparable because of clinical or methodological heterogeneity, the results were not pooled to include in the meta-analysis. Every outcome underwent a separate meta-analysis, divided into two groups: placebo and EPO. Stata 14.0 (StataCorp LLC, USA) was used to conduct a random-effect model meta-analysis for each outcome for which two or more studies were available. Odds ratios (ORs) and expected absolute effects with their 95% confidence intervals (CIs) were used to compute the comparative effect sizes. Standardized mean differences (SMD) with their 95% confidence interval (CI) were used to compute the continuous effect sizes. Effect size (ES) with 95% confidence intervals (CIs) were used to compute the pooled prevalence rate. The RevMan 5.3 software (Cochrane Statistical Package) defined the risk of bias. The Cochrane Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) was used in conjunction with the description of findings and outcomes of the meta-analyses to evaluate the LOE. The GRADE method was used by the writers (first and second authors) to assess the LOE independently. Discussion and agreement helped to settle the differences.

**Reporting bias assessment**
The quality evaluation of diagnostic accuracy studies (QUADAS 2) tool was used to rank each property as “low risk,” “unclear risk,” or “high risk.” The results are shown graphically as a graph and summary figure. Consequently, we assessed the publication bias using the funnel plot combined with Egger’s test.

**Certainty assessment**
Meta-analysis will be conducted to combine and synthesize results data across studies. A fixed model will be conducted if the variance is low, but if the variance is moderate, a random effects model will be conducted. P value <0.05 is considered statistically significant. Heterogeneity will be calculated using the χ² test, and if the p-value is less than 0.10, it will be considered evidence of heterogeneity. The I² statistic will be used to assess the percentage of variation. If the I² value is more than 30%, it will be considered moderate heterogeneity; if the I² value is more than 50%, it will be considered severe. If both I² <40% and p >0.05 were met, we regarded this as not indicating significant heterogeneity. If any of those mentioned above were true, then there was heterogeneity, and either subgroup analysis or metaregression analysis was required.

**RESULTS**

**Study selection**
We identified 1,396 studies (Figure 1), 64 duplicate studies were removed, titles and abstracts screened 45 citations, and 9 were assessed for full-text review (Figure 1). Three studies were eliminated after reading the full-text review (not RCT=3). In the meta-analysis, we included 6 trials with 812 newborn patients.

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Study characteristics
Table 1 shows the study characteristics. Three research\textsuperscript{12,27,28} comprising 80.05\% of the six included trials (all published as RCTs) used a double-blind randomized control with 650 patients. Single-blinded was utilized in three other studies\textsuperscript{29-31} (19.95\%, with 162 neonatal patients), but the quality of evidence was low since it only blinded the outcome investigators—doctor and nurse blinding did not occur throughout the treatment. Three out of six studies, or 75.74\%, involving 615 patients, were categorized based on the degree of HIE. Of the 379 patients who received EPO therapies, 329 received high-dose therapy (≥1000 U/kg/week), and the other patients had low-dose therapy (<1000 U/kg/week). Twelve, twenty-seven, and twenty-nine studies reported follow-up for ≥18 months, while the remaining studies reported follow-up between three and twelve months. Each of the six articles had a different start time for the EPO treatment. One study\textsuperscript{29} reported that EPO was used the first 4 hours after birth, one within 6 hours after birth,\textsuperscript{27} three within 24 hours,\textsuperscript{28,30,31} and two within 26 hours.\textsuperscript{12}

Risk of bias in studies
Figure 2 displays the risk of bias assessed by the JBI critical appraisal of RCT and categorized according to the Cochrane Handbook for Systematic Reviews of Intervention criteria. Regarding selection bias, outcome bias, participation retention bias, and statistical validity, all trials had a low risk of bias; however, two trials\textsuperscript{29,30} had a significant risk of bias with regard to intervention bias. Two trials have a significant chance of intervention bias since the doctors, nurses, and research participants were not blinded.\textsuperscript{29,30} Generally, there was little risk of reporting, attrition, and detection bias.

