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Association of Hypocalcemia with Persistent Pulmonary Hypertension in Neonates: A Prospective Observational Cohort Study in a Tertiary Care Hospital in Lahore, Pakistan

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ABSTRACT

Objective: To determine the frequency of hypocalcemia among neonates with persistent pulmonary hypertension of the newborn (PPHN) and to assess its correlation with disease severity and short-term clinical outcomes.

Methodology: This Prospective Observational Cohort Study was conducted in the Neonatal Intensive Care Unit (NICU) of Fatima Memorial Hospital, Lahore. A total of 203 neonates with echocardiographically confirmed PPHN were enrolled consecutively. Ionized calcium was measured within 48 hours of echocardiography. Hypocalcemia was defined as ionized calcium <1.10 mmol/L for birth weight ≥1500 g and <0.90 mmol/L for <1500 g. PPHN severity was graded as moderate or severe. Statistical comparisons were performed using Mann-Whitney U, independent t-test, and chi-square tests, with p ≤0.05 considered significant.

Results: Hypocalcemia was present in 59.6% (121/203) of neonates. Severe PPHN occurred in 53.2% of cases. Ionized calcium levels were lower in severe compared to moderate PPHN (0.992 ± 0.148 vs 1.047 ± 0.149 mmol/L; p = 0.017). Severe PPHN was associated with increased need for invasive ventilation (50.0% vs 27.4%, p = 0.002) and high-frequency oscillatory ventilation (25.9% vs 11.6%, p = 0.016). Mortality was higher in severe PPHN (25.9% vs 15.8%), although not statistically significant (p = 0.087). NICU stay and inotrope use were comparable.

Conclusion: Hypocalcemia is common in neonates with PPHN and lower ionized calcium levels are associated with greater disease severity. These findings suggest a potential role of calcium homeostasis in the pathophysiology and clinical course of PPHN, warranting further evaluation as a modifiable risk factor.

Keywords: Echocardiography, Inotropes, Ionized hypocalcemia, Mechanical ventilation, Neonatal hypocalcemia, Neonatal intensive care unit, Persistent pulmonary hypertension of the newborn, PPHN, PPHN severity

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [3,4]Critical analysis and manuscript review; [5,6]Data analysis; Manuscript Editing.

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Introduction

Persistent pulmonary hypertension of the newborn (PPHN) is a serious neonatal condition characterized by the failure of the normal circulatory transition at birth, resulting in persistent elevated pulmonary vascular resistance and subsequent right-to-left shunting of deoxygenated blood.[1] This causes severe hypoxemia and can lead to multi-organ dysfunction, usually appearing within the first 48 to 96 hours of life.[2] The reported incidence of PPHN is about 1-2 per 1,000 live births, and it remains associated with high mortality and long-term neurodevelopmental complications among survivors.[3] Several risk factors contribute to PPHN, including perinatal asphyxia,

meconium aspiration syndrome (MAS), neonatal sepsis, pneumonia, congenital diaphragmatic hernia, and pulmonary hypoplasia.[4] Standard management strategies involve supportive ventilation, surfactant therapy, inotropes, inhaled nitric oxide (iNO), Sildenafil, Bosentan, and, in resistant cases, extracorporeal membrane oxygenation (ECMO).[5] Despite these treatments, mortality and morbidity remain significant, highlighting the need to explore additional contributing factors.

One such factor is neonatal hypocalcemia, which is one of the most common metabolic disturbances in critically ill neonates and defined as ionized calcium concentration < 4.4 mg/dL (≈ 1.10 mmol/L) in neonates ≥ 1500 g and in low birth-weight neonates (<1500 g), as ionized calcium < 3.6 mg/dL.[6] Calcium plays a vital role in vascular smooth muscle relaxation, myocardial contractility, and neurotransmission.[7] The pathophysiology involves calcium's role in regulating pulmonary vascular tone. Inadequate calcium availability decreases smooth muscle relaxation and promotes vasoconstriction, thereby sustaining high pulmonary artery pressures. At the same time, reduced myocardial contractility from hypocalcemia may further compromise systemic and pulmonary circulation.[8] In neonates with PPHN, hypocalcemia can worsen pulmonary hypertension and contribute to poorer clinical outcomes.

