Effects of EPO combined with mild hypothermia on oxidative stress and neuroprotection in neonates with hypoxic-ischemic encephalopathy

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ABSTRACT

After perinatal asphyxia, hypoxic-ischemic encephalopathy (HIE) in term infants produces long-term neurologic sequelae or death. Obtaining a reliable, evidence-based prognosis is critical. Therapeutic hypothermia is a suggested treatment for newborn babies with moderate-to-severe HIE at or near term. However, this treatment is unsuccessful in a significant proportion of newborns. This sparked a worldwide hunt for neuroprotectants that may enhance the effects of mild hypothermia. We look at erythropoietin (EPO) as a possible possibility. This research aimed to see how EPO paired with moderate hypothermia affects oxidative stress and neuroprotection in newborns with HIE. Children with HIE diagnosed and treated at the hospital were first recruited as research participants and split into two groups using a random number system. The control group got mild hypothermia therapy as part of their standard treatment, whereas the EPO group received EPO therapy in addition to mild hypothermia therapy. Statistical analysis techniques such as the Mann-Whitney U test, Chi-squared test, and t-test were used to examine the effects. The data show that the efficacies of combination therapy of mild hypothermia and EPO for infant HIE seem to be promising right now.

Introduction

Hypoxic-ischemic encephalopathy (HIE) is the name for the various physiological, cellular, and molecular alterations that occur after severe anoxic brain damage in the newborn period. In industrialized nations, the incidence of HIE is about 1.5 cases per 1000 live births, but in poor and middle-income countries, the incidence is over 1020 cases per 1000 live births (1). Hypoxia-ischemia is not the only cause of neonatal encephalopathy. Seizures are one of the most visible and frequent symptoms of encephalopathy, and they may be used to diagnose the disease. Both newborn encephalopathy and seizures are prevalent in premature babies, although the seizures are usually asymptomatic (2). Fig.1 illustrates the complications of Hypoxic-ischemic encephalopathy.

The principal hematopoietic growth factor is erythropoietin (EPO). The kidney is the principal location of EPO production throughout embryonic development. EPO receptors (EPOR) are found in glial cells, neurons, and endothelial cells throughout the brain, including the hippocampus, cortex, internal capsule, and midbrain. Low dosages of EPO (250U/kg) used to treat anemia do not often raise EPO concentrations in the cerebrospinal fluid. In the case of hypoxic ischemia, however, the BBB (Blood–Brain Barrier) permeability is enhanced, and dosages of 300 to 500 U/kg are linked with a considerable increase in EPO levels in the cerebrospinal fluid (3). In addition to being a hemopoietic growth factor, erythropoietin is a cytokine with numerous functions. Neurons, glia, and endothelial cells all have erythropoietin receptors, which play a role in cell proliferation and differentiation both during normal brain development and after hypoxia. Hypoxia and pro-inflammatory cytokines stimulate the production of erythropoietin and receptors via activating the hypoxia-inducible factor. Erythropoietin promotes anti-apoptotic, anti-oxidative, and anti-inflammatory responses, which offer neuroprotection. Additionally, the production of matrix metalloproteinases by erythropoietin promotes neuronal and glial migration surrounding the damaged location (4). Epo is created by the liver throughout fetal development, but it is gradually synthesized in the peritubular cells of the kidney after birth. The hematological activities of erythropoietin are the most well-known. The transcription of the Hypoxia-

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Inducible Transcription Factor, which is activated by hypoxia, determines an increase in Epo hormone synthesis in the kidney after a 30-minute ischemia insult. When EPO binds to receptors on erythroid progenitor cells, the mass of red blood cells increases. The blood's increased oxygen-carrying capacity suppresses Epo expression, completing the feedback loop. After hypoxic exposure, endogenous Epo levels have been shown to rise in the brain for four hours. Hypoxia triggers the production of hypoxia-inducible factor-1, which regulates the expression of growth factors such as VEGF and EPO. Vascular Endothelial Growth Factor (VEGF) has long been recognized as an important growth factor for the vascular endothelium. Epo's immediate action is indicated by increased hemoglobin expression, which enhances oxygen intake and storage in hypoxic tissue.

