Investigating the Effect of Melatonin on Short-Term Prognosis of Neonatal Hypoxic-Ischemic Encephalopathy: A Randomized Controlled Trial

Nahid Kiani[1], Maryam Shokouhi Solgi[1,2], Mohammad Kazem Sabzehei[1], Fatemeh Eghbalian[1], Nasrin Jiryaee[3], Behnaz Basiri[1,2*]

[1] Department of Pediatrics, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[2] Clinical Research Development Unit of Fatemieh Hospital, Hamadan University of Medical Sciences, Hamadan, Iran
[3] Department of Community Medicine, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Received: 23 Jun. 2025; Accepted: 21 Nov. 2025

Abstract- Hypoxic-ischemic encephalopathy (HIE) is a leading cause of neonatal mortality with limited therapeutic options. This study evaluated the effect of melatonin on short-term outcomes in neonates with HIE. To determine whether adjunctive oral melatonin improves short-term clinical outcomes, including in-hospital survival, in neonates with HIE. A randomized controlled trial enrolled 80 neonates with HIE at Fatemieh Hospital, Hamadan, Iran (2021-2023). The intervention group (n=32) received standard treatment plus 10 mg/kg oral melatonin daily for 5 days, while the control group (n=48) received standard treatment alone. Outcomes included seizure frequency, hospital stay duration, time to initiate oral feeding, time to regain consciousness, adverse effects, and in-hospital survival. Baseline characteristics, HIE severity, medications for seizure control, and paraclinical/imaging findings were comparable between groups (all $P>0.05$). No significant adverse effects were observed. The intervention group had a higher survival rate (81.2%, 26/32) than the control group (52.1%, 25/48) ($P=0.04$). There were no significant differences in hospital length of stay, time to start feeding, or time to regain consciousness among surviving neonates (all $P>0.05$). Adjunctive melatonin may improve in-hospital survival in neonates with HIE and merits further evaluation as an additive therapy.

Acta Med Iran 2026;64(1):23-27.
https://doi.org/10.18502/acta.v64i1.21036

Keywords: Neonates; Hypoxic-ischemic encephalopathy; Melatonin; Survival rate; Hospitalization

Introduction

Hypoxic-ischemic encephalopathy (HIE) is a condition resulting from insufficient oxygen and blood flow, occurring in term and near-term infants and leading to significant morbidity and mortality (1,2). This condition substantially contributes to neonatal mortality, especially in resource-limited settings, and is a major cause of permanent brain damage, resulting in long-term issues such as cerebral palsy or developmental delays (3,4). Approximately 15-20% of infants with HIE do not survive the neonatal period, and 25-30% of survivors face neurodevelopmental challenges (1,5,6), imposing a substantial burden on families and healthcare systems (3). Efforts to mitigate complications or improve treatment outcomes are therefore essential. The National Center for Health Statistics reports that neonatal asphyxia contributes to mortality in the United States (4). The World Health Organization and other sources indicate that neonatal asphyxia is among the leading causes of neonatal death in developing countries, with millions of cases annually (5,6). Melatonin (N-acetyl-5-methoxytryptamine) is a hormone synthesized by the pineal gland in response to the light-dark cycle (7,8). It exhibits diverse biological functions, including protection against oxidative stress, regulation of energy

Corresponding Author: B. Basiri
Department of Pediatrics, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
Tel: +98 9188121848, E-mail address: behnazbasiri@yahoo.com

metabolism, enhancement of immune function, and potential neuroprotective effects (9-11). In animal models, melatonin demonstrates neuroprotection against hypoxic and ischemic brain injuries (11). In adult stroke, intravenous melatonin has been associated with improvements in electrophysiological and neurological outcomes, reduction in infarct size, and attenuation of inflammatory responses (12). In fetal sheep, maternal melatonin during asphyxia reduced inflammation and cell death (13). Given these findings, we examined the impact of melatonin on short-term outcomes in Iranian neonates diagnosed with HIE.

Materials and Methods

Study design and setting

The clinical trial was conducted from 2021 through the end of 2023 at Fatemieh Hospital in Hamedan, western Iran. The study received approval from the Ethics Committee of Hamadan University of Medical Sciences (ID: IR.UMSHA.REC.1400.452) and was registered with the Iranian Registry of Clinical Trials (IRCT) (ID: IRCT20160523028008N19). Written informed consent was obtained from parents or legal guardians.

Participants

Infants who met moderate or severe HIE criteria based on the modified Sarnat staging system were included. The six categories evaluated were level of consciousness, spontaneous activity, posture, tone, primitive reflexes, and autonomic responses (14). Exclusion criteria included congenital anomalies, gestational age< 36 weeks, imperforate anus, or suspected intracranial hemorrhage. Clinical data were extracted from medical records and parental histories.