Some studies described hypocalcemia as a compounding factor in PPHN, linked with poor response to oxygen and cardiac dysfunction.[9,10] Despite these observations, there is limited systematic evidence exploring the association between hypocalcemia-particularly ionized calcium-and PPHN severity, especially in low- and middle-income countries where co-morbidities such as birth asphyxia and sepsis are more common.

Given the potential link between hypocalcemia and PPHN, identifying this correlation in our setting will have significant clinical implications. Early recognition and correction of hypocalcemia could serve as a simple and cost-effective intervention to improve oxygenation, hemodynamic adaptation and outcomes in neonates with PPHN. Therefore, this cohort study aims to determine the frequency of hypocalcemia among neonates with PPHN and evaluate its correlation with severity and short-term outcomes in a tertiary neonatal intensive care unit setting.

Methodology

This Prospective Observational Cohort Study was conducted in the NICU of Fatima Memorial Hospital, Lahore, from 16th December 2025 to 15th May 2026, and was designed and reported in accordance with the STROBE guidelines for observational studies. The sample size of 203 neonates was calculated using the WHO/Lwanga and Lemeshow single-proportion formula, taking a reference prevalence of persistent pulmonary hypertension of the newborn (PPHN) of 5% reported by Abdel Mohsen & Amin as the reference proportion (p = 0.05), with a 95% confidence level (Z = 1.96) and an absolute precision of 3% (d = 0.03). No interim analysis or sample size modification was performed, and non-probability consecutive sampling was employed to enroll all eligible neonates admitted during the study period until the required sample size was achieved.

Neonates were selected based on predefined inclusion criteria: all neonates aged 0-7 days admitted to the NICU who underwent echocardiography for any clinical indication according to unit protocol and were diagnosed with PPHN, including both term and preterm neonates provided a baseline ionized calcium level was available within 48 hours of echocardiography and written informed consent was obtained from parents or guardians. Exclusion criteria comprised neonates with known congenital anomalies or syndromes, major structural congenital heart disease (excluding PDA, PFO, or small ASD), those born to mothers with documented bone metabolic

disorders, and cases lacking parental consent or missing essential baseline data.

The demographic, perinatal, and clinical data of all the enrolled patients were recorded using a structured proforma. Variables collected included age, gender, gestational age (GA), birth weight, mode of delivery, maternal risk factors (such as gestational diabetes mellitus and hypertension/pregnancy-induced hypertension), and perinatal factors including birth asphyxia, MAS, and sepsis. Laboratory investigations included ionized calcium, serum magnesium, and C-reactive protein (CRP) levels. Ionized hypocalcemia was defined as a decrease in the physiologically active free calcium fraction in the blood below the normal neonatal range, which is considered a more accurate indicator than total serum calcium as it is not influenced by albumin levels or blood pH. In this study, hypocalcemia was defined as ionized calcium <1.10 mmol/L in neonates with birth weight ≥1500 g, and <0.90 mmol/L in low-birth-weight neonates (<1500 g), based on which neonates were categorized into hypocalcemic and normocalcemic groups.

Supportive care and treatment modalities, including oxygen therapy, mode of ventilation, surfactant administration, inotrope use, and sildenafil therapy, were documented. Neonates were followed prospectively until discharge or death to assess short-term outcomes, including duration of invasive ventilation, length of NICU stay, and survival.

Potential bias was minimized through consecutive enrolment and standardized ionized calcium measurement. However, residual confounding from differences in GA, birth weight, and illness severity, and limitations of single-time calcium measurement may persist.

Data were analyzed using SPSS v26. Continuous variables were assessed for normality using the Shapiro–Wilk test and compared using t-test or Mann–Whitney U test, as appropriate; categorical variables were compared using chi-square or Fisher's exact test. Multivariable logistic regression adjusted for GA, PPHN severity, MAS, and sepsis, selected a priori based on clinical relevance. Results were reported as odds ratios/relative risks with 95% CIs; p≤0.05 was considered significant. Model performance was assessed using AUC, VIF, and Hosmer–Lemeshow test. No multiple-comparison correction was applied, and p-values were considered exploratory.

Ethical approval was obtained from the Institutional Review Board prior to study initiation with IRB certificate No. FMH-19/9/2025-IRB-1776. Written informed consent was obtained from parents or guardians before enrolment. Patient confidentiality was strictly maintained, and participation was voluntary, with the right to withdraw at any stage without affecting clinical care.