The remainder of the description is divided into five parts: Segment 2: related works and problem definition, Segment 3: the methodology used, Segment 4: result and discussion, and Segment 5: conclusion.
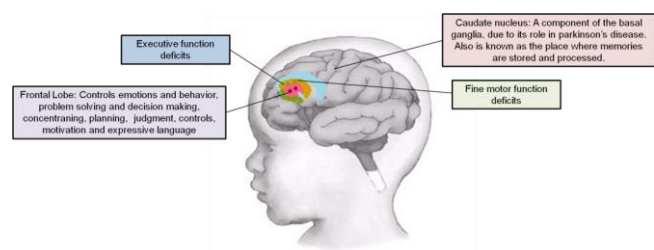

Figure 1. Complications of Hypoxic-ischemic encephalopathy

Related works

Many of the disorders have multiple odontogenic keratocysts (6). A 12-year-old female youngster had several odontogenic keratocysts. The studies found no other anomalies indicative of a condition. In a study (7), personalized medicine employs fine-grained data to identify specific deviations from normal. These developing data-driven health care methods were conceptually and ethically investigated using 'Digital Twins' within engineering. Physical artifacts were coupled using digital techniques which continuously represent their state. Moral differences can be observed based on data structures and interpretations imposed on them. Digital Twins' ethical & sociological ramifications are examined. The

Healthcare system has become increasingly data-driven. This technique could be a social equalizer by providing efficient equalizing enhancing strategies. In a research (8), allergic rhinitis would be a long-standing worldwide epidemic. Taiwanese doctors commonly treat it with either traditional Chinese or Chinese–Western drugs. Outpatient traditional Chinese medicine therapy of respiratory illnesses was dominated by allergic rhinitis. They compare traditional Chinese medicine with western medical therapies in treating allergic rhinitis throughout Taiwan. In a report (9), the usage of high-dose-rate (HDR) brachytherapy avoids radioactivity, allows for outpatient therapy, and reduces diagnosis timeframes. A single-stepping source could also enhance dosage dispersion by adjusting latency at every dwell location. The shorter processing intervals need not permit any error checking, and inaccuracies could injure individuals. Hence HDR brachytherapy therapies should be performed properly. In a study (10), this study presented a treatment as well as the technology of domestic sewage to improve the rural surroundings. In a report (11), soil samples from chosen vegetable farms throughout Zamfara State and Nigeria have been tested for physicochemical & organochlorine pesticides. Testing procedure and data were analyzed using QuEChERS with GC-MS. In (12), the absence of cerebral palsy, to assess the long-term cognitive and behavioral outcomes of infants with newborn HIE. In (13), the newborns with hypoxic-ischemic encephalopathy, and therapeutic hypothermia lower the risk of mortality or moderate to severe neurodevelopment impairment ("NDI"). There are few reports on its safety and effectiveness in preterm newborns. The goal of (14) was a systematic review to find out what was currently known about reported outcomes in babies with moderate HIE. In (15), find out more about term babies with persistent pulmonary hypertension of the newborn ("PPHN") and moderate or severe HIE. The researchers (16) intended to discover whether children with "HIE" who were given therapeutic hypothermia ("TH") performed better than their peers on tests of fine motor abilities, executive function, language, and general cognitive capacity all of which are important for school readiness. The goal of (17) was to look at the prevalence of placental abnormalities in a multicenter cohort of newborns with HIE and see whether there

was a link between placental abnormality acuity and HIE clinical features. In a research (18), the efficacy of the previously discovered candidate metabolites to predict HIE both separately and in combination with clinical data. In (19), Evoked potentials (EP) were evaluated for their predictive value in newborns with normal magnetic resonance imaging ("MRI") after therapeutic hypothermia (TH) for HIE. The results of studies (20) on infants with HIE have been mixed. Erythropoietin administration in HIE, with or without therapeutic hypothermia, seems to be safe and beneficial. In a study (21), the goal was to use machine learning algorithms to analyze freely accessible clinical data that may help doctors identify children who will develop HIE early and accurately.

