Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14,Issue.33s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Melatonin Effects on Persistent Pulmonary Hypertension Induced Hypoxia

Sarah Mohamed Nofal [1]*, Amany Mohamed Abouelenain [2], Abdel-Rahman Mohamed El-Mashad [1], Mostafa Mohamed Awny [1], Asmaa Mahmoud Elmesiry [1]

1 Pediatrics and Neonatology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

[2] Clinical Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

***Corresponding Author:**

Sarah Mohamed Nofal

Assistant lecturer of Pediatrics Affiliation: Faculty of Medicine, University of Tanta, Tanta, Egypt, Pediatrics and Neonatology Department, Tanta University Hospitals.

Email: sara.elgharably@med.tanta.edu.eg

ORCID ID: 0000-0002-2016-7874

.Cite this paper as Sarah Mohamed Nofal , Amany Mohamed Abouelenain , Abdel-Rahman Mohamed El-Mashad , Mostafa Mohamed Awny , Asmaa Mahmoud Elmesiry (2025) Melatonin Effects on Persistent Pulmonary Hypertension Induced Hypoxia .Journal of Neonatal Surgery, 14.(33s) 268-275

ABSTRACT

Background: Tissue hypoxia and insufficient perfusion can cause end organ damage in the systemic condition known as persistent pulmonary hypertension of neonates (PPHN), which has several causes. To diagnose hypoxia caused by PPHN and evaluate its improvement, changes in the high-mobility group box-1 (HMGB-1) protein can be used as a preliminary diagnostic marker. As a biomarker for disease severity and treatment response prediction, it may be pivotal in the hypoxia- and inflammatory-related pathophysiology of PPHN. The purpose of this study was to measure serum levels of high mobility group box-1 protein to determine the effects of melatonin in PPHN-induced hypoxia.

Methods: This randomized-control study was conducted on 80 neonates ≥ 36 weeks of gestation, diagnosed with persistent pulmonary hypertension. The patients were split into two equal groups: Group I was given Melatonin, whereas group II got a placebo.

Results: Melatonin significantly decreased serum HMGB levels compared to the control group. The melatonin group had a much shorter duration of mechanical breathing, oxygen support, and Neonatal Intensive Care Unit (NICU) stay compared to the control group. Also, Estimated Systolic Pulmonary Arterial Pressure (ESPAP) was significantly lower in the melatonin group than in control group.

Conclusions: Melatonin can be used as an adjuvant therapy in PPHN and Hypoxic respiratory failure (HRF). Serum HMGB-1 levels can be used to prove melatonin's positive effects in PPHN**...**

Keywords: *Melatonin, Newborn, Persistent Pulmonary Hypertension, High-mobility group box-1, Hypoxia.*

1. INTRODUCTION

Hypoxemic respiratory failure and persistent pulmonary hypertension of newborn (PPHN) can occur if the pulmonary vascular resistance (PVR) does not decrease throughout the transition from the fetal to the neonatal phase [1].

Several factors contribute to systemic hypotension in PPHN, including peripheral vasodilation, preload, and left ventricular function. Poor perfusion can cause harm to end organs. Babies born with prenatal asphyxia often experience PPHN [2].

Melatonin has effective protection against Reactive Oxygen Species (ROS). This defense is based on three well-documented features: it is a direct scavenger of ROS, it stimulates antioxidant enzymes, and it negatively modulates ROS production in pro-oxidant agents [3].

Melatonin has been shown in a small number of studies to improve endothelial function and increase vasodilator capacity, two important mechanisms involved in the control of newborn pulmonary circulation during chronic hypoxia. However, melatonin's effects on pulmonary artery prostanoid pathway modulation remain unexplored [4].

.

For the purpose of early identification and therapy of hypoxia-induced PPHN, alterations in High-mobility group box-1 (HMGB-1) can be utilized. When hypoxia-induced PPHN develops, there is considerable evidence that inflammation is a causal factor [5].

The purpose of this research was to determine whether melatonin was beneficial for infants with hypoxic respiratory failure and PPHN by monitoring their blood levels of the high mobility group box-1 protein

Patients and Methods

This experimental prospective randomized-control study was conducted at the Neonatal Intensive Care Unit, Tanta University Hospital, Tanta, Egypt, over two years duration, from January 2023 to January 2025. The study was conducted on 80 neonates ≥ 36 weeks of gestation, presented with Persistent Pulmonary Hypertension [6, 7].

