Research Square

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Status of Non-Thiamine B-vitamins in Children with Septic Shock from a Lower Middle Income Country: A Case-Control study

Ajay Kumar
Postgraduate Institute of Medical Education and Research, Chandigarh, India
Arun Kumar Baranwal
baranwal1970@gmail.com
Postgraduate Institute of Medical Education and Research, Chandigarh, India https://orcid.org/0000-0003-4334-2652
Savita Verma Attri
Postgraduate Institute of Medical Education and Research, Chandigarh, India
Praveen Kumar-M
Postgraduate Institute of Medical Education and Research, Chandigarh, India
Arushi Gehlot Saini
Postgraduate Institute of Medical Education and Research, Chandigarh, India
Jayashree Muralidharan
Postgraduate Institute of Medical Education and Research, Chandigarh, India

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Abstract

Objectives

B-vitamins are increasingly being studied in management of septic shock, particularly in patients with deficiencies. However, evidence beyond thiamine remains limited. We aimed to assess status of riboflavin, niacin, pyridoxine, folate and cobalamin among children with septic shock in a lower middle-income country, and to explore their potential association with outcome.

Methods

Seventy-nine consecutive patients (age, 6 month − 12 years) with fluid-refractory septic shock were enrolled (period, July 2018 to December 2019) along with 22 sibling controls and 64 external controls from an immunization clinic for comparison. Children with chronic illnesses or receiving vitamin supplementation were excluded. Plasma concentrations of riboflavin, niacin, pyridoxine, folate, and cobalamin were measured at enrolment, and survival was assessed as primary outcome.

Results

Patients and their siblings were more stunted than external controls, both in terms of prevalence ($p = 0.014$) and severity ($p = 0.012$). Plasma levels of all five B-vitamins were significantly lower in patients than in sibling controls (all $p < 0.001$), which in turn had lower levels of riboflavin ($p < 0.001$), niacin ($p < 0.001$), pyridoxine ($p = 0.072$), folate ($p = 0.070$) and cobalamin ($p < 0.001$) compared to external controls. Among patients, non-survivors had lower levels of riboflavin ($p = 0.20$) and pyridoxine ($p = 0.04$), while folate levels were unexpectedly higher ($p = 0.037$).

Conclusions

Findings suggest that children with septic shock often come from families with pre-existing B-vitamin deficiencies, which worsen further during illness. This highlights an opportunity to evaluate adjunctive therapeutic role of B-vitamins for pediatric septic shock in low- and middle- income countries.

Lay Summary

B-complex vitamins are increasingly being studied in management of septic shock, particularly in patients with deficiencies. However, evidence beyond thiamine remains limited. To explore this, we evaluated riboflavin, niacin, pyridoxine, folate, and cobalamin in 79 children (6 months–12 years) with fluid-refractory septic shock in a lower middle-income country, alongside 22 sibling controls and 64 external controls from an immunization clinic. We found that both patients and their siblings were more stunted than external controls, and that plasma levels of all five B-vitamins were significantly lower in

patients compared to siblings, who themselves had lower levels than external controls. Among patients, non-survivors had lower levels of riboflavin and pyridoxine. Overall, our findings suggest that children with septic shock often come from families with pre-existing B-vitamin deficiencies, which worsen during illness. This highlights an opportunity to evaluate adjunctive therapeutic role of B-vitamins for pediatric septic shock in low- and middle-income countries.

INTRODUCTION

Sepsis, a state of dysregulated immune response to infection, leads to life-threatening multi-organ dysfunction. It affects approximately 25 million children globally [1]. Burden and mortality of pediatric sepsis are disproportionately higher in low- and middle-income countries (LMICs) driven by multiple socioeconomic challenges, including malnutrition and micronutrient deficiencies [1–5]. Nearly one-third of children admitted to pediatric intensive care units (PICUs) in tertiary hospitals are malnourished [4, 6]. Associated impairments in cellular, immune, and metabolic functions may contribute to the higher morbidity and mortality observed in this setting [7, 8].