Primary outcome: Cerebral Palsy
Figure 3 shows a forest plot of the odds ratio of cerebral palsy in the treatment group with EPO compared to the placebo group. Heterogeneity test showed Chi-squared = 4.29, p = 0.672, I²=76.7\%. The results show that EPO alone can be beneficial to the newborn with HIE and could prevent cerebral palsy than the control group (OR=0.90, 95\%CI was 0.57-1.44), which was statistically significant (z = 0.42, p = 0.038). We graded the level of evidence for this outcome as low (Table 2). Finally, the funnel plot and Egger's test were conducted to test publication biases by using STATA 14. The result suggested no published bias in this pooled outcome (symmetrical funnel plot). Sensitivity was conducted to assess the robustness of the synthesized results. The result suggested there was the robustness of the synthesized results (OR = 0.95, 95\%CI was 0.75-1.19).

Figure 4 shows a forest plot of the prevalence rate of cerebral palsy in the treatment group with EPO compared to the placebo group. Heterogeneity test showed Chi-squared = 0.02, p= 0.88, I²=86.38\%. The results show that EPO alone could decrease cerebral palsy event rate in the intervention group rather than the control group (overall event rate 17.72\%, which EPO vs. placebo was 13.03\% vs. 13.43\%), which was statistically significant (z = 6.27, p = <0.001). We graded the level of evidence for this outcome as low (Table 2). Finally, the funnel plot and Egger's
### Table 1. Summary of included studies comparing EPO (or its analogs) with placebo for infants with moderate to severe hypoxic-ischemic encephalopathy

<table>
<thead>
<tr>
<th>Study No.</th>
<th>Study ID</th>
<th>Location</th>
<th>Study period</th>
<th>Setting</th>
<th>Erythropoietin dose</th>
<th>Study design</th>
<th>Sample size</th>
<th>Estimated variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>El Shimi (2014)30</td>
<td>Cairo, Egypt</td>
<td>September 2007 to February 2010</td>
<td>Neonatal Intensive Care Units (NICU), Ain Shams University Hospitals</td>
<td>Single subcutaneous 1500 U/kg rEPO at day-1</td>
<td>RCT</td>
<td>45</td>
<td>Thompson's score, Neuromuscular function scale (NMS)</td>
</tr>
<tr>
<td>3</td>
<td>Wu (2016)31</td>
<td>USA</td>
<td>Not reported</td>
<td>Children's National Health System</td>
<td>1000 U/kg intravenously on days 1, 2, 3, 5, and 7</td>
<td>RCT</td>
<td>50</td>
<td>Albert Infant Motor Scale (AIMS), Warner Initial Developmental Evaluation (WIDEA)</td>
</tr>
<tr>
<td>4</td>
<td>Malla (2017)32</td>
<td>Srinagar, India</td>
<td>December 2012 to November 2015</td>
<td>Neonatal intensive care unit of the Sheri Kashmir Institute of Medical Sciences (SKIMS)</td>
<td>EPO 500 U kg⁻¹ per dose in 2 ml saline intravenously on alternate days for a total of five doses, with the first dose given by 6 h of age</td>
<td>RCT</td>
<td>100</td>
<td>Gross Motor Function Classification (GMFCS)</td>
</tr>
<tr>
<td>5</td>
<td>Wu (2020)33</td>
<td>USA</td>
<td>Not reported</td>
<td>Children's National Health System</td>
<td>Epo 1000 U/kg intravenously on days 1, 2, 3, 5, and 7</td>
<td>RCT</td>
<td>50</td>
<td>Albert Infant Motor Scale (AIMS), Warner Initial Developmental Evaluation (WIDEA)</td>
</tr>
<tr>
<td>6</td>
<td>Wu (2022)34</td>
<td>California, USA</td>
<td>January 25th, 2017, through October 9th, 2019</td>
<td>17 sites involving 23 hospitals</td>
<td>Erythropoietin (1000 U per kilogram of body weight) was administered intravenously within 26 hours after birth, as well as at 2, 3, 4, and 7 days of age.</td>
<td>RCT</td>
<td>500</td>
<td>Gross Motor Function Classification (GMFCS)</td>
</tr>
</tbody>
</table>

**Figure 2.** Risk of bias summary: a review of authors' judgments about each risk of bias item for each included study.

**Figure 3.** Forest plots for comparison-erythropoietin vs. placebo. The pooled outcome was EPO had a decreased risk of cerebral palsy in moderate-severe HIE patients. Sensitivity test were conducted to test publication biases by using STATA 14. The result suggested no publication bias in the pooled outcome (Egger's test 0.134). The result suggested the robustness of the synthesized results (ES = 14.99%, 95%CI was -52.95-82.94).
Figure 4. Forest plots for comparison-erythropoietin vs. placebo. The pooled outcome of the events of cerebral palsy was 13.03% vs 13.43% (erythropoietin vs placebo). The overall event rate was 17.72%.