All patients' legal guardians gave their signed informed permission. All procedures followed the guidelines established by the Declaration of Helsinki and best practices in clinical research. The Ethics Committee of Tanta University's Faculty of Medicine gave its approval to the project. Approval Code: **36173/12/22.**

The neonates, who were included, were split into two equal groups: Melatonin was given to Group I, whereas a placebo was given to Group II.

Melatonin administration

A total of five doses of 10 mg/kg/day of melatonin were administered. In terms of safety, this dosage was comparable to those used in earlier investigations on neonates. The oral route was selected due to the easy availability of over-the-counter oral supplements. An orogastric tube was used to give crushed melatonin tablets (10 mg each; Puritan's Pride, Oakdale, NY, USA) diluted in 5 ml of normal saline [8].

Inclusion criteria

Neonates of ≥ 36 weeks of gestational age were admitted with persistent pulmonary hypertension. In addition to prenatal history, clinical symptoms, and laboratory testing, the diagnosis of PPHN were confirmed by color Doppler Echocardiography [6].

Exclusion criteria

Preterm infants < 36 weeks of gestation, infants with congenital heart diseases, secondary causes of PPHN as MAS, CDH and congenital pneumonia, patients received sildenafil., infants with dysrhythmia, chromosomal abnormalities, severe pathological jaundice, metabolic or endocrinal disorders, and major congenital malformations.

A complete history taking and clinical examination were performed on all patients, also laboratory investigations, including complete blood counts (CBC), C-reactive protein (CRP), renal and liver function tests, serum electrolytes' levels (Na^+, K^+, and Ca^{+2}), and arterialized/capillary blood gases (ABG\CBG).

Randomization

Randomization and allocation concealment were executed utilizing computer-generated software (randomizer.org) with a 1:1 ratio; assignments were disguised within sequentially numbered, opaque, sealed envelopes to maintain blinding.

All patients were examined by conventional color doppler Echocardiography to assess the ESPAP on admission for diagnosis of PPHN and follow up after melatonin administration.

Machine: Using commercially accessible ultrasound transducers and equipment, echocardiographic examinations were conducted (Vivid 7, GE Healthcare, Horten, Norway).

Transducers: Using a 6S-D probe (frequency 2.7 -8 MHz), data were acquired.

Tricuspid regurgitation peak velocity (TR jet) was used to quantify pulmonary artery pressure [9].

Research work investigations

Serum HMGB1 protein testing: in accordance with the manufacturer's instructions, blood samples were collected when PPHN had been decreased, and the Melatonin course was complete. For detection by Enzyme-linked immunosorbent assays (ELISA) (Wuhan Huamei Biotech, China), the samples were centrifuged into serum and then frozen at -20°C.

Serum HMGB1 protein testing:

Following the addition of 100 µL of each standard, blank, and sample to their respective wells, the plate was sealed and incubated at 37°C for two hours. Prior to being incubated at 37°C for 1 hour, the 100 µL of Detection Reagent A must be sealed. The next day, the wells were washed three times with 300.0 µL of 1× wash buffer. Following each rinse, they were allowed for 1-2 minutes before being blotted dry.

Furthermore, Detection Reagent B, 100 µL, was subsequently included. After washing the wells five times using the preceding technique, the mixture was incubated at 37°C for 1 hour. Upon adding 90 µL of Substrate Solution and letting it

incubate at 37°C (in the absence of light) for 15-25 minutes, the solution became blue. Once all the components were mixed, 50 µL of Stop Solution was added, which turned the mixture yellow. The plate was then tapped to ensure even distribution. Water droplets, fingerprints, and bubbles were removed before reading absorbance at 450 nm immediately.

Study Outcomes

The primary outcome was to evaluate effects of Melatonin in PPHN by measuring the HMGB-1 protein levels after melatonin administration.

The secondary outcome was the clinical correlation including duration of invasive mechanical ventilation (MV), the duration of oxygen support, and NICU stay.