Current guidelines for resuscitation of septic shock focus primarily on correcting imbalance between oxygen demand and delivery [9]. However, sepsis-induced mitochondrial dysfunction, which develops early in the disease course, impairs cellular oxygen utilization and contributes to multi-organ failure [10, 11]. The concept of 'microcirculatory and mitochondrial distress syndrome' was introduced to highlight this pathophysiology and guide development of mitochondria-targeted therapies [12]. Interventions aimed at improving mitochondrial function, and thus cellular oxygen utilization, termed as 'metabolic' or 'mitochondrial' resuscitation are increasingly being explored as adjuncts to the standard care [13]. Research to date has largely been emphasized on thiamine, ascorbic acid, calciferol, and tocopherol, with or without hydrocortisone [14–16]. Non-thiamine B-vitamins also play critical roles in mitochondrial energy metabolism, while many of them have immunomodulatory and antioxidant effects as well. Cobalamin may further provide vasoactive effects by countering endogenous vasodilators such as nitric oxide (NO) and hydrogen sulfide (H_2S) [17].

Critical illness with its accompanying hypermetabolic state predisposes patients to deficiencies of B-vitamins [6, 18–20]. Potential contribution of sepsis-induced absolute or relative B-vitamin deficiency to mitochondrial dysfunction has prompted consideration of these vitamins for mitochondrial resuscitation in septic shock, even in well-nourished populations [13, 17, 19–21]. This potential may be particularly relevant for children in LMICs, where micronutrient deficiencies are highly prevalent and worsen further during septic shock [6, 22]. However, data on the role of non-thiamine B-vitamins in pediatric septic shock remain scarce. We therefore conducted an exploratory cross-sectional case–control study to evaluate plasma levels of riboflavin, niacin, pyridoxine, folate, and cobalamin in children with septic shock in an LMIC setting, and to examine their association, if any, with clinical outcomes. Findings from this study may inform future interventional trials in resource-limited contexts.

MATERIAL AND METHODS

Study Design, Study Setting and Ethical Clearance

This single-center, cross-sectional case–control study was conducted in Pediatric Emergency Room (PER) and Pediatric Intensive Care Unit (PICU) of the Department of Pediatrics at a tertiary care teaching hospital in an LMIC as a time-bound dissertation project. Research methods adhered to the Declaration of Helsinki, as amended by 75th General Assembly of World Medical Association in 2024, and conformed to ethical standards of the Institutional Ethics Committee. Study protocol was approved vide letter no. INT/IEC/2018/001948 dated 29.11.2018, and the Departmental Review Board approved the manuscript. Written free and informed consent, along with assent when applicable, was obtained from parents or legal guardians prior to enrolment. Participants' confidentiality regarding personal and clinical information was strictly maintained.

Eligibility Criteria of Patients and Controls

Children aged > 6 months to < 12 years admitted between July 2018 and December 2019 (an 18-month period) with fluid-refractory shock and clinically suspected infection were enrolled within the first 24 hours of septic shock documentation. Fluid-refractory shock was defined as persistent hypotension (systolic blood pressure below the 5th centile for age) or the need for vasoactive support despite administration of > 40 mL/kg isotonic fluid bolus in the first hour [23]. A convenience sample of 150 consecutive patients was planned. For comparison, one apparently healthy sibling of each patient (aged 6 months to 12 years, from the same household, sharing the same kitchen) was enrolled as a 'sibling controls', while apparently healthy children attending the immunization clinic were enrolled as 'external controls'. Exclusion criteria included lack of informed consent, multivitamin supplementation within preceding three months, raised intracranial pressure, and conditions associated with elevated lactate, such as seizures, liver dysfunction, known or suspected mitochondrial disorders, or cardiac arrest/cardiopulmonary resuscitation before enrolment.

Data Collection and Outcome Variable

For enrolled patients, demographic and clinical data —including nutritional status [24], Pediatric Sequential Organ Failure Assessment (pSOFA) [25], Pediatric Logistic Organ Dysfunction (PeLOD) scores [26], fluid resuscitation, and vasoactive inotrope score (VIS) [27] —were recorded at enrolment. Pediatric Risk of Mortality III (PRISM III) and Pediatric Index of Mortality 2 (PIM 2) scores were documented at admission to PER and upon transfer to PICU. Serial measurements of lactate, pH, and anion gap were recorded for up to 36 hours. Blood samples for assessment of B-vitamins were drawn within 24 hours of septic shock identification. Patients were resuscitated according to standard guidelines in PER and were subsequently transferred to PICU as and when beds became available. They were followed until death or hospital discharge. Patients who left the hospital after counselling about futility of further care, categorized as Leave Against Medical Advice (LAMA), were considered equivalent to in-hospital deaths, and both outcomes were combined as adverse outcome [28]. For sibling and external controls, demographic data were recorded and blood samples collected for assessment of B-vitamin as per convenience.