Figure 5. Forest plots for comparison-erythropoietin vs. placebo. The pooled outcome was EPO had a decreased risk of neurodevelopmental impairments in moderate-severe HIE patients.

Figure 6. Forest plots for comparison-erythropoietin vs. placebo. The pooled outcome of the events of neurodevelopmental impairments was 19.76% vs 51.86% (erythropoietin vs placebo). The overall event rate was 35.72%.

Figure 7. Forest plots for comparison-erythropoietin vs. placebo. The pooled outcome was EPO had decreased AIMS and WIDEA scores in moderate-severe HIE patients, but it was not significant. A) AIMS score; B) WIDEA-communication score; C) WIDEA-mobility score; D) WIDEA-self-care score; E) WIDEA social score; and F) WIDEA total score.

Reduced the risk of neurodevelopmental impairments (p = 0.014, OR 0.50, 95%CI 0.36-0.70) (Figure 5). Neurodevelopmental impairments were reported in 19.76% (95%CI 15.59-24.25) of the erythropoietin group and 51.86% (95%CI 29.01-74.33) of the control group (overall event rate 35.72% [95%CI 24.92–47.24]) (shown in Figure 6). We graded the level of evidence for this outcome as low (Table 2). Finally, the funnel plot and Egger’s test were conducted to test publication biases by using STATA 14. The result suggested published bias in this pooled outcome (Egger’s test 0.026). Sensitivity was conducted to assess the robustness of the synthesized results. The result suggested there was the robustness of the synthesized results (OR = 0.69, 95%CI was 0.58-0.84).

Neurodevelopmental impairments were measured with AIMS and WIDEA. There were two studies that reported AIMS and WIDEA scores. WIDEA contained 5 assessments: communication, mobility, self-care, social, and total. Erythropoietin administration not significantly reduced the AIMS (p = 0.154, SMD 0.45, 95%CI -0.01-0.91), WIDEA-communication (p = 0.767, SMD 0.23, 95%CI -0.22-0.68),
Figure 8. Forest plots for comparison-erythropoietin vs. placebo. The pooled outcome was that EPO had decreased the risk of seizures in moderate-severe HIE patients, but it was not significant.

Figure 9. Forest plots for comparison-erythropoietin vs. placebo. The pooled outcome of the events of seizures was 26.75% vs 37.52% (erythropoietin vs placebo). The overall event rate was 31.70%.

Figure 10. Forest plots for comparison-erythropoietin vs. placebo. The pooled outcome was that EPO had decreased brain injuries in moderate-severe HIE patients, but it was not significant. A) white matter region; B) subcortical region; C) cortical region; D) brainstem region; and E) cerebellar region.

Figure 11. Forest plots for comparison-erythropoietin vs. placebo. The pooled outcome was the events of brain injuries, white matter, subcortical, cortical, brainstem, and cerebellar, were 52.38% vs. 61.00%, 28.55% vs. 68.33%, 25.63% vs. 31.58%, 6.85% vs 14.53%, 4.73% vs 29.01%, respectively. The overall event rate was 29.01%.

WIDEA-mobility (p = 0.156, SMD 0.39, 95%CI -0.07-0.85), WIDEA-self-care (p = 0.844, SMD 0.49, 95%CI 0.03-0.95), WIDEA-social (p = 0.649, OR 0.34, 95%CI -0.11-0.80), and WIDEA-total (p = 0.382, OR 0.31, 95%CI -0.14-0.77) based on its score (Figure 7).