Statistical analysis

A statistical analysis was performed using SPSS v26, a program created and maintained by IBM Inc. of Chicago, IL, USA. Quantitative variables used here were mean and standard deviation (SD). For that reason, we used a student's t-test with two tails and no pairing. Suitable statistical tests, like Fisher's exact or Chi-square, were used to analyze the percentages and frequencies of the qualitative variables. For the purpose of finding correlations, the Pearson moment correlation equation was utilized. A ROC curve was used to depict the sensitivity, specificity, PPV, and NPV, which allowed for the evaluation of testing results. As a matter of statistical significance, we considered a two-tailed P value below 0.05.

The flow chart of the study: Figure 1

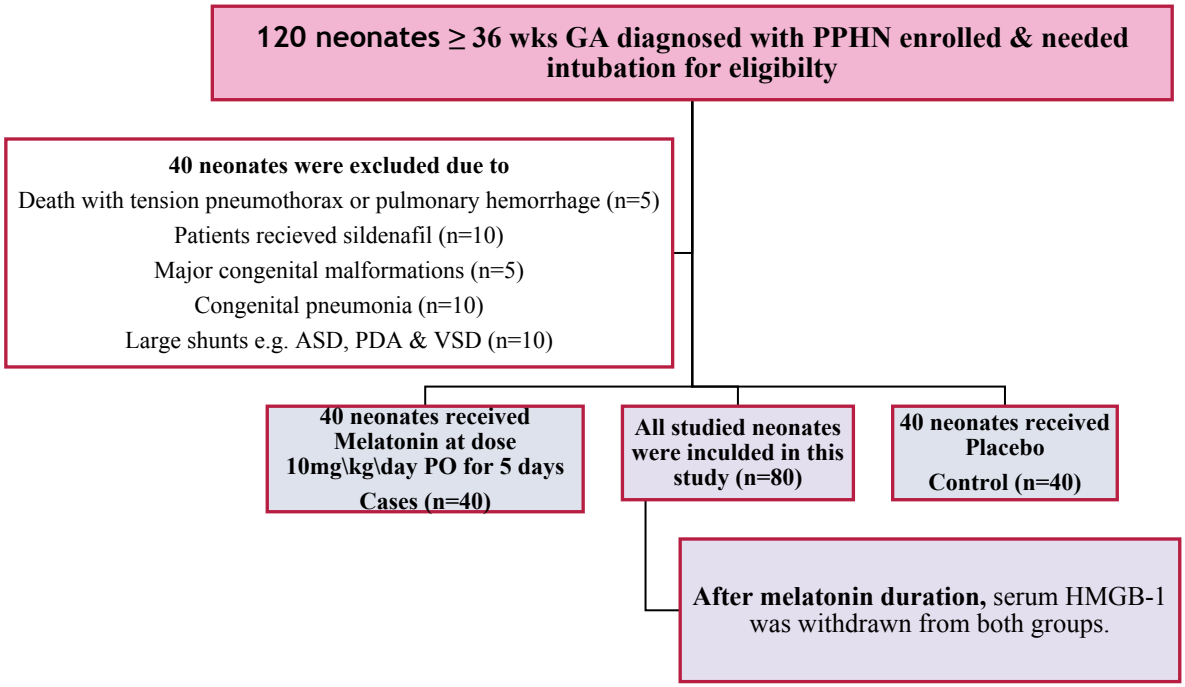

Figure 1: The study flow chart.

Results:

A comparison of the groups' demographics, vital signs, and Down's scores revealed no statistically significant differences. Infants in the cases group had significantly lower APGAR scores at 1st and 5th minutes postpartum compared to those in the control group. Time spent requiring invasive MV, oxygen support, and intensive care unit stays was significantly lower in the melatonin group. **Table 1**

Table 1: Comparison between the two groups studied according to demographic data, vital signs, APGAR and Downe's scores and secondary outcomes of Melatonin administration

		Melatonin (n = 40)	Control (n = 40)	Test of Sig.	P
GA (weeks)		38.03 ± 1.17	38.40 ± 1.28	t=1.372	0.174
Sex	**Male**	26(65.0%)	28(70.0%)	χ^2=0.228	0.633
	Female	14(35.0%)	12(30.0%)		