Problem statement

Lack of oxygen to the fetus at any stage during pregnancy or delivery is the most common cause of Neonatal Encephalopathy (NE). When NE is caused by a lack of oxygen, the illness is known as HIE. The brain is damaged by a lack of oxygen, but it may also harm other internal organs.HIE is caused by a sudden decrease in blood and oxygen supply to a baby's brain after delivery during pregnancy. The lack of oxygen and blood leads cells in the growing brain of the infant to quickly degrade and die. Infants with moderate HIE usually survive and do not suffer major, long-term consequences. Injuries to the brain caused by HIE often result in physical and cognitive problems.

Materials and methods

The purpose of this research is to see how EPO paired with moderate hypothermia affects oxidative stress and neuroprotection in newborns with HIE. Children with HIE who had been diagnosed and treated at the hospital were first recruited as research participants and split into two groups using a random number system. The control group got mild hypothermia therapy as part of their standard treatment, whereas the EPO group received EPO therapy in addition to mild hypothermia therapy. Fig. 2 indicates the proposed methodology.

Data collection

The ethics committee at Yan Tai Yu Huang-ding Hospital has accepted this retrospective investigation. Clinical data from 90 neonates with HIE admitted to

our hospital between May 2020 and May 2021 were analyzed (23). To divide the data into two groups, the random number table approach is used.
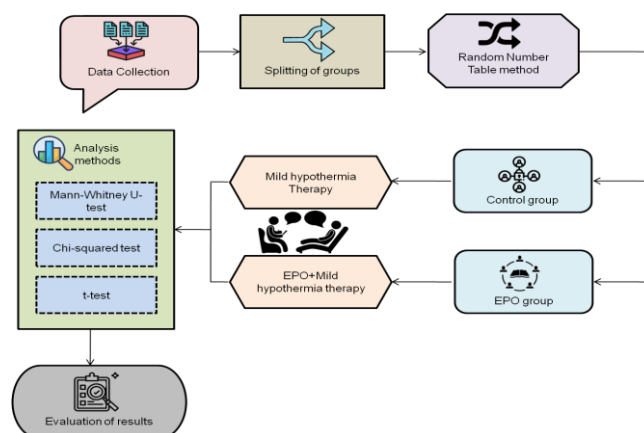

Figure 2. Proposed methodology

Random Number Table Method

A total of 90 children with HIE who were diagnosed and treated in the hospital between May 2020 and May 2021 were selected as research participants and split into two groups using the random number table method: control (n=45) and EPO (n=45). The control group got moderate hypothermia treatment based on conventional therapy, whereas the EPO group received EPO therapy based on the control group's therapy.

Control group

In the control group, 45 infants were chosen and moderate hypothermia therapy was administered.

Mild hypothermia treatment

The control group received mild hypothermia treatment based on conventional treatment, which was as follows: temperature-variable circulation liquid was poured into the hypothermia cushion in the mild hypothermia therapy apparatus, the children were placed above the hypothermia cushion, the anus temperature was monitored and controlled at 33-34 °C for 3 days, and then natural rewarming was performed.

EPO group

In the EPO group, 45 babies were selected and given EPO treatment along with mild hypothermia treatment.

EPO with Mild Hypothermia Treatment (MHT)

EPO group received EPO treatment based on the treatment of the control group, which was as follows: EPO 1 000 IU/kg, intramuscular injection, once every other hour, for a total of 7 times.