Results

A total of 203 neonates with echocardiographically confirmed PPHN were enrolled during the study period. All neonates had ionized calcium measured within 48 hours of echocardiography. The study cohort comprised 115 males (56.7%) and 88 females (43.3%). The mean GA was 35.1 ± 3.4 weeks and 121 neonates (59.6%) were preterm (<37 weeks). Mean birth weight was 2.41 ± 0.87 kg. The predominant mode of delivery was caesarean section (170/203; 83.7%). Meconium-stained liquor was present in 34 (16.7%) neonates, and bacteraemia (positive blood culture) was documented in 21 (10.3%) cases. Maternal diabetes mellitus was present in 64 (31.5%) and maternal hypertension/PIH in 48 (23.6%) cases.

All 203 neonates had ionized calcium measured, with a cohort mean of 1.018 ± 0.150 mmol/L. Applying weight-adjusted ionized calcium thresholds (< 1.10 mmol/L for neonates ≥ 1500 g; < 0.90 mmol/L for low-birth-weight neonates < 1500 g), 121 of 203 neonates (59.6%) were classified as hypocalcemic. The remaining 82 neonates (40.4%) were normocalcemic.

Table I. Comparison of Baseline Demographic and Perinatal Characteristics by PPHN Severity (n=203)			
Variable	**Moderate PPHN (n=95)**	**Severe PPHN (n=108)**	**p-value**
Continuous variables — Median (IQR) or Mean ± SD			
Gestational age (weeks)	36.0 (33.0–37.0)	36.0 (33.8–37.0)	0.594*
Birth weight (kg)	2.3 (1.8–2.9)	2.6 (2.0–3.2)	0.169*
Maternal age (years)	28.97 ± 3.65	27.95 ± 4.37	**0.076†**
APGAR score at 1 min	6 (4–7)	7 (5–7)	0.573*
APGAR score at 5 min	8 (7–9)	8 (7–9)	0.844*
Ionized calcium (mmol/L)	1.1 (0.9–1.1)	1.1 (0.9–1.1)	**0.017***
Categorical variables — n (%)			
Male gender	53 (55.8%)	62 (57.4%)	0.928‡
Caesarean section delivery	78 (82.1%)	92 (85.2%)	0.687‡
Meconium-stained liquor	18 (18.9%)	16 (14.8%)	0.550‡
Sepsis (positive blood culture)	11 (11.6%)	10 (9.3%)	0.756‡
Maternal diabetes mellitus	32 (33.7%)	32 (29.6%)	0.639‡
Maternal hypertension / PIH	20 (21.1%)	28 (25.9%)	0.516‡
Hypocalcemia,	52 (54.7%)	69 (63.9%)	0.237‡

* Mann–Whitney U test; † Independent t-test; ‡ Chi-square test. Values are median (IQR) for non-normally distributed continuous variables and mean ± SD for normally distributed variables. Categorical values expressed as n (%).

Baseline demographic and perinatal characteristics were comparable between neonates with moderate and severe PPHN (Table 1). There were no significant differences in GA, birth weight, mode of delivery, APGAR scores, or maternal risk factors between the two groups (all p > 0.10). The only parameter that differed significantly between groups was ionized calcium level, which was lower in severe PPHN compared to moderate PPHN (p = 0.017), supporting a graded relationship between calcium levels and disease severity. The frequency of categorical hypocalcemia was numerically higher in severe PPHN (63.9% vs 54.7%) but did not reach statistical significance (p = 0.237). Echocardiographically, PPHN was graded as severe in 108 (53.2%) and moderate in 95 (46.8%) neonates. Among hypocalcemic neonates, 69 (57.0%) had severe PPHN compared to 39 (47.6%) among normocalcemic neonates; this difference did not reach statistical significance (χ^2 = 1.758, p = 0.185; unadjusted OR 1.46, 95% CI 0.83–2.57). Following logistic regression adjusting for GA, sepsis, and MAS, hypocalcemia was not an independent predictor of echocardiographic PPHN severity (aOR 1.46, 95% CI 0.80–2.67; p = 0.219). However, a significant difference in mean ionized calcium was observed when neonates were stratified by severity: those with severe PPHN had lower mean ionized calcium (0.992 ± 0.148 mmol/L) compared to those with moderate PPHN (1.047 ± 0.149 mmol/L; mean difference 0.055 mmol/L, 95% CI 0.014–0.096; p = 0.017). This suggests an inverse association between ionized calcium levels and PPHN severity.