Postnatal Age (days)		1.60 ± 0.63	1.90 ± 0.78	U=634.00	0.083
Birth weight (kg)		3.12 ± 0.33	3.18 ± 0.28	t=0.961	0.339
Length (cm)		48.31±1.12	48.48±0.82	t=0.742	0.460
Mode of delivery	**Normal vaginal delivery**	7(17.5%)	8(20.0%)	χ^2=0.082	0.775
	CS	33(82.5%)	32(80.0%)		
Vitals signs	**HR (bpm)**	133.6±12.80	133.6±12.80	0.0	1.000
	SBP (mmHg)	77.70±3.87	77.70±3.87	0.0	1.000
	MBP (mmHg)	43.58±3.35	44.90±2.92	1.884	0.063
APGAR score	**At 1st min**	2.33±0.47	3.03±0.89	U=449.00*	**<0.001***
	At 5th min	5.15±0.89	6.55±0.93	U= 250.50*	**<0.001***
Downe's score		8.23 ± 0.66	8.20 ± 0.69	t= 0.166	0.869
Secondary outcomes of Melatonin administration					
MV duration (days)		7.28±0.75	11.45±1.75	0.0*	**<0.001***
Oxygen duration (days)		9.43±0.64	15.80±1.73	0.0*	**<0.001***
NICU stay (days)		11.95±1.04	18.53±1.93	0.0*	**<0.001***

Data are presented as mean ± SD or frequency (%). * Significant P value <0.05. c2: Chi square test, t: Student t-test, U: Mann Whitney test, GA: Gestational age, SBP: systolic blood pressure, MBP: mean blood pressure, HR: Heart rate, CS: Cesarean section, NICU: neonatal intensive care unit, MV: Mechanical ventilation.

The two groups did not vary substantially with respect to total blood count, arterialized/capillary blood gas, ionized calcium, potassium, or sodium and liver functions tests. When comparing the melatonin group to the control group, renal function tests revealed substantially increased levels of serum urea and creatinine. **Table 2**

Table 2: Comparison between the two groups studied according to laboratory parameters

	Melatonin (n = 40)	**Control (n = 40)**	**t**	**P**
HB (g/dl)	15.24±1.26	15.61±1.47	1.223	0.225
TLC (x10^9/L)	11.08±2.21	11.57±1.88	1.073	0.287
PLTs (x10^9/L)	357.0±96.28	357.0±96.28	0.00	1.000
PH	7.28±0.11	7.28±0.11	t=0.000	1.000
PCO_2 (mmHg)	50.08±12.64	49.70±12.56	U=777.500	0.827
HCO_3 (mmol/L)	18.98±2.11	18.98±2.11	t=0.000	1.000
Na^+ (mEq/L)	139.3±6.63	139.3±6.63	t=0.00	1.000
K^+ (mEq/L)	4.46 ± 0.94	4.46±0.94	U=800.00	1.000
Ionized Ca^{+2} (mg/dl)	1.03±0.16	1.02±0.17	U=719.00	0.432
TSB (mg/dl)	5.97±2.36	5.97±2.36	t=0.000	1.000
DSB (mg/dl)	0.88±0.52	0.88±0.52	U=800.00	1.000

ALT (IU/L)	31.70±6.43	31.70±6.43	U=800.00	1.000
AST (IU/L)	30.63±6.24	30.63 ± 6.24	U=800.00	1.000
Albumin (g/dl)	3.14±0.19	3.14±0.19	t=0.000	1.000
Urea (mg/dl)	55.43±17.27	36.14±8.99	t=6.265*	**<0.001***
Creatinine (mg/dl)	0.81±0.28	0.41±0.16	t=7.984*	**<0.001***

Data are presented as mean ± SD. * Significant P value <0.05. c2: Chi square test, t: Student t-test, U: Mann Whitney test, HB: Hemoglobin, TLC: Total leukocytic count, PLTs: Platelets, Na: Sodium, K: Potassium, Ca: Calcium, TSB: Total Serum Bilirubin, DSB: Direct serum bilirubin, ALT: Alanine transaminase, AST: Aspartate aminotransferase.