Estimation of B-vitamins: Blood samples were collected in heparinized vacutainers and centrifuged for 10 min at 3000 rpm to separate plasma immediately after collection. Plasma samples were then stored at 20°C. Individual standard stock solutions of riboflavin, niacin, pyridoxine, folate and cobalamin (3.125ng/ml to 600 ng/ml) were prepared in 0.01% formic acid and were stored at -80^{0}C until use. 100 µL plasma was mixed with 400 µL of MeOH/ACN/Acetone, 1:1:1 (v/v/v), vortexed and incubated at 4°C for 10 min, revortexed and centrifuged for 5 min at 16,000 rpm. The supernatant was dried under nitrogen stream and analytes were reconstituted in 100 µL of 0.1% formic acid in water. 10µL was injected into analytical column for analysis. Analyses were done using Nexera X2 high-performance liquid chromatograph (M/s Shimadzu Corporation, Japan) and QTRAP 4500 LC-MS/MS System (M/s SCIEX, Canada).

Statistical Analysis

Statistical analyses were performed using R software (v3.6.1, R Core Team, 2019) [29]. Continuous variables were compared between two independent groups with Mann–Whitney U test, and among more than two groups with Kruskal–Wallis test, followed by Dunn's *post hoc* test for pairwise comparisons. Categorical variables were analyzed with Chi-square test or Fisher's exact test, as appropriate, with standardized residuals for *post hoc* comparisons in tables larger than 2 × 2. A p-value < 0.05 was considered statistically significant.

RESULTS

A total of 79 children with fluid-refractory septic shock were enrolled. Siblings of 22 patients meeting the defined criteria were enrolled as sibling controls; the remaining patients either did not have eligible siblings or their parents were unable to bring them despite repeated requests. Sixty-four apparently healthy children were enrolled as external controls. Demographic characteristics, nutritional status, and plasma B-vitamin levels of patients and controls are summarized in Table 1. Approximately one-fourth of patients and sibling controls were underweight, while a lower proportion were underweight among external controls (p3 = 0.072). Patients and sibling controls were significantly more stunted than external controls, both in terms of prevalence (X^2 test, p = 0.013) and severity (p2 = 0.021; p3 = 0.003). Patients were symptomatic for a median of 5 days (interquartile range [IQR]; 4, 7) prior to admission, and 20 of them were referred to us after a median stay of 5 days (IQR; 3, 7) in referring hospitals. Clinical characteristics are summarized in Table 2. Acute pyogenic infections (18, 22.8%), pneumonia (18, 22.8%), toxic shock syndrome (15, 18.9%), and scrub typhus (14, 17.7%) were the common diagnoses; one patient with pneumonia tested positive for H1N1.

Table 1
Demographic profile, nutritional status and levels of B-vitamins among patients, internal and external controls.

Variables	Patients (n = 79)	Sibling Controls (n = 22)	External Controls (n = 64)	p-value[a]	p-value[b]
Age (month)	60 (24, 101)	83.5 (51.5, 108.8)	72 (36, 108)	0.4	p1 = 0.230 p2 = 0.453 p3 = 0.457
Gender (M:F)	44:35	17:5	47:17	0.039[c]	For males,[c] Patients: -2.53 SibCon: 1.25 ExtCon: 1.72
Nutritional Status					
WAZ	-1.2 (-2.1, 0.3)	-1.2 (-2.1, -0.8)	-0.7 (-1.8, 0.1)	0.2	p1 = 0.193 p2 = 0.734 p3 = 0.072
Normal Weight (WAZ >-2), n (%)	58 (73.4)	15 (68.2)	49 (76.6)	p = 0.73[c]	-----
Underweight (WAZ <-2), n (%)	21 (26.6)	7 (31.8)	15 (23.4)		
HAZ	-0.7 (-1.4, 0.2).	-1.4 (-2.3, -0.4)	-0.2 (-1.4, 1.1)	0.012	p1 = 0.08 p2 = 0.021 p3 = 0.003
Not Stunted (HAZ >-2), n (%)	66 (83.5)	14 (63.6)	58 (90.6)	p = 0.013[c]	For stunted,[c] Patients: 0.03 SibCon: 2.72 ExtCon: -1.93
Stunted (HAZ <-2), n (%)	13 (16.5)	8 (36.4)	6 (9.4)		
Plasma Levels of B-Vitamins at Admission					
Riboflavin (ng/ml) (4.5–18.1)[d]	11.4 (8.1, 16.2)	35.3 (30.1, 38.8)	71.7 (61.4, 79.2)	< 0.001	p1 < 0.001 p2 < 0.001 p3 < 0.001
Niacin (ng/ml) (500–8900)[e]	255 (180.5, 37.5)	511 (489.0, 574.2)	625.4 (555.9, 710.5)	< 0.001	p1 < 0.001 p2 < 0.001 p3 < 0.001
Pyridoxine (ng/ml) (< 7.4)[f]	5.4 (3.5, 6.6)	23.7 (19.8, 26.3)	26.8 (23.8, 30.6)	< 0.001	p1 < 0.001 p2 < 0.001 p3 = 0.072
Folate (ng/ml) (1.8-9.0)[f]	7.0 (4.4, 8.4)	14.6 (12.4, 17.4)	18.0 (15.8, 19.9)	< 0.001	p1 < 0.001 p2 < 0.001 p3 = 0.070