Table II. PPHN Severity and Ionized Calcium Levels According to Hypocalcemia Status			
Variable	**Hypocalcemic (n=121)**	**Normocalcemic (n=82)**	**p-value**
PPHN severity distribution — n (%)			
Severe PPHN	69 (57.0%)	39 (47.6%)	0.185‡
Moderate PPHN	52 (43.0%)	43 (52.4%)	
Unadjusted OR for severe PPHN (hypocalcemic vs. normocalcemic)		1.46 (95% CI 0.83–2.57)	
Adjusted OR for severe PPHN (aOR)		1.46 (95% CI 0.80–2.67), p = 0.219	
Ionized calcium by PPHN severity — Mean ± SD (mmol/L)			
Severe PPHN	0.992 ± 0.148	—	**0.017***
Moderate PPHN	1.047 ± 0.149	—	(vs. moderate)
Mean difference (moderate – severe)	0.055 mmol/L, 95% CI 0.014–0.096†	—	—

* Mann–Whitney U test (ionized calcium, severe vs. moderate PPHN); ‡ Chi-square test (severity distribution). aOR adjusted for gestational age, sepsis, and meconium aspiration. OR = odds ratio; CI = confidence interval. † Mean difference and 95% CI by Welch's t-test; Hodges–Lehmann median difference 0.04 mmol/L, approximate 95% CI 0.00–0.08, paired with the Mann-Whitney U test used for the primary comparison.

Table III. Mortality, Respiratory Support, Duration of NICU Stay, and Inotrope Use According to PPHN Severity (n = 203)				
Variable	**Moderate PPHN** (n = 95)	**Severe PPHN (n = 108)**	**OR (95% CI)**	**p-value**
1. In-Hospital Mortality				
Deaths, n (%)	15 (15.8%)	28 (25.9%)	1.87 (0.93–3.76)	0.087[b]
Survived, n (%)	80 (84.2%)	80 (74.1%)	—	—
2. Respiratory Support				
Low Flow Nasal Cannula (LFNC), n (%)	34 (35.8%)	48 (44.4%)	1.44 (0.82–2.53)	0.267[a]
CPAP, n (%)	63 (66.3%)	61 (56.5%)	0.66 (0.37–1.17)	0.197[a]
Conventional Mechanical Ventilation (MV), n (%)	25 (26.3%)	39 (36.1%)	1.58 (0.87–2.89)	0.178[a]
High Frequency Oscillatory Ventilation (HFOV), n (%)	11 (11.6%)	28 (25.9%)	2.67 (1.25–5.73)	**0.016[a]**
Any Invasive Ventilation (MV or HFOV), n (%)	26 (27.4%)	54 (50.0%)	2.65 (1.47–4.78)	**0.002[a]**
3. Duration of NICU Stay				
Median (IQR) (days)	5.0 (3–10)	6.0 (3–9)	—	0.759[c]
Range (days)	2–40	2–26	—	—
4. Inotrope Use				
Any inotrope use, n (%)	30 (31.6%)	44 (40.7%)	1.49 (0.84–2.66)	0.227[a]
Dobutamine, n (%)	4 (4.2%)	11 (10.2%)	2.58 (0.79–8.39)	0.176[b]
Nor-Epinephrine, n (%)	17 (17.9%)	42 (38.9%)	2.92 (1.52–5.60)	**0.002[a]**
Milrinone, n (%)	26 (27.4%)	51 (47.2%)	2.37 (1.32–4.28)	**0.006[a]**

*Values expressed as n (%), mean ± SD, or median (IQR) as appropriate. OR = odds ratio (severe vs moderate PPHN); CI = 95% confidence interval (Woolf method). RR = relative risk. [a] Chi-square test [b] Fisher's exact test [c] Man-Whitney U test (data non-normally distributed; Shapiro-Wilk p<0.001 in both groups).

To further evaluate the relationship between hypocalcemia and PPHN severity the findings are summarized in Table II. Overall, in-hospital mortality was 43/203 (21.2%). Mortality was higher among neonates with severe PPHN (25.9%, n=28) compared to those with moderate PPHN (15.8%, n=15), corresponding to a relative risk of 1.64 (95% CI 0.94–2.88) and an odds ratio of 1.87 (95% CI 0.93–3.76). Although this difference did not reach statistical significance (Fisher's exact p=0.087), the observed trend is clinically meaningful and consistent with the expected pathophysiological relationship between PPHN severity and mortality outcomes.