Secondary outcome: Seizures
The secondary outcome of seizures was reported in four of the six included studies. Erythropoietin administration has not significantly reduced the risk of seizures (p = 0.270, OR 0.71, 95%CI 0.51-0.98) (Figure 8). Seizures were reported in 26.75% (95%CI 14.71-40.65) of the erythropoietin group and 37.52% (95%CI 21.53-54.93) of the control group (overall event rate 31.70% [95%CI 22.79–41.28]) (shown in Figure 9). We graded the level of evidence for this outcome as moderate (Table 2). Finally, the funnel
plot and Egger’s test were conducted to test publication biases by using STATA 14. The result suggested no published bias in this pooled outcome (Egger’s test 0.574). Sensitivity was conducted to assess the robustness of the synthesized results. The result suggested the robustness of the synthesized results (OR = 0.84, 95% CI was 0.71-0.99).

Secondary outcome: Brain injuries
The secondary outcome of brain injuries was subgrouped based on its anatomical regions (white matter, subcortical, cortical, brainstem, cerebellar) reported in two of the six included studies. Erythropoietin administration did not significantly reduce the risk of brain injuries: white matter (p = 0.923, OR 0.70, 95%CI 0.29-1.68), subcortical (p = 0.808, OR 0.19, 95%CI 0.07-0.48), cortical (p = 0.130, OR 0.76, 95%CI 0.29-1.95), brainstem (p = 0.429, OR 0.44, 95%CI 0.10-1.92), cerebellar (p = 0.655, OR 0.25, 95%CI 0.05-1.27) (Figure 10). Brain injuries, including white matter, subcortical, cortical, brainstem, cerebellar, were reported in 52.38% (95%CI 36.88-67.67), 28.55% (95%CI 15.46-43.55), 25.63% (95%CI 13.10-40.35), 6.85% (95%CI 0.54-17.38), 4.73% (95%CI 0.14-30.10), respectively, of the erythropoietin group and 61.00% (95%CI 45.21-75.78), 68.33% (95%CI 52.85-82.13), 31.58% (95%CI 17.79-46.05), 14.53% (95%CI 4.75-27.64), 29.01% (95%CI 19.45-39.50), respectively, of the control group (overall event rate 29.01% [95%CI 19.45–39.50]) (shown in Figure 11). We graded the level of evidence for these outcomes as low except subcortical as moderate (Table 2).

Secondary outcome: Death
The secondary outcome of death was reported in all included studies. Erythropoietin administration also significantly reduced the risk of death (p = 0.018, OR 0.81, 95%CI 0.54-1.20) (Figure 12). Death was reported in 14.31% (95%CI 6.09-24.80) of the erythropoietin group and 20.67% (95%CI 9.84-33.85) of the control group (overall event rate 16.75% [95%CI 10.64–23.77]) (shown in Figure 13). We graded the level of evidence for this outcome as low (Table 2). Finally, the funnel plot and Egger’s test were conducted to test publication biases by using STATA 14. The result suggested no published bias in this pooled outcome (Egger’s test 0.026). Sensitivity was conducted to assess the robustness of the synthesized results. The result suggested the robustness of the synthesized results (OR = 0.69, 95% CI was 0.58-0.84).

DISCUSSION
In this systematic review and meta-analysis, we found that newborns treated with EPO had a lower risk of cerebral palsy and neurodevelopmental deficits than infants with moderate to severe HIE. For newborns receiving EPO, there was a tendency toward a decreased risk of death. In newborns treated with EPO, the event rate of cerebral palsy, neurodevelopmental abnormalities, seizures, brain injury on MRI, and death was reduced.

While numerous clinical trials have assessed the safety and effectiveness of EPO in a range of conditions (preterm infants, liver function impairment, renal dysfunction, HIE), only two randomized controlled trials have examined EPO’s clinical benefits in preventing cerebral palsy in infants with HIE,12,27 as well as its association with brain injury on MRI,28,31 and neurodevelopmental measurements using AIMS and WIDEA.28,31 Few studies are unable to analyze biased publications. The majority of the trials in this review were tiny. Trials were divided into half single-centered and half multi-centered. The risk of bias in the included studies was influenced by potential selection bias and the lack of blinding of participants and personnel, which impacted the LOE.