Variables	Patients (n = 79)	Sibling Controls (n = 22)	External Controls (n = 64)	p-value[a]	p-value[b]
Cobalamin (pg/ml) (200–800)[f]	155.2 (129.9, 188.4)	559.9 (510.7, 612.5)	812.0 (741.0, 897.2)	< 0.001	p1 < 0.001 p2 < 0.001 p3 < 0.001

Abbreviations: IQR: Interquartile range; WAZ: Weight for Age z-score; HAZ: Height for Age z-score; SibCon: Sibling Controls; ExtCon: External Controls.

All values are in median (IQR) unless specified otherwise.

[a]Comparative analysis was done with Kruskal-Wallis test unless specified otherwise.

[b]p1 (patients *vs* sibling controls), p2 (patients *vs* external controls) and p3 (sibling controls *vs* external controls) represents *post-hoc* intergroup comparisons by Dunn's test.

[c]Chi-square test with standardized residuals.

Reference plasma levels of Riboflavin [52][d], Niacin [53][e], Pyridoxine, Folate and Cobalamin [54][f].

Table 2
Baseline Clinical Profile of patients (n = 79).

Primary Clinical Diagnosis	
Acute Pyogenic Infections, n (%)	18 (22.8)
Pneumonia, n (%)	18 (22.8) (H1N1 positive, n = 1)
Toxic Shock Syndrome, n (%)	15 (18.9)
Scrub Typhus, n (%)	14 (17.7)
Acute Gastroenteritis, n (%)	3 (3.8)
Acute Meningoencephalitis, n (%)	3 (3.8)
Complicated vivax malaria, n (%)	1 (1.3)
Enteric Fever, n (%)	1 (1.3)
Unspecified Sepsis, n (%)	6 (7.6)
Positive Culture at Admission, n (%)	**8 (10.1)**
MSSA (Blood and/or Pus), n (%)	2 (2.5)
MRSA (Blood and/or Pus), n (%)	5 (6.3)
Salmonella typhi, n (%)	1 (1.3)
Outcome	
Adverse Outcome, n (%)	37 (46.8%) (Deaths, 33; LAMA, 4)

Thirty-seven patients (46.8%) experienced adverse outcome (deaths, 33; LAMA, 4). Non-survivors had significantly higher illness severity scores at admission compared with survivors, including PRISM III ($p < 0.001$), PIM 2 ($p < 0.001$), pSOFA ($p < 0.001$), and PeLOD ($p < 0.001$), and required substantially greater inotropic support throughout their clinical course (VIS at 6 hours, $p < 0.001$; VIS at 24 hours, $p = 0.0014$; maximum VIS, $p < 0.001$). At admission, non-survivors also exhibited lower blood pH [7.3 (7.2, 7.4) vs 7.4 (7.3, 7.4); $p = 0.007$], lower serum bicarbonate [17.2 (14, 20.3) vs 19.9 (17.4, 22.1); $p = 0.019$], and higher lactate [2.8 (2.1, 5.2) vs 2.3 (1.8, 3.0); $p = 0.087$] compared to survivors (Table 3).

Table 3
Comparison of critical care characteristics and plasma levels of B-vitamins between survivors and non-survivors.