Respiratory support requirements escalated significantly with PPHN severity. Any form of invasive ventilation (conventional MV or HFOV) was required in 50.0% of neonates with severe PPHN compared to 27.4% with moderate PPHN (p=0.002). Specifically, HFOV used as rescue ventilation for refractory respiratory failure was significantly more frequent in the severe group (25.9% vs 11.6%; p=0.016). Conventional mechanical ventilation showed a trend toward higher use in severe PPHN (36.1% vs 26.3%, p=0.178) that did not reach statistical significance. Non-invasive support with CPAP and LFNC was comparable between both groups (p>0.05). There was no significant difference in duration of NICU stay between the two groups, with comparable median lengths of stay (6 vs. 5 days; p = 0.759).

To adjust for potential confounders, binary logistic regression was performed with mortality as the dependent variable. Covariates included hypocalcemia status, GA, PPHN severity (severe vs. moderate), meconium aspiration, and blood culture positivity. Results are presented in Table IV and Figure 1.

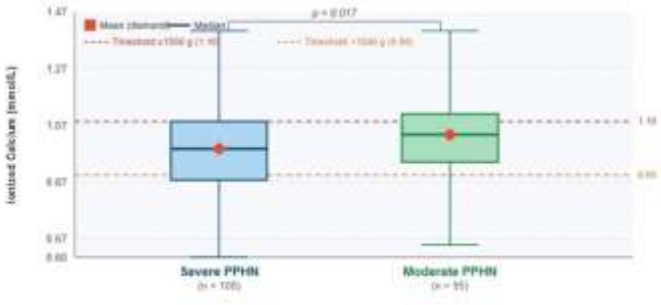

Figure 1. Distribution of ionized calcium (iCa) by PPHN severity

Boxes represent IQR with median line; whiskers extend to minimum and maximum values. Red diamonds indicate group means. Dashed horizontal lines denote the weight-adjusted hypocalcemia thresholds. Neonates with severe PPHN had significantly lower iCa (0.992 ± 0.148 mmol/L) than those with moderate PPHN (1.047 ± 0.149 mmol/L; Mann-Whitney U, *p* = 0.017).

On multivariable analysis, hypocalcemia was associated with lower mortality (aOR 0.11, 95% CI 0.04–0.30; p < 0.001); as discussed below, this is not interpreted as a protective biological effect and most likely reflects residual confounding by prematurity. Decreasing GA was a significant independent predictor of death (aOR 0.74 per week, 95% CI 0.64–0.85; p < 0.001), confirming that the mortality differential between the two calcium groups was substantially driven by prematurity confounding. Severe PPHN was independently associated with a nearly four-fold increased mortality risk (aOR 3.88, 95% CI 1.52–9.89; p = 0.004). MAS was the strongest independent predictor of death (aOR 10.85, 95% CI 3.50–33.67; p < 0.001). Sepsis did not independently predict mortality after adjustment (aOR 0.35, p = 0.231).

The mortality model showed good discrimination (AUC 0.856, 95% CI 0.795–0.930). Variance inflation factors ranged from 1.02 to 1.26 across all covariates, indicating no meaningful multicollinearity. The Hosmer-Lemeshow test indicated statistically significant lack of fit at conventional grouping ($\chi 2 = 26.9$, df = 8, p <0.001), which we attribute to the high proportion of tied predicted probabilities arising from four binary covariates and only one continuous covariate (GA) which is a recognised limitation of this test rather than evidence against the model's discrimination or effect estimates.

The observed lower adjusted mortality in hypocalcemic neonates should be interpreted with caution. Although the association remained statistically significant after adjustment, it is likely influenced by residual confounding, particularly differences in GA, birth weight, and illness severity between groups. The normocalcemic group included a higher proportion of extremely preterm and critically ill neonates, which independently increases mortality risk. Therefore, this finding does not imply a protective effect of hypocalcemia but reflects the complex interaction between metabolic status and underlying disease severity.