Throughout the included trials, the median cumulative dose of EPO was 1000 U/kg (range: 500–1500 U/kg). Only one trial administered EPO subcutaneously, and the other trials used intravenous
Erythropoietin compared to placebo or no intervention for moderate to severe hypoxic-ischemic encephalopathy

**Table 2. Summary of findings**

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Anticipated absolute effects (95%CI)</th>
<th>Odds effect (Random, 95%CI)</th>
<th>No. of participants (studies)</th>
<th>Heterogeneity (I²)</th>
<th>Certainty of the evidence (GRADE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral palsy</td>
<td>134 per 1000 (9.69-17.66)</td>
<td>OR 0.90 (0.57-1.44)</td>
<td>150 (2 RCTs)</td>
<td>76.7%</td>
<td>⊕⊕ΟΟ Low&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neurodevelopmental impairs</td>
<td>519 per 1000 (15.59-24.25)</td>
<td>OR 0.50 (0.36-0.70)</td>
<td>762 (5 RCTs)</td>
<td>67.8%</td>
<td>⊕⊕ΟΟ Low&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Seizures</td>
<td>375 per 1000 (14.71-40.65)</td>
<td>OR 0.71 (0.51-0.98)</td>
<td>712 (4 RCTs)</td>
<td>23.5%</td>
<td>⊕⊕ΟΟ Moderate&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brain injury on MRI</td>
<td></td>
<td></td>
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<tr>
<td>White matter</td>
<td>610 per 1000 (36.88-67.67)</td>
<td>OR 0.70 (0.29-1.68)</td>
<td>100 (2 RCTs)</td>
<td>0%</td>
<td>⊕⊕ΟΟ Low&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Subcortical</td>
<td>683 per 1000 (15.46-43.55)</td>
<td>OR 0.19 (0.07-0.48)</td>
<td>100 (2 RCTs)</td>
<td>0%</td>
<td>⊕⊕ΟΟ Moderate&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cortical</td>
<td>316 per 1000 (13.10-40.35)</td>
<td>OR 0.76 (0.29-1.95)</td>
<td>100 (2 RCTs)</td>
<td>56.5%</td>
<td>⊕⊕ΟΟ Low&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
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<td>Brainstem</td>
<td>145 per 1000 (0.54-17.38)</td>
<td>OR 0.44 (0.10-1.92)</td>
<td>100 (2 RCTs)</td>
<td>0%</td>
<td>⊕⊕ΟΟ Low&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cerebellar</td>
<td>169 per 1000 (0-14.30)</td>
<td>OR 0.25 (0.05-1.27)</td>
<td>100 (2 RCTs)</td>
<td>0%</td>
<td>⊕⊕ΟΟ Low&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Death</td>
<td>207 per 1000 (6.09-24.80)</td>
<td>OR 0.81 (0.54-1.20)</td>
<td>812 (6 RCTs)</td>
<td>63.5%</td>
<td>⊕⊕ΟΟ Low&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

<sup>a</sup>The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the odds effect of the intervention (and its 95% CI). CI, confidence interval; OR, odds ratio.

**GRADE Working Group grades of evidence**

**High certainty**: We are very confident that the true effect is close to the effect estimates.

**Moderate certainty**: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

**Low certainty**: Our confidence in the effect estimate is limited. The true effect may be substantially different from the estimate of the effect.

**Very low certainty**: We have very little confidence in the effect estimate: The true effect is likely to differ substantially from the estimate of effect.

<sup>b</sup>Unclear allocation concealment, non-blinded to care providers in two trials.

<sup>c</sup>CI wide enough to downgrade the evidence.

<sup>d</sup>Other, than any publication bias in one trial, there was no publication bias analysis due to the limited study number (two trials).

delivery. Neonatal practice has assessed both administration methods; nevertheless, there is insufficient data to recommend one. In animal studies, the documented neuroprotective dosage of EPO varies between 1000 and 30,000 U/kg. It has been demonstrated that the 5000 U/kg EPO dose increases neuronal proliferation and reduces inflammation and stops neuronal apoptosis. The same dosage schedule authorized for the treatment of anemia was used in the previous trials. A higher dose of EPO, 1000 U/kg/day (cumulative dose = 5000 U/kg), was employed in the subsequent RCT, which concluded that it was safe to treat newborn HIE. The high dose regimen (1000 U/kg/day) for treating neonatal HIE has also been included in the ongoing trials. This review makes it difficult to determine whether the therapeutic advantages of EPO are dose-dependent and whether the subcutaneous and intravenous routes of administration have equal doses and efficacies.