Variables	Total (n = 79)	Survivors (n = 42)	Non-Survivors (n = 37)	p-value
Critical Care Characteristics				
Blood pH	7.34 (7.24, 7.41)	7.4 (7.3, 7.4)	7.3 (7.2, 7.4)	0.007
Blood Lactate	2.0 (1.9, 3.8)	2.3 (1.8, 3.0)	2.8 (2.1, 5.2)	0.087
Serum Bicarbonate (mEq/L)	19 (15.9, 22.0)	19.9 (17.4, 22.1)	17.2 (14.0, 20.3)	0.019
Serum Creatinine (mg/dL)	0.5 (0.3–0.9)	0.5 (0.2, 0.6)	0.8 (0.5, 1.0)	0.004
PRISM III at PER admission	18 (11.8, 24.0)	15 (10, 18)	23.5 (18.8, 28)	< 0.001
PIM 2 at PER admission	9.7 (7.5, 17.2)	7.9 (7.1, 10.6)	17 (9.5, 24.9)	< 0.001
PRISM III at PICU admission	16 (10–23)	13 (9.2, 17.8)	22.5 (15, 30.5)	< 0.001
PIM 2 at PICU admission	9.2 (7.2, 14.8)	7.8 (6.8, 9.7)	15.3 (9.8, 24.2)	< 0.001
pSOFA score on Day 1	11 (7, 15)	8 (7, 11)	14 (11, 15.2)	< 0.001
PeLOD score on Day 1	12 (10, 22)	10 (10, 12)	21 (11, 31)	< 0.001
Fluid Bolus by 6 hours (ml)	40 (20, 40)	40 (20, 40)	40 (20, 40)	0.7
Fluid Bolus by 24 hours (ml)	40 (20, 60)	40 (20, 50)	40 (30, 60)	0.4
VIS at 6 hours	20 (10, 40)	20 (10, 28.8)	30 (20, 60)	< 0.001
VIS at 24 hours	30(10, 55)	20 (10, 30)	47 (20, 67)	0.0014
Maximum VIS	50 (30, 92)	20 (10, 30)	60 (30, 63)	< 0.001
Time to Reversal of Shock (hours)[a]	85 (48.8, 145.5) (n = 46)	77.5 (46.5, 135.3) (n = 42)	214.5 (157.5,302.2) (n = 4)[b]	0.026
PER Stay (hours)	17 (12, 22)	17.5 (13, 23.5)	17 (8, 20)	0.266
PICU Stay (days)	6 (3.75, 9.75)	6 (3, 11.2)	5.5 (4, 8.2)	0.11
Hospital Stay (days)	9 (5, 19)	14 (9.2, 23.5)	6 (2, 9)	< 0.001
Culture-positive Healthcare Associated Infections (n = 7, 8.9%)				
MSSA, n (%)	1 (1.3%)	0	1	-----
Escherichia coli, n (%)	3 (3.8%)	0	3	-----

Variables	Total (n = 79)	Survivors (n = 42)	Non-Survivors (n = 37)	p-value
Critical Care Characteristics				
Acinetobacter baumanii, n (%)	1 (1.3%)	0	1	-----
Klebsiella pneumoniae, n (%)	1 (1.3%)	1	0	-----
Staphylococcus hemolyticus and *Pseudomonas aeruginosa*, n (%)	1 (1.3%)	0	1	-----
Plasma Levels of B-Vitamins				
Riboflavin (ng/ml) (4.5–18.1)[c]	11.4 (8.1, 16.2)	13.0 (9.2, 16.2)	9.8 (6.8, 16.2)	0.2
Niacin (ng/ml) (500–8900)[d]	255 (180.5, 337.5)	248.5 (179.8, 356.5)	255 (185.0, 317.0)	> 0.9
Pyridoxine (ng/ml) (< 7.4)[e]	5.4 (3.5, 6.6)	5.9 (4.0, 7.8)	4.6 (3.1, 6.2)	0.040
Folate (ng/ml) (1.8-9.0)[e]	7.0 (4.4, 8.4)	6.2 (4.1, 7.8)	7.6 (5.1, 9.9)	0.037
Cobalamin (pg/ml) (200–800)[e]	155.2 (129.9,188.4)	156.9 (133.3,185.6)	149.2 (129.8,192.5)	> 0.9
All values are in median (IQR) and at admission unless specified otherwise.				
Abbreviations:				
PRISM, the paediatric risk of mortality; PIM, paediatric index of mortality; pSOFA, paediatric sequential organ failure assessment score; PeLOD score, paediatric logistic organ dysfunction score; VIS, vasoactive inotrope score; PER, paediatric emergency room; PICU, paediatric intensive care unit; MSSA, Mthicillin-Sensitive *Staphylococcus aureus*.				
[a]Reversal of septic shock was defined as off inotropes for 24 hours.				
[b]These patients (n = 4) had shock reversal initially, but died of some other organ failure later on.				
Reference plasma levels of Riboflavin [52][c], Niacin [53][d], Pyridoxine, Folate and Cobalamin [54][e].				