Melatonin significantly reduced serum HMGB1 levels compared to the control group. **Table 3**

Table 3: Comparison between the two groups studied according to serum HMGB-1 levels:

	Melatonin (n = 40)	**Control (n = 40)**	**t**	**P**
HMGB-1 concentration (pg/ml)	3082.3±606.1	4973.4±900.8	t= 11.016*	**<0.001***

Data are presented as mean ± SD. * Significant P value <0.05. c2: Chi square test, t: Student t-test, U: Mann Whitney test, HMGB-1: high mobility group box-1.

Also, this study showed that ESPAP was significantly higher in the melatonin group than in control group on admission then became significantly lower in cases than in control group after melatonin administration. **Table 4**

Table (4): Comparison between the two groups studied according to estimated systolic pulmonary arterial pressure (ESPAP) (on admission & after melatonin administration):

	Melatonin (n = 40)	**Control (n = 40)**	**t**	**p**
ESPAP1 On admission (mmHg)				
Min. – Max.	60.0 – 90.0	55.0 – 90.0	2.066*	**0.043***
Mean ± SD.	74.25 ± 6.89	70.25 ± 10.12		
Median (IQR)	75.0 (70.0 – 80.0)	70.0(62.50 – 75.0)		
ESPAP2 after melatonin (mmHg)				
Min. – Max.	37.0 – 55.0	45.0 – 60.0	6.690*	**<0.001***
Mean ± SD.	43.60 ± 4.30	50.50 ± 4.91		
Median (IQR)	43.0 (40.0 – 45.0)	50.0 (45.0 – 55.0)		

IQR: Inter quartile range, SD: Standard deviation, t: Student t-test, p: p value for comparing between the two studied groups, *: Statistically significant at p ≤ 0.05, ESPAP: Estimated systolic pulmonary arterial pressure.

Our study showed that HMGB-1 was a good predictor for PPHN improvement as HMGB-1 showed a sensitivity of 92.5%, a specificity of 80% to predict cardiac affection after melatonin in both groups at a cutoff ≤4079.3 (pg./ml). **Table 5**

Table (5): Diagnostic performance for serum HMGB-1 protein concentration:

	AUC	p	95% C. I	Cut off	Sensitivity	Specificity	PPV	NPV
H M G B - 1 (pg\ml)	0.960	**<0.001***	0.924 – 0.996	≤4079.3	92.50	80.0	82.2	91.4

AUC: Area Under a Curve, p value: Probability value, CI: Confidence Intervals, NPV: Negative predictive value, PPV: Positive predictive value, *: Statistically significant at $p \leq 0.05$

Discussion

Failure to transition from the fetal to the neonatal period with a reduction in PVR can lead to hypoxemic respiratory failure and PPHN [1].

Our study showed a significantly shorter duration MV in cases than in control group after melatonin administration, oxygen support and NICU stay which can prove positive effects of melatonin in management of hypoxic respiratory failure HRF and PPHN. Additionally, this study comes somehow in agreement with Figueroa et al. [4] found that neonates fed 1 mg/kg of melatonin orally between 4 and 11 days after birth have altered expression of vasoactive prostanoid pathways in their hypertensive pulmonary circulation when exposed to chronic hypoxia. And the expression of prostanoid signaling molecules that act as both vasodilators and vasoconstrictors is influenced by this modulation.

In our study, there was no significant difference in the Downe's score, however the APGAR score at 1st and 5th minutes after delivery was considerably lower in the cases compared to the control group. In the cases, APGAR score's mean was (2.33 ± 0.47) at the 1st min. and (5.15 ± 0.89) at the 5th min., while in the control group, it was (3.03 ± 0.89) at 1st min. and (6.55 ± 0.93) at 5th min. This was in harmony with Nchabeleng et al. [10] discovered low APGAR score in PPHN patients (<6) at the 1st minute and 5th minute. In addition to that, Alhumaid et al. [11] The APGAR scores at 1 and 5 minutes for neonates with PPHN ranged from 0 to 3, 4, and 6 for 132 (61.6% of the total), and 7 to 10 for 62 (28.7% of the total).