A breach in the blood-brain barrier caused by hypoxia-ischemia allows more components, including epoxide, to enter the CSF and possibly even reach neuronal structures in the brain. In these circumstances, EPO neuroprotection may also have systemic benefits, such as improved erythropoiesis, which raises iron utilization and lowers free iron levels, lowering oxidative brain injury. In light of this, EPO has reduced the systemic inflammatory response in both term and preterm newborns. EPO’s systemic effects, which include lowering inflammation, regulating oxygen availability, and reducing free iron, enhance its direct neuroprotective benefits. This could account for why lower dosage regimens also lead to better results. The acute effects of EPO have the overall effect of reducing apoptosis. EPO also enhances the brain’s ability to heal itself over the long term following an insult by promoting neurogenesis and enhancing angiogenesis, two processes that increase the body’s capacity to carry oxygen.
in infants receiving EPO for moderate to severe HIE. The study's strengths lie in the importance of the issue it poses and in its comprehensive literature evaluation, which will assist medical professionals in treating newborns with HIE. The LOE for the pooled estimations is evaluated in the study. This review also offers a quantitative synthesis; nevertheless, the pooled effect for several outcomes had fewer than two participants overall, making it impossible to examine publication bias—a finding that one would anticipate from a well-powered study—because of this. The review examined the effectiveness of EPO used alone. A thorough search, clear inclusion and exclusion criteria, and a completed meta-regression analysis to establish homogeneity are among the other strong points.

The fact that only six studies were included for the targeted outcomes may be one of this review's limitations. Just two trials examined the efficacy of EPO as a preventative measure against cerebral palsy in patients with HIE,12,27 as well as its correlation with MRI brain injury.28,31 and neurodevelopmental assessments using AIMS and WIDEEA.28,31 This prevented the performance of a biased publication analysis in a limited number of trials. The lack of a plan for the secondary search for pertinent publications (non-systematic reviews and conference abstracts) is one of the main limitations; that being said, it is doubtful that our search overlooked any RCTs. Finally, a small number of non-blinded RCTs and possible selection bias impact the LOE. As explained earlier, from a database search, only two journals examine the correlation of EPO to CP. More RCT research is needed on the role of EPO in CP development.

HIE is common in nations with limited resources. The likelihood of survival is decreased, and the risk of morbidity is elevated in the absence of facilities for therapeutic hypothermia. Numerous studies, including newborns with HIE, have demonstrated the efficacy of EPO in lowering morbidity and enhancing neurodevelopmental outcomes, even in those infants who did not receive therapeutic hypothermia.12,44-46 Erythropoietin's efficacy as a stand-alone treatment for HIE is anticipated to be further supported or refuted by the ongoing research. In any case, in resource-constrained environments, it will support parents' decision-making and counseling when neonatal care providers treat newborns with HIE. In addition, it is important to support evidence-based, economical interventions that improve the quality of life for HIE survivors while reducing mortality.

CONCLUSION

Erythropoietin may be able to prevent cerebral palsy, according to the pooled data from this meta-analysis. Additionally, erythropoietin may enhance neurodevelopmental outcomes and avert neonatal HIE deaths. When used as a monotherapy, erythropoietin has proven to be effective. It has also shown safety and efficacy at various doses, with no negative side effects. Conducting appropriately powered clinical trials to further assess erythropoietin is imperative, considering only 6 trials were included. It will be essential for researchers and clinicians to work with specialists and create interest groups to define primary and secondary outcomes and acceptable guidelines for using erythropoietin in future trials. As a neuroprotective drug, erythropoietin has thus far shown promise, and its continued use may significantly improve outcomes for newborns with HIE.

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CONFLICT OF INTEREST

There is no conflict of interest in conducting and reporting the study.

AUTHORS CONTRIBUTION

The authors have equal contributions in conducting and reporting the study.

REFERENCES