Plasma concentrations of all studied B-vitamins—riboflavin, niacin, pyridoxine, folate, and cobalamin—were significantly lower in patients compared to their siblings ($p < 0.001$) and external controls ($p < 0.001$). Among patients, levels ranged from approximately half (niacin and folate) to less than one-fourth (pyridoxine and cobalamin) of those observed in sibling controls (Table 1, Fig. 1). Sibling controls also exhibited lower levels of riboflavin ($p < 0.001$), niacin ($p < 0.001$), pyridoxine ($p = 0.072$), folate ($p = 0.070$) and cobalamin ($p < 0.001$) than external controls (Table 1, Fig. 1). Within patients, non-survivors had

lower riboflavin [9.8 (6.8, 16.2) vs 13 (9.2, 16.2); p = 0.20] and pyridoxine levels [4.6 (3.1, 6.2) vs 5.9 (4, 7.8); p = 0.04] compared to survivors, while folate levels were unexpectedly higher among non-survivors [7.6 (5.1, 9.9) vs 6.2 (4.1, 7.8); p = 0.037]. Niacin and cobalamin levels did not differ significantly between survivors and non-survivors (p > 0.9) (Table 3, Fig. 2).

DISCUSSION

This pilot study explored the status of riboflavin, niacin, pyridoxine, folate, and cobalamin in critically ill children with fluid-refractory septic shock in an LMIC. Lower plasma B-vitamin levels observed in their siblings compared with external controls reflect the higher prevalence and severity of micronutrient malnutrition in these families, suggesting that patients had pre-existing deficiencies that worsened during septic shock. Apart from pyridoxine, there were no significant differences in plasma B-vitamin levels between survivors and non-survivors. These findings are broadly consistent with prior observations regarding thiamine in similar populations [6]. The observed high rate of adverse outcome (47%) aligns with findings from contemporary studies in India [4–6], and likely reflects factors such as delayed presentation to healthcare facilities, suboptimal acute care, incomplete implementation of evidence-based resuscitative protocols at referring centers, late referral to our institution, and pre-existing macro- and micronutrient deficiencies [2, 3].

B-vitamins are water-soluble nutrients that are not synthesized endogenously and have short biological half-lives, ranging from approximately one hour for riboflavin and niacin, to a few days for pyridoxine and cobalamin, and up to three months for folate. Consequently, the human body has limited B-vitamin stores and requires daily intake to maintain optimal function of cellular and subcellular enzyme systems. These vitamins are essential cofactors in metabolic pathways responsible for energy production, with riboflavin, niacin, pyridoxine, and cobalamin directly participating in the Krebs cycle while folate contributing indirectly. Even brief periods of critical illness deplete these already limited stores due to increased metabolic demand, reduced intake, and impaired intestinal absorption [6, 18, 19, 30, 31], a situation that is likely to get exacerbated in individuals with pre-existing deficiencies as seen in case of thiamine [6].

Cobalamin deficiency has been reported in 2% of critically ill adults in high-income countries (HICs) [18]. A systematic review suggests that cobalamin has immunomodulatory effects [32], while a prospective cohort study (n = 99) demonstrated an association between cobalamin deficiency and development of sepsis in adults with culture-positive infections [33]. In a retrospective case series of 26 patients with refractory septic shock [median age, 41 years (range, 13–82.5)], high-dose hydroxocobalamin significantly improved blood pressure and reduced VIS at one and six hours post-administration, with benefits persisting up to 24 hours [34]. Similarly, a randomized controlled trial (RCT) [n = 20; baseline VIS: hydroxocobalamin group, 29 (20, 36); placebo group, 34 (24, 51)] showed a significant percentage reduction in VIS 30 minutes after high-dose hydroxocobalamin compared with placebo [-36% (-48%, -31%) vs 4% (-5%, 13%), p < 0.001], with effects sustained for three hours [-28% (-67%, -12%) vs + 10% (-14%, 49%), p = 0.01] [35]. However, intervention did not affect vasopressor-free days or overall survival

as both these studies are from a population likely to have normal cobalamin status [34, 35]. In contrast, the pre-existing low cobalamin levels observed in our patients, its further depletion during septic shock and high vasoactive requirements suggest that cobalamin supplementation, particularly early rather than as rescue treatment, could be beneficial [17].