Regarding the vital indicators (heart rate, systolic blood pressure, and mean blood pressure), our study found no significant difference between the two groups. However, the mean SBP was (77.70 ± 3.87) mmHg in both groups, MBP was (43.58 ± 3.35 in cases and 44.90 ± 2.92 mmHg in control group) and the mean ESPAP on admission was (74.25 ± 6.89) mmHg in cases and (70.25 ± 10.12) mmHg in control, which means that ESPAP was higher than systemic BP in both groups and it was severe PPHN. This comes in agreement with Dillard et al. [12] who included 99 neonates diagnosed with PPHN, who found mean SBP was (56±6.4) mmHg in inhaled nitric oxide (iNO) responders and (65.8±6.7) mmHg in non-responders requiring milrinone introduction.

In our study, CBC in both groups were of normal ranges because all included neonates were diagnosed with primary PPHN only. On the contrary, Kaveh et al. [13] who performed their study on 49 neonates suffering from PPHN, 40.8% of cases showed leucocytosis or leukopenia, 8.2% showed polycythaemia and positive blood culture due to secondary causes of PPHN as MAS, RDS, congenital pneumonia and sepsis.

This study showed no significant difference between both studied groups as regards the arterialized/capillary blood gases (mean PH was 7.28±0.11, PCO2 was 50±12.64) this is typically explained by the systemic manifestations of PPHN including respiratory acidosis, while our study showed normal ranges of serum sodium, potassium and ionized calcium levels. However, Kaveh et al. [13] found that 18.4% of cases had hypocalcaemia in PPHN cases.

Our study showed a significantly shorter duration of MV in cases than in control group after melatonin administration (of mean 7.28 ± 0.75 days in cases & mean 11.45 ± 1.75 days in control), oxygen support (of mean 9.43 ± 0.64 days in cases & mean 15.80 ± 1.73 days in control) and NICU stay (of mean 11.95 ± 1.04 days in cases & mean 18.53 ± 1.93 days in control) that can demonstrate melatonin's beneficial effects in treatment of HRF and PPHN. There are a number of mechanisms in which oxidative stress reduces NO bioavailability in living organisms. The development of pulmonary arterial hypertension and diminished endothelium-derived relaxing factors are two of its many consequences. Thus, melatonin offers a number of significant advantages over other drugs, leading to its suggestion for use in antioxidant treatments in perinatal medicine [4].

Melatonin was shown to have extra positive benefits on PPHN/HRF, as this study demonstrated that serum HMGB-1 levels were substantially lower (P<0.001) in the melatonin group compared to the control group.

Our findings are consistent with Tang et al. [14] who found that the levels of serum HMGB-1 were 33190±9450 pg/ml on diagnosis of PPHN, 13420±2140 pg/ml after PPHN resolution, and 2370±880 pg/ml in control group than healthy controls, with a significant decrease after PPHN resolution.

Our study showed that the serum HMGB-1 protein was a good predictor for improvement of PPHN after melatonin administration as the HMGB-1 showed AUC 0.960, a sensitivity of 92.5%, a specificity of 80%, 95% CI (0.924 – 0.996), and at a cutoff ≤4079.3 (pg/ml) which is proving the beneficial effects of melatonin in PPHN-induced hypoxia.

Limitations of the study

The study's sample size was small, which was one of its limitations. Research took place in a single center.

Conclusions

Melatonin can be used as adjuvant therapy in management of PPHN and HRF. Serum HMGB-1 levels can be used to prove melatonin's positive effects in PPHN.

Funding

None.

Conflicting Interest

None.

Declaration on the use of AI

None.

Author Contributions

In terms of the study's idea and layout, all writers were involved. Paper writing, data analysis, and material preparation were all done by Asmaa Elmesiry and Sarah Nofal. Amany Abouelenain: laboratory data analysis revision and validation, and approval of the findings. Abdel-Rahman El-Mashad and Mostafa Awny: critical revision of the manuscript and final version approval.

REFERENCES

[1] S. Timberline, A. Bhatt, S. Sunderji, D. J. Tancredi, S. Lakshminrusimha, and H. Siefkes, "Novel scoring tool of hypoxemic respiratory failure and pulmonary hypertension for defining severity of persistent pulmonary hypertension of newborn," J. Perinatol., vol. 43, no. 10, pp. 1281–1287, 2023, doi: 10.1038/s41372-023-01762-w.