Table IV. Multivariable Logistic Regression Analysis for In-Hospital Mortality (n=203*)

Variable	aOR	95% Confidence Interval	p-value
Hypocalcemia (vs. normocalcemia)	0.11	0.04 – 0.30	**< 0.001**
Gestational age (per week increase)	0.74	0.64 – 0.85	**< 0.001**
Severe PPHN (vs. moderate)	3.88	1.52 – 9.89	**0.004**
Meconium aspiration syndrome	10.85	3.50 – 33.67	**< 0.001**
Sepsis (positive blood culture)	0.35	0.06 – 1.96	0.231

*aOR = adjusted odds ratio; CI = confidence interval. Reference categories: normocalcemia (for hypocalcemia), moderate PPHN (for severe PPHN). Model adjusted for gestational age (continuous), PPHN severity, meconium aspiration, and sepsis. The adjusted OR for hypocalcemia should not be interpreted as a protective effect; see Discussion for the confounding explanation.

Discussion

This study included 203 neonates with echocardiographically confirmed PPHN. Ionized hypocalcemia was common, seen in 59.6% of cases. Lower ionized calcium levels were significantly associated with greater PPHN severity on continuous analysis (p = 0.017). Neonates with severe PPHN required more invasive respiratory support and vasopressor requirements.

On multivariable analysis, three factors independently predicted in-hospital mortality. These were lower GA, severe PPHN, and MAS. These findings are discussed in the context of current evidence below.

Ionized hypocalcemia was present in 59.6% of our cohort. This is much higher than in healthy neonates

and reflects the metabolic instability of critically ill infants. Neonatal calcium homeostasis relies on placental transfer, parathyroid hormone activity, and vitamin D metabolism. These mechanisms are often impaired in prematurity, perinatal hypoxia, and systemic illness.[12]

Cheng et al. reported that early-onset hypocalcemia is common, often asymptomatic, and strongly associated with prematurity, perinatal asphyxia, and maternal diabetes, factors frequently observed in our cohort.[12] The high rate of caesarean delivery (83.7%) may have contributed. Neonates delivered by caesarean section lack the catecholamine surge of vaginal birth, which normally supports postnatal calcium regulation, predisposing them to hypocalcemia.[12]

In PPHN, ionized calcium is essential for nitric oxide production and regulation of pulmonary vascular tone[8]. As established by Liu et al., disrupted calcium signalling contributes to persistent pulmonary vasoconstriction[8]. Maintaining normal ionized calcium is part of standard PPHN management[9,10]. The high prevalence observed (59.6%) suggests this issue is common and likely underrecognized in resource-limited settings.

A key finding in our study was the inverse relationship between ionized calcium and PPHN severity. Neonates with severe PPHN had lower calcium levels than those with moderate disease ($p = 0.017$). Low calcium levels impair smooth muscle relaxation and nitric oxide mediated vasodilation, sustaining pulmonary vasoconstriction.[1,8] This supports a potential pathophysiological role of calcium dysregulation in PPHN rather than a simple association.

It is notable that categorical hypocalcemia (defined by weight-adjusted thresholds) did not differ between severity groups ($p = 0.237$, OR 1.46). In contrast, continuous calcium levels showed a significant association. Dichotomizing a continuous variable reduces information and statistical power, masking dose response relationships.[13] Therefore, continuous analysis is more informative for assessing severity.

In-hospital mortality in our cohort was 21.2%, consistent with reported global ranges and similar to the 21.0% reported by Sahin et al. in a Turkish tertiary NICU over six years.[4] Higher mortality in Low- and middle-income country (LMIC) settings is well recognized[4,14]. Prematurity was a key determinant. Chuaikaew et al. in a 13-year retrospective study from Thailand, identified GA <28 weeks as the strongest predictor of mortality[14]. Our findings align, with decreasing GA independently predicting death (aOR 0.74 per week, $p < 0.001$).

MAS emerged as the strongest predictor of death (aOR 10.85, $p < 0.001$). This is expected, as MAS causes airway obstruction, inflammation, surfactant inactivation, and severe PPHN.[15] Olicker et al. emphasised that MAS associated PPHN is often refractory and carries high mortality.[15]

While Lin et al. identified sepsis as an independent predictor[16], it was not significant in our cohort after adjustment ($p = 0.231$), likely due to differences in case mix and aetiology distribution between the two cohorts.

Severe PPHN independently predicted mortality (aOR 3.88, $p = 0.004$), supporting its prognostic value. Although mortality was higher in severe cases (25.9% vs 15.8%), this was not statistically significant ($p = 0.087$). The RR of 1.64 and OR of 1.87 indicate a clinically important trend, but limited subgroup power likely reduced statistical significance. This trend is hypothesis-generating rather than confirmatory, unlike significant associations with invasive ventilation, HFOV, norepinephrine, and milrinone use by severity. The paradoxical lower adjusted mortality in hypocalcemic neonates (aOR 0.11, $p < 0.001$) likely reflects confounding rather than protection. The normocalcemic group included more extremely preterm, low-birth-weight infants with inherently higher mortality. Lower cutoff values in this group may have classified high-risk infants as normocalcemic. Mortality was mainly driven by GA,

PPHN severity, and meconium aspiration rather than calcium status alone.