EPO is a glycoprotein that controls the synthesis of red blood cells (i.e., erythropoiesis). EPO, in general, offers a mechanism for all other cells to retain or re-establish function under difficult physiological situations (e.g., hypoxia). Fig.3 indicates the restorative effects of EPO.
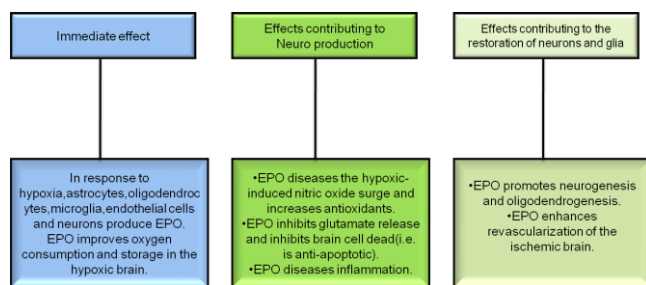

Figure 3. Restorative effects of EPO

EPO seems to have an impact outside of the hematological system, especially on the neurological system, according to preliminary research. The paucity of widely accessible polyclonal antibodies targeting the EPO receptor impeded our study. In the human autonomic nervous system, EPO and/or its transmitter activation were found in axons, hepatocytes, endothelium, macrophages, and neurogenesis. All commercially available EPO receptor antibodies, however, were limited by non-specific cross-reactivities.

The Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes ("NEATO") approach were used to study 45 newborn newborns with moderate to severe HIE. Five doses of EPO or an equal volume of normal saline were administered to the newborns (placebo). On days one, two, three, five, and seven of life, the doses were administered. At 6 hours after birth, both groups got MHT of the entire body or head cooling to the internationally accepted standard of 33.5 degrees Celsius for 72 hours. The first dose of r-Hu-EPO (Procrit or epoetin alfa) was given less than 24 hours after the infant was delivered, according to the clinical study number NCT 01913340. MRI was used to detect ischemic brain damage in 45 neonates, and it was shown that those who received HT with EPO had a statistically significantly lower volume of acute brain injury than those who received HT saline.

Analysis methods

Mann-Whitney U test, Chi-squared test, and t-tests are used to evaluate the data show that the efficacy of combination therapy of mild hypothermia and EPO for infant HIE seems to be promising right now. The oxidative stress levels are measured in the control and EPO group using the Mann-Whitney U test, Chi-squared test, and t-tests.

Mann-Whitney U test

The Mann-Whitney U test is often used to 2 autonomous groups has a difference in the predictor variables. It examines if the interdependent variable's dispersion is the same with the two groups, implying that they are from the identical community.

$$a=As(B_{11}>B_{21})+\frac{1}{2}As(B_{11}=B_{21})=-\int_0^\infty T_1^{\pm}(b)eT_2(b) \quad [1]$$

Where $T_1^{\pm}(b)=\{T_1(b+)+T_1(b-)\}/2$ is the normalized version. The hypothesis $H_0^t:T_1(b)=T_1(b)\forall b$ implies $q=1\backslash2$.

$$S_0^q:a=1\backslash2 \quad Vs \quad S_1^a:a=1\backslash2 \quad [2]$$

In order to test S_0^a, one can use Efron's estimator of q given by

$$W_o(a)=\sqrt{\frac{o_1o_1}{o}}(\hat{a}-a)\xrightarrow{e}O(0,\sigma^2) \text{ as } o\to\infty \quad [3]$$

$$\sigma_{jl}^2=\int_0^\infty\int_0^\infty\Gamma_j^{\pm\pm}(v,w)eT_1(v)eT_1(w) \quad [4]$$

$$DJ=\left[\hat{a}-c_{\alpha/2}\frac{\hat{\sigma}}{\sqrt{o_1o_1/o}},\hat{a}+c_{\alpha/2}\frac{\hat{\sigma}}{\sqrt{o_1o_1/o}}\right] \quad [5]$$

Where c_α is the upper percent point of N (0, 1).