Intervention

All neonates received standard care (including therapeutic hypothermia). The intervention group received standard care plus oral melatonin (10 mg/kg once daily for five consecutive days). Admission labs included complete blood count (CBC), blood gas analysis, liver function tests (LFT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), and serum electrolytes. On day 5, follow-up CBC, electrolytes, LDH, CPK, and LFT were obtained.

Outcome measures

Primary outcomes: seizure frequency, duration of hospitalization, time to initiation of oral feeding, and time to regain alertness. These were assessed via clinical examination, medical history, medical record review, and paraclinical tests/imaging.

Secondary outcomes: adverse drug effects (e.g., thrombocytopenia), need for ventilator, and paraclinical/imaging abnormalities.

Randomization and blinding

Simple randomization was performed using allocation cards (equal numbers of cards labeled "intervention" and "control" were drawn without replacement for each patient; the cards were then returned and reshuffled for the next draw). The outcome assessor/statistical analyst was blinded to group allocation, constituting single blinding.

Sample size and statistical analysis

Using G*Power (α=0.05, power=80%, effect size=0.9 for survival difference), based on previous studies (15), the required sample size was 32 per group. Due to lower-than-expected enrollment in the intervention arm (n=32), the control arm was expanded (n=48) to preserve statistical power. Data were analyzed using SPSS v20, with $P<0.05$ considered statistically significant. Means±standard deviations (SD) described quantitative variables; counts and percentages described qualitative variables. Independent-samples t-tests compared quantitative variables; chi-square (χ^2) or Fisher's exact tests compared qualitative variables.

Results

Baseline demographic and clinical characteristics were similar between groups (Table 1). There were no significant differences in side effects, ventilator requirement, or seizures (Table 2). Bradycardia was the most common adverse event in both groups. The intervention group had a higher in-hospital survival rate (26/32; 81.2%) compared to the control group (25/48; 52.1%) (P=0.04). Among surviving neonates, there were no significant differences in hospital length of stay, time to start feeding, or time to regain consciousness (Table 3). Paraclinical and imaging findings did not differ significantly between groups (Table 4). No serious or notable adverse effects were observed in either group.

Table 1. Comparison of baseline characteristics between study groups

Variable	Control group (n = 48)	Intervention group (n = 32)	*P*
Gender (Male/Female)	17/31	18/14	0.066
Severity of HIE (Moderate/Severe)	31/17	22/10	0.669
Drug for Seizure – Levetiracetam, n (%)	39 (81.2)	20 (62.5)	0.065
Drug for Seizure – Phenobarbital, n (%)	5 (10.4)	10 (31.2)	
Drug for Seizure – Both, n (%)	4 (8.3)	2 (6.2)	
Gestational Age (weeks), Mean ± SD	38.6 ± 1.3	38.7 ± 1.2	0.843
Birth Weight (g), Mean ± SD	2938.5 ± 342.9	3108.4 ± 628.3	0.175
Apgar Score (1 min), Mean ± SD	2.9 ± 1.9	2.9 ± 1.7	0.907
Apgar Score (5 min), Mean ± SD	5.2 ± 1.9	5.7 ± 1.1	0.327

HIE: Hypoxic-Ischemic Encephalopathy; SD: Standard Deviation

Table 2. Side effects, ventilator requirement, and seizures

Variable	Control group (n = 48), n (%)	Intervention group (n = 32), n (%)	*P*
Thrombocytopenia	21 (43.7)	12 (37.5)	0.578
Bradycardia	45 (93.7)	30 (93.7)	1.000
Skin Necrosis	1 (2.1)	2 (6.2)	0.561
Seizures	41 (85.4)	22 (68.7)	0.097
Need for ventilator	32 (66.7)	26 (81.2)	0.204

Table 3. Length of hospital stay, time to start feeding, and time to regain consciousness (survivors only)

Variable	Control Group (n = 48), Mean ± SD	Intervention Group (n = 32), Mean ± SD	*P*
Length of Hospital Stay (days)	15.5 ± 11.0	20.5 ± 12.9	0.219
Time to Start Feeding (days)	4.8 ± 1.3	4.3 ± 1.2	0.301
Time to Regain Consciousness (days)	4.8 ± 5.1	4.7 ± 5.1	0.933

SD: Standard Deviation

Table 4. Imaging and EEG findings

Variable	Control group (n = 48), n (%)	Intervention group (n = 32), n (%)	*P*
MRI abnormalities	28 (58.3)	18 (56.3)	0.583
aEEG abnormalities	45 (93.7)	26 (81.3)	0.083

MRI: Magnetic Resonance Imaging; a-EEG: amplitude integrated Electroencephalogram

Discussion

This clinical trial evaluated the impact of adding melatonin to standard treatment in neonates with HIE at a university center in Iran. To our knowledge, no prior study has examined melatonin as an adjunct to standard neonatal HIE care in an Iranian cohort. Consistent with the Results, we report survival rather than mortality metrics to avoid confusion: the survival rate was 52.1% in the standard care group and 81.2% in the melatonin group (P=0.04). Expressed as mortality, this corresponds to 47.9% vs. 18.8%; the relative risk of death is approximately 2.6 (47.9/18.8≈2.55), not "four times higher," and has been corrected here for numeric accuracy.