Severe PPHN required more invasive ventilation (50.0% vs 27.4%) and HFOV (25.9% vs 11.6%). This supports echocardiographic grading as a reliable marker of severity. HFOV is typically used as rescue therapy when conventional ventilation fails, indicating more severe respiratory compromise.[13] This stepwise escalation is consistent with current management approaches for severe PPHN.[13]

Severe PPHN required more cardiovascular support, with higher use of nor-epinephrine (38.9% vs 17.9%, p = 0.002) and milrinone (47.2% vs 27.4%, p = 0.006), reflecting greater haemodynamic instability. Nor-epinephrine was mainly needed in refractory hypotension with right ventricular failure. Milrinone, through pulmonary vasodilation and inotropy via cAMP pathways, is increasingly used in PPHN, especially where iNO is not available[17,18,19]. A Cochrane review by Bassler et al. showed that although RCT evidence is lacking, observational studies consistently report improved oxygenation and haemodynamics with milrinone in severe cases[18]. Choobdar et al. found milrinone and sildenafil both effective and safe alternatives in settings without iNO[17], relevant to LMIC practice. The MINT 1 pilot RCT by El-Khuffash et al. also confirmed feasibility of a milrinone protocol in neonates ≥34 weeks with PPHN[19].

NICU stay was similar between groups (median 6 vs 5 days, p = 0.759). This likely reflects two opposing effects: higher early mortality in severe PPHN shortening stay among non-survivors, and longer recovery in survivors of moderate disease. Similar patterns have been reported in other PPHN studies, where illness severity does not directly correlate with length of stay once mortality is considered.[14,16]

Study Strengths and Limitations: This study's strengths include its prospective design, use of ionized calcium, weight-adjusted thresholds, consecutive enrolment, and multivariable confounder adjustment. It also provides data from an LMIC setting where evidence on calcium homeostasis and PPHN is limited.

Limitations include the absence of a non-PPHN control group, precluding assessment of hypocalcemia as a risk factor for PPHN development. Single-time calcium measurement may not capture dynamic changes during illness. Residual confounding by GA, birth weight, and illness severity remains possible, particularly with 43 deaths and wide confidence intervals. Birth weight was excluded due to collinearity with GA, while illness severity was represented by PPHN severity, meconium aspiration syndrome (MAS), and sepsis. As a single-centre study from a tertiary NICU in Lahore, generalizability is limited. Although discrimination was good (AUC 0.856) and multicollinearity was absent (VIF 1.02–1.26), formal calibration could not be firmly established. Finally, unavailability of iNO may have influenced management and mortality outcomes.

Recommendations: First, ionized calcium should be checked routinely in all PPHN neonates at admission, using weight-adjusted cut-offs, and repeated every 24-48 hours in the acute phase. With a 59.6% prevalence in our cohort, hypocalcemia should be expected and corrected early.

Second, prospective studies with a non-PPHN control group are needed to clarify whether hypocalcemia is linked to PPHN specifically or reflects general critical illness. Third, randomized trials assessing early calcium correction as an adjunct therapy are justified, given its low cost and feasibility in LMICs. Fourth, future studies should stratify analyses by the underlying etiology of PPHN (MAS, sepsis, idiopathic) since the relationship between hypocalcemia and pulmonary vascular tone may differ by pathophysiological mechanism.

Conclusion

Ionized hypocalcemia is highly prevalent among neonates with PPHN, affecting nearly 60% of cases in this cohort. Lower ionized calcium levels were

significantly associated with greater PPHN severity. However, categorical hypocalcemia was not an independent predictor of PPHN severity after multivariable adjustment, the severity association was demonstrated for continuous ionized calcium values, not the dichotomized hypocalcemia classification. Severe PPHN was independently associated with increased need for invasive ventilation, HFOV, nor-epinephrine, and milrinone, as well as a trend toward higher mortality. On multivariable analysis, decreasing GA, severe PPHN, and MAS were independent predictors of in-hospital death.

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