Chi-square test

Pearson's chi-squared test is a statistical process used to determine whether or not any measurable variance across collections of category data is random.

It investigates if the frequency distribution of certain occurrences observed in a collection is consistent with an analytical distribution. The alternatives considered should be mutually exclusive and have a one-to-one cumulative probability. This is a common circumstance where all of the episodes have a categorical data result. The idea that a standard six-sided die is "fair" is, in fact, a simplification.

In three sets of relationships, Pearson's chi-squared assessment measures convenience, uniformity, and independence.

➢ A convenient test estimates when a frequency distribution measured differs from the analytical distribution.

➢ Using the same type parameter, a uniformity test evaluates the distribution of values among multiple groups.

➢ Analysis of independent determines if findings comprised of 2 factors' measurements, as represented in a contingency table, were independent of one another.

$$Pearson's \ chi\text{-}squared \ test = \sum_{j=1}^{n} \frac{(N_j\text{-}F_j)^2}{F_j} \qquad [6]$$

Here, N_j=measurements of type j

N=sum of measurements

F_j=predicted count of type j

N=amount of cells

Whether or not they had been treated. Expected Chi-Square values are determined as follows:

$$E = \frac{N_V \times N_C}{o} \qquad [7]$$

Where: E = reflects the work value of the unit,

N_C = denotes that cell nucleus row edge,

N_V = denotes that cell's row edge, and

o = reflects the sample group as a whole.

The sample size is split by the product of the row marginal and the column marginal for each cell.

$$y^2 = \frac{(Q\text{-}E)^2}{E} \qquad [8]$$

Correlation measures are statistical assessments of the strength of a relationship. The Cramer's V test is the most often utilized Chi-square strength test. Using the formula below, it's easy to calculate out:

$$\sqrt{\frac{y^2/o}{(l\text{-}1)}} = \sqrt{\frac{y^2}{o(l\text{-}1)}} \qquad [9]$$

Useful for analyzing data, the Chi-square is an excellent tool for discovering the nature of research data.

T-test

The assumption that there is no variation across the three groups is tested using the Student's t-test. It's used in a variety of situations:

To conclude if a test denotes (as an estimate of a group mean) deviates considerably from a particular group mean

$$m = \frac{x\text{-}s}{VE} \qquad [10]$$

Where X = sample mean, S = population mean and VE = standard error of mean

$$m = \frac{X_1\text{-}X_2}{VE_{X_1\text{-}X_2}} \qquad [11]$$

Wherein X1-X2 signifies the distinction

It is determined to predict the data shown from the two variable samples differ considerably. Whenever variables are measured on the same participants during a drug, a paired t-test is commonly used.

The paired t-test equation is:

$$m = \frac{g}{VE_g} \qquad [12]$$

Where g stands for the overall mean and VE stands for the standard error of the variance.

To evaluate component variations, the F-test can be utilized. The F-test employs the proportion of deviations. The subgroup variances are presumed to be distinguishable if E is distinguishable from 1.0.

Results and discussion

The purpose of this research is to see how EPO paired with moderate hypothermia affects oxidative stress and neuroprotection in newborns with HIE. Children with HIE who had been diagnosed and treated at the hospital were first recruited as research participants and split into two groups using a random number system. Existing methods include the Neuroprotective Effect of Sovateltide (NES), Cell-Based Treatment (CBT), Biomarkers, and Mild Hypothermic Therapy (MHT). The performance matrices are frequency of alive, complications, and dead scores of control and EPO group are analyzed, post-intervention, efficiency, amplitude, latency, the velocity of sensory conduction nerve and motor conduction nerves are analyzed.

Frequency

The frequency of alive, complications, and dead are analyzed using the control group and EPO group. Alive, complications and death rates of the EPO group are comparatively higher than the control group. Fig.4 depicts the frequency of control and EPO group.