Current therapeutic measures for HIE include rapid

resuscitation and supportive care—temperature regulation, fluid/electrolyte management, blood glucose control, and seizure management (16). Even with optimal management, a substantial proportion of neonates with moderate to severe encephalopathy may die or suffer severe disabilities (17). Therapeutic hypothermia remains the only approved intervention, yet outcomes remain suboptimal in many settings (18), motivating research into additive therapies.

The neonatal brain is particularly susceptible to oxidative stress because of its immature antioxidant defenses (19). Excess free radical generation via oxidative stress, apoptosis, and inflammation contributes to HIE pathogenesis (20,21). Melatonin's antioxidant, anti-inflammatory, and immunomodulatory effects, along with excellent safety and BBB penetration, make it a biologically plausible adjunct (9-12,22. Prior work spans animal studies (11,13,23,24,31) and small human trials (25-27), supporting potential benefit yet underscoring the need for larger clinical investigations.

Animal models first established melatonin's neuroprotection. Cuzzocrea *et al*., demonstrated reduced cerebral edema after ischemia in gerbils with melatonin (28). In fetal sheep, melatonin reduced inflammation and cell death following umbilical cord occlusion (13). In mice, melatonin and derivatives protected against excitotoxic injury and supported secondary repair, axonal growth, and cognitive/sensorimotor functions (29). In human neonates, Fulia *et al*. reported decreased serum malondialdehyde and nitrite/nitrate levels after early melatonin administration following asphyxia (30). Other studies found elevated levels of oxygen-free radicals in asphyxiated neonates relative to controls, with significant reductions by 12-24 hours post-melatonin (31). Regarding survival, a randomized trial from Pakistan (n=80) showed 35% mortality in standard care vs. 13% with melatonin 10 mg via NGT (26). Aly *et al*., reported improved survival with early melatonin administration in neonates with HIE (27), in line with our findings. Collectively, these data suggest melatonin may augment neuroprotection when added to hypothermia-based care (22,31).

Limitations include the single-center design and modest sample size (n=80), which may limit generalizability and statistical precision. The focus on short-term outcomes without longitudinal neurodevelopmental follow-up is another constraint. Selection and reporting biases cannot be ruled out. Future studies should incorporate multicenter designs, standardized dosing/timing, pharmacokinetics, and long-term neurodevelopmental assessments.

Adjunctive melatonin (10 mg/kg daily for 5 days) added to standard care was associated with improved in-hospital survival among neonates with HIE. Given unresolved questions about optimal dosing, route, and timing, larger multicenter trials with long-term follow-up are warranted.

Acknowledgments

The study was approved by the Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1400.452). The authors thank the Vice-Chancellor for Research at Hamadan University of Medical Sciences and Clinical Research Development Unit of Fatemieh Hospital for supporting this project.

References

1. Papazian O. Neonatal hypoxic-ischemic encephalopathy. Medicina (B Aires) 2018;78:36-41.
2. Aneja S, Sharma S. Hypoxic ischaemic encephalopathy in low resource settings—time to stop cooling? Lancet Glob Health 2021;9:e1187–8.
3. Naburi H, Pillay S, Houndjahoue F, Bandeira S, Kali G, Horn A, et al. A survey on the diagnosis and management of neonatal hypoxic ischaemic encephalopathy in sub-Saharan Africa. Sci Rep 2024;14:22046.
4. Murphy SL, Kochanek KD, Xu J, Arias E. Mortality in the United States, 2023. NCHS Data Brief, no. 521. Hyattsville, MD: National Center for Health Statistics; 2024.
5. Cainelli E, Vedovelli L, Mastretta E, et al. Long-term outcomes after neonatal hypoxic-ischemic encephalopathy in the era of therapeutic hypothermia: a longitudinal, prospective, multicenter case-control study in children without overt brain damage. Children (Basel) 2021;8:1076.
6. Peeples ES, Rao R, Dizon ML, Johnson YR, Joe P, Flibotte J, et al. Predictive models of neurodevelopmental outcomes after neonatal hypoxic-ischemic encephalopathy. Pediatrics 2021;147:e2020024837.
7. Lynch H, Wurtman R, Moskowitz MA. Daily rhythm in human urinary melatonin. Science 1975;187:169-71.
8. Lewy AJ, Wehr TA, Goodwin FK. Light suppresses melatonin secretion in humans. Science 1980;210:1267-9.
9. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007;42:28-42.
10. Reiter RJ, Tan DX, Fuentes-Broto L. Melatonin: a multitasking molecule. Prog Brain Res 2010;181:127-51.