[2] H. M. L. S. Siefkes, "Management of systemic hypotension in term infants with persistent pulmonary hypertension of the newborn: an illustrated review," Arch. Dis. Child. Fetal Neonatal Ed., vol. 106, no. 4, pp. 446–455, Jul. 2021, doi.org/10.3390/children11040490.

[3] D. Liu, R. P. Ceddia, W. Zhang, F. Shi, H. Fang, and S. Collins, "Discovery of another mechanism for the inhibition of particulate guanylyl cyclases by the natriuretic peptide clearance receptor," Proc. Natl. Acad. Sci. U. S. A., vol. 120, no. 28, p. e2307882120, Jul. 2023, doi: 10.1073/PNAS.2307882120.

[4] E. G. Figueroa et al., "Beneficial effects of melatonin on prostanoids pathways in pulmonary hypertensive neonates," Vascul. Pharmacol., vol. 138, no. March, 2021, doi: 10.1016/j.vph.2021.106853.

[5] T. Nakamura et al., "Measurement of plasma concentration of high mobility group box1 (HMGB1) in early neonates and evaluation of its usefulness," Clin. Chim. Acta, vol. 61, no. 5, pp. 1–28, 2022, doi: 10.1016/j.plabm.2023.e00327.

[6] B. Mathew and S. Lakshminrusimha, "Review persistent pulmonary hypertension in the newborn," Children, vol. 4, no. 8, 2017, doi: 10.3390/children4080063.

[7] M. Jankowich, B. A. Maron, and G. Choudhary, "Mildly elevated pulmonary artery systolic pressure on echocardiography: bridging the gap in current guidelines," Lancet Respir. Med., vol. 9, no. 10, pp. 1185–1191, 2021, doi: 10.1016/S2213-2600(21)00072-2.

[8] D. P. Cardinali, "Are melatonin doses employed clinically adequate for melatonin-induced cytoprotection?," Melatonin Res., vol. 2, no. 2, pp. 106–132, 2019, doi: 10.32794/mr11250025.

[9] I. J. C. Heart et al., "Assessment of pulmonary artery pressure by echocardiography — A comprehensive review," IJCHA, vol. 12, pp. 45–51, 2016, doi: 10.1016/j.ijcha.2016.05.011.

[10] M. J. Nchabeleng, M. H. K. Hamese, and T. S. Ntuli, "Prevalence and outcomes of persistent pulmonary hypertension of the newborn in a neonatal unit, mankweng hospital, Limpopo Province, South Africa," SAJCH South African J. Child Heal., vol. 15, no. 2, pp. 103–106, 2021, doi: 10.7196/SAJCH.2021.v15.i2.1773.

[11] S. Alhumaid et al., "International treatment outcomes of neonates on extracorporeal membrane oxygenation (ECMO) with persistent pulmonary hypertension of the newborn (PPHN): a systematic review," J. Cardiothorac. Surg., vol. 19, no. 1, 2024, doi: 10.1186/s13019-024-03011-3.

[12] J. Dillard, L. R. Pavlek, S. Korada, and B. Chen, "Worsened short-term clinical outcomes in a cohort of patients with iNO-unresponsive PPHN: a case for improving iNO responsiveness," J. Perinatol., vol. 42, no. 1, pp. 37–44, 2022, doi: 10.1038/s41372-021-01228-x.

[13] M. Kaji Yazdi et al., "Prospective Study of Neonates with Persistent Pulmonary Hypertension of the Newborn:

Prevalence, Clinical Outcomes, and Risk Factors," Iran. J. Neonatol., vol. 2, no. 2, p. 13, 2022, doi: 10.22038/IJN.2022.59465.2128.

[14] Z. Tang, M. Jiang, Z. Ou-Yang, H. Wu, S. Dong, and M. Hei, "High mobility group box 1 protein (HMGB1) as biomarker in hypoxia-induced persistent pulmonary hypertension of the newborn: a clinical and in vivo pilot study," Int. J. Med. Sci., vol. 16, no. 8, p. 1123, 2019, doi: 10.7150/IJMS.34344.

.