Post-intervention

After treatment, the mild, moderate, and severe HIE are assessed using the control and EPO groups. Post-intervention of treatment in mild, moderate, and severe HIE shows the best outcomes for the EPO group. Fig. 5 depicts the post-intervention of the control and EPO group.

Sensory and motor nerve transmission are altered in HIE infants

Conduction of information between the senses and the nervous system. Fig. 6a, b, and c stand for amplitude, latency, and nerve conduction velocity, respectively. The ulnar, radial superficial, sural, and superficial peroneal nerves are the sensory nerves studied. (d–f): Conduction between the motor and nervous systems. Fig.6d, e, and f stand for amplitude, latency, and nerve conduction velocity, respectively. The ulnar, peroneal, and tibial motor nerves are all represented. Neonates with HIE are referred to as control and EPO groups, respectively.

Efficiency

The efficiency of the EPO group is evaluated in Fig. 7. The proposed method has a high efficiency when compared to the existing methods such as NES, CBT, biomarkers, and MHT.

Analysis methods
Mann-Whitney test results for oxidative stress level

Glutathione peroxidase ("GSH-Px"), Superoxide dismutase ("SOD"), advanced oxidation protein products ("AOPP"), Reactive oxygen species ("ROS") are measured using Mann-Whitney U-test. Table 1 depicts the oxidative stress level using the Mann-Whitney test.
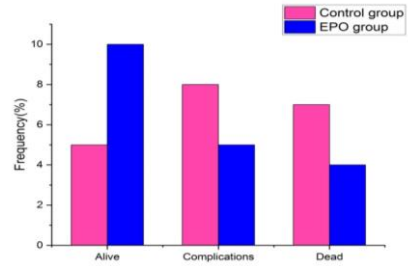

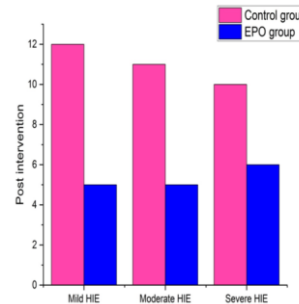**Figure 4.** Frequency of control and EPO group

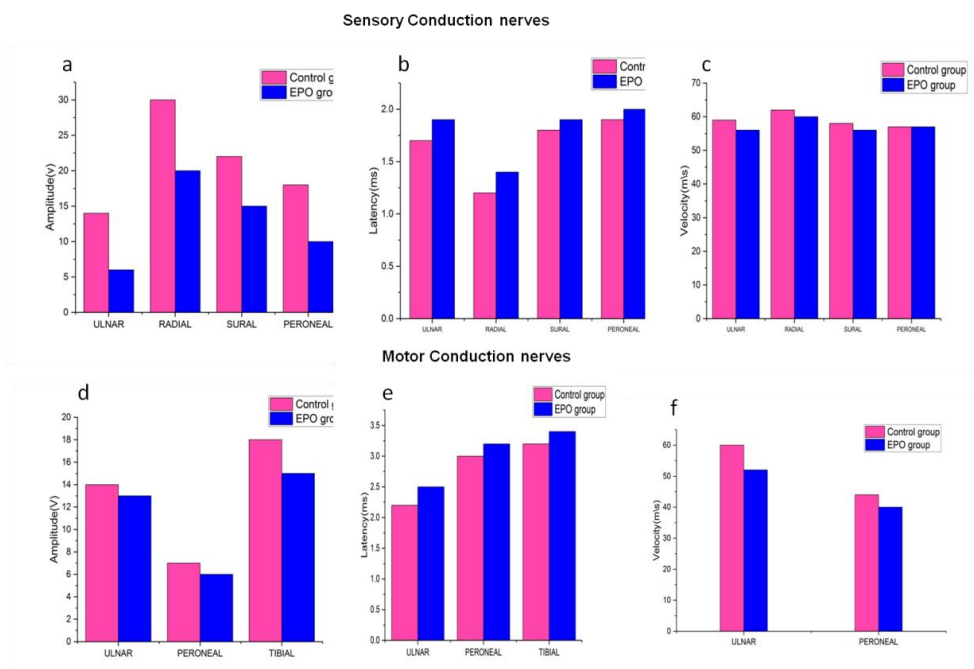**Figure 5.** Post-intervention of control and EPO group

Figure 6. Sensory and motor nerve transmission is altered in HIE infants.