11. Perrone S, Carloni S, Dell'Orto VG, Filonzi L, Beretta V, Petrolini C, et al. Hypoxic ischemic brain injury: animal models reveal new mechanisms of melatonin-mediated neuroprotection. Rev Neurosci 2024;35:331-9.
12. Sadanandan N, Cozene B, Cho J, Park YJ, Saft M, Gonzales-Portillo B, et al. Melatonin—a potent therapeutic for stroke and stroke-related dementia. Antioxidants (Basel) 2020;9:672.
13. Welin AK, Svedin P, Lapatto R, Sultan B, Hagberg H, Gressens P, et al. Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. Pediatr Res 2007;61:153-8.
14. Prechtl HFR. Assessment methods for the newborn infant: a critical evaluation. In: Stratton P, editor. Psychobiology of human newborn. Chichester: Wiley; 1982: 21-52.
15. Wu YW, Gonzalez FF. Erythropoietin: a novel therapy for hypoxic–ischaemic encephalopathy? Dev Med Child Neurol 2015;57:34-39.
16. Babbo CCR, Mellet J, van Rensburg J. Neonatal encephalopathy due to suspected hypoxic ischemic encephalopathy: pathophysiology, current, and emerging treatments. World J Pediatr. 2024;20:1105-14.
17. Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev 2013;1:CD003311.
18. Ouwehand S, Smidt LC, Dudink J, Benders MJ, de Vries LS, Groenendaal F, et al. Predictors of outcomes in hypoxic-ischemic encephalopathy following hypothermia: a meta-analysis. Neonatology 2020;117:411-27.
19. D'Angelo G, Cannavò L, Reiter RJ. Melatonin administration from 2000 to 2020 to human newborns with hypoxic-ischemic encephalopathy. Am J Perinatol 2020;37:1082-8.
20. Sinha B, Wu Q, Li W, Tu Y, Sirianni AC, Chen Y, et al. Protection of melatonin in experimental models of newborn hypoxic-ischemic brain injury through MT1 receptor. J Pineal Res 2018;64:e12443.
21. Tütüncüler F, Eskiocak S, Başaran UN, Ekuklu G, Ayvaz S, Vatansever U, et al. The protective role of melatonin in experimental hypoxic brain damage. Pediatr Int 2005;47:434-9.
22. Ahmed J, Robertson NJ, More K. Melatonin for neuroprotection in neonatal encephalopathy: a systematic review & meta-analysis of clinical trials. Eur J Paediatr Neurol 2021;31:38-45.
23. Pluta R, Furmaga-Jabłońska W, Januszewski S, Tarkowska A. Melatonin: a potential candidate for the treatment of experimental and clinical perinatal asphyxia. Molecules 2023;28:1105
24. Seabra ML, Bignotto M, Pinto LR, Tufik S. Randomized, double-blind clinical trial, controlled with placebo, of the toxicology of chronic melatonin treatment. J Pineal Res 2000;29:193-200.
25. Merchant N, Azzopardi D, Counsell S. Melatonin as a novel neuroprotectant in preterm infants—a double-blinded randomized controlled trial (MINT study). Arch Dis Child 2014;99:A43.
26. Ahmad QM, Chishti AL, Waseem N. Role of melatonin in management of hypoxic ischaemic encephalopathy in newborns: a randomized control trial. J Pak Med Assoc 2018;68:1233-1237.
27. Aly H, Elmahdy H, El-Dib M, Rowisha M, Awny M, El-Gohary T, et al. Melatonin use for neuroprotection in perinatal asphyxia: a randomized controlled pilot study. J Perinatol 2015;35:186-91.
28. Cuzzocrea S, Costantino G, Gitto E, Mazzon E, Fulia F, Serraino I, et al. Protective effects of melatonin in ischemic brain injury. J Pineal Res 2000;29:217-27.
29. Bouslama M, Renaud J, Olivier P. Melatonin prevents learning disorders in brain-lesioned newborn mice. Neuroscience 2007;150:712-9.
30. Fulia F, Gitto E, Cuzzocrea S, Reiter RJ, Dugo L, Gitto P, et al. Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: reduction by melatonin. J Pineal Res 2001;31:343-9.
31. Robertson NJ, Faulkner S, Fleiss B, Bainbridge A, Andorka C, Price D, et al. Melatonin augments hypothermic neuroprotection in a perinatal asphyxia model. Brain 2013;136:90-105.