Chi-square test results for oxidative stress level

The Chi-square test is used to detect GSH-Px, SOD, AOPP, and ROS. The oxidative stress level is shown in Table 2 using the Chi-square test.

T-test

To detect GSH-Px, SOD, AOPP, and ROS, the t-test is performed. Table 3 uses the t-test to determine the amount of oxidative stress.
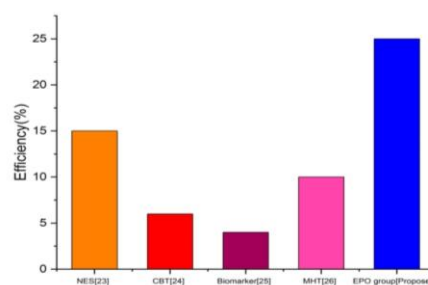

Figure 7. Comparison of efficiency

Table 1. Oxidative stress level using Mann-Whitney test

Group	n	GSH-Px		SOD		AOPP		ROS	
		Before therapy	After therapy ended	Before therapy	After therapy ended	Before therapy	After therapy ended	Before therapy	After therapy ended
Control	45	5.16±0.65	8.28±0.97	40.28±5.17	62.74±6.9*	5.94±0.68	3.26±0.39*	744.2±89.66	493.14±53.39*
EPO	45	5.23±0.66	13.19±1.78*	40.54±4.8	85.45±9.19*	5.81±0.64	1.78±0.25*	739.64±85.24	216.07±27.49*
t		0.183	8.483	0.215	16.492	0.163	7.394	0.231	23.051
p		＞0.04	＜0.04	＞0.04	＜0.04	＞0.04	＜0.04	＞0.04	＜0.04

Table 2. Oxidative stress level using Chi-square test

Group	n	GSH-Px		SOD		AOPP		ROS	
		Before therapy	After therapy ended	Before therapy	After therapy ended	Before therapy	After therapy ended	Before therapy	After therapy ended
Control	45	5.17±0.64	8.27±0.96	40.38±5.18	62.84±6.9*	5.84±0.78	3.36±0.49*	754.2±79.66	493.24±54.39*
EPO	45	5.43±0.66	13.2±1.78*	40.56±4.8	85.46±9.2*	5.86±0.61	1.74±0.28*	740.67±85.25	218.07±28.49*
t		0.184	8.484	0.216	16.495	0.167	7.398	0.235	23.056
p		＞0.03	＜0.04	＞0.05	＜0.04	＞0.05	＜0.04	＞0.03	＜0.04

Table 3. Oxidative stress level using t-test

Group	n	GSH-Px		SOD		AOPP		ROS	
		Before therapy	After therapy ended	Before therapy	After therapy ended	Before therapy	After therapy ended	Before therapy	After therapy ended
Control	45	6.17±0.64	8.37±0.96	40.39±5.18	65.84±6.9*	5.87±0.79	3.39±0.51*	757.2±8.66	494.24±55.39*
EPO	45	5.53±0.66	13.3±1.78*	40.58±4.8	85.43±9.2*	5.89±0.61	2.74±0.28*	770.67±85.25	218.08±28.5*
t		0.284	8.284	0.212	16.295	0.267	7.298	0.225	23.057
p		＞0.02	＜0.03	＞0.04	＜0.06	＞0.04	＜0.03	＞0.02	＜0.04

In children with HIE, mild hypothermia may help to some degree by slowing neuron metabolism, as well as other mechanisms, but many experts feel that EPO may further increase clinical therapeutic effects and preserve the children's lives. Human EPO is an endogenous glycoprotein hormone that rises in hypoxia, but the synthesized quantity cannot entirely compensate for neuron hypoxia, thus, exogenous EPO is needed. Children with HIE were treated with exogenous EPO supplementation in this research to see whether it had any beneficial effects on nerve function, myocardial damage, or overall oxidative stress. One of the important causes of neurological disorders is an oxidative stress imbalance that occurs when the hypoxia condition in children with HIE leads to systemic aerobic metabolism disorders, ROS synthesis increasing and stimulating the response to oxidative stress, the synthesis of oxidative metabolic AOPPs increasing and antioxidant GSH-Px and SOD consumption increasing. EPO and mild hypothermia therapy may effectively restrain the systemic oxidative stress response in children with HIE, and this is one of the core mechanisms for it to protect brain function and myocardial function. Despite its effectiveness of NES (existing), NES has some drawbacks, including a lack of skilled people, equipment, and pediatric neurology support in most NICUs (newborn ICUs). Furthermore, it has low effectiveness in avoiding neurological issues in HIE infants since more than 40% of neonates that are hypothesized still have neurological problems. Moreover, the effect of this treatment on neurodevelopment in children over the long term (>2 years) is unknown (23). In CBT (existing) some

clinical experiments employing umbilical cord blood cells, placenta-derived stem cells, mesenchymal stem cells (MSCs), and other stem cells have shown promising outcomes, while additional research and multi-center trials are required to establish reduced safety and inefficacy. Most cell-based treatments' therapeutic benefits are now thought to be due to the bystander impact of donor cells. Transplanting stem cells after HIE reduces the abnormal inflammatory cascade and creates a better environment for endogenous neurogenesis and repair (24). In biomarkers (existing), the shortcoming may be owing to the limited number of newborns tested, rendering it insufficiently powered to detect associations between biomarkers and infant survival. Finally, chosen markers may be used to assess the severity of HIE; however, their levels do not correlate with neonatal prognosis (25). In MHT (existing), despite these restrictions, the treatment regimen is continually improving because of advances in clinical practice. Mild hypothermia treatment, for example, may successfully enhance neurological function and decrease the inflammatory response in children with HIE. Furthermore, regular hypothermia treatment for HIE children carries the risk of respiratory failure, which is linked to oxygen deficiency caused by ischemic hypoxia episodes (26). So that the results show that treating newborn HIE with a combination of mild hypothermia and EPO seems to be successful.

Conclusions

Hypoxic-ischemic encephalopathy (HIE) is one of the most serious birth complications affecting full-term infants.1 It occurs in 1.5 to 2.5 per 1000 live births in developed countries. The study objectives are to investigate the effects of EPO and moderate hypothermia on oxidative stress and neuroprotection in newborns with HIE. By using a random number table, children who had been diagnosed and treated at the hospital were first selected as research volunteers. The EPO group got EPO treatment in conjunction with mild hypothermia therapy, whereas the control group received conventional therapy plus mild hypothermia therapy. Mann-Whitney U test, Chi-squared test, and t-tests were used to assess the results, and they found that oxidative stress levels improved. Existing methods include the Neuroprotective Effect of Sovateltide (NES), Cell-

Based Therapy (CBT), Biomarkers, and Mild Hypothermia Therapy (MHT) is shown the less efficient when compared to the EPO group. Analyzed are the frequency of alive, complications and death scores of the EPO group, as well as the efficiency and amplitude of sensory conduction nerve and motor conduction nerve latency and velocity shows the better outperforms for the EPO group. Mild Hypothermia treatment and EPO treatment may preserve heart function and minimize the systemic oxidative stress response in children with HIE. This therapy is successful and can be used in the treatment of similar children in the future.

Authors' contribution

This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents.

Interest conflict

The authors declare that they have no conflict of interest.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Statement and Declarations

The author declares that no conflict of interest is associated with this study.

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