Hypofolatemia can lead to acute megaloblastic anemia, pancytopenia, and impaired phagocytic function, potentially compromising the body's ability to control infections and influencing outcomes in septic shock. It has been reported in 19–75% of critically ill adults in HICs, with febrile patients—both with and without confirmed sepsis—having significantly lower folate levels than non-sepsis patients, and levels declining further in sepsis [19, 20]. Sepsis appears to be a major risk factor due to increased folate utilization, altered metabolism, poor oral intake, and decreased absorption. Notably, these observations come from populations likely to have normal baseline folate levels; pre-existing deficiencies may exacerbate the impact. Historically, folate supplementation was recommended in critically ill patients even from nutritionally sufficient populations to mitigate risks of acute hypofolatemia and related hematological complications [19, 20]. However, contemporary clinical data on role of hypofolatemia in morbidity and mortality of septic shock, and potential of folate as an adjunct in mitochondrial resuscitation, are lacking—especially in LMIC settings. The observation of higher folate levels among non-survivors in our study warrants further investigation.

Riboflavin, niacin, and pyridoxine possess antioxidant, anti-inflammatory, and antimicrobial properties. Experimental studies have demonstrated that these vitamins can enhance macrophage function, improve staphylococcal clearance, and attenuate reperfusion-related oxidative injury, collectively contributing to improved outcomes in sepsis [36–43]. One study reported suboptimal riboflavin levels in 56% of critically ill adult patients [31], while another found it to be associated with higher ICU mortality [44]. Undetectable niacin levels was observed to be associated with severe hypotension and hypoglycemia [45], while pyridoxine supplementation is shown to improve total lymphocyte count, T-helper and T-suppressor cells among critically ill adult patients [46]. However, data on prevalence and severity of their deficiencies, and their specific role in septic shock remain limited [47].

Although advances in septic shock management and critical care support have reduced interest in the resuscitative role of micronutrients, persistently high mortality in LMICs, stagnating outcomes in HICs, and emerging insights into the metabolic and immunomodulatory effects of micronutrients have renewed attention to their potential role [9, 47, 48]. Given mechanistic potential, evaluating adjunctive therapeutic role of B-vitamin supplementation in mitochondrial resuscitation for pediatric septic shock appears to be a promising avenue. Impact is likely to be greatest in LMICs, where children frequently exhibit undernutrition, and micronutrient deficiencies. Lack of significant differences in B-vitamin levels between survivors and non-survivors in our study may reflect pre-existing deficiencies that limit further differentiation of outcomes. It remains conjectural whether low vitamin levels predispose to severe sepsis. Large community-based studies are needed to establish reference intervals and determine prevalence and severity of subclinical B-vitamin deficiencies in children in LMICs. RCTs are warranted to evaluate adjunctive role of individual or combined B-vitamins in mitochondrial resuscitation for pediatric

sepsis in LMICs, given their low cost, safety, and tolerability even at higher doses [47, 48]. While a survival benefit may be difficult to demonstrate, potential outcome benefits such as reduced PICU transfers, lower utilization of critical care resources, longer inotrope-free days, ventilator-free days and PICU-free days may be clinically productive in the setting of LMICs [28, 49, 50, 51].

This study provides preliminary data on plasma levels of non-thiamine B-vitamins in children with fluid-refractory septic shock. Longitudinal assessment during the course of septic shock and follow-up after discharge would have yielded additional insights into dynamics of these vitamins. Limited data on reference intervals for the studied B-vitamins may have introduced some ambiguity in interpretation; however, inclusion of external and sibling controls offered a comparative perspective on vitamin status in the general population and within patients' families. Relatively small number of sibling controls may be a potential source of bias. Measurement of erythrocyte folate levels would have provided a more robust indicator of hypofolatemia, as plasma folate is sensitive to short-term dietary intake. Additionally, pre-analysis storage of samples for approximately one year may have influenced the measured values.

CONCLUSIONS

Siblings of children with fluid-refractory septic shock exhibit significantly lower levels of non-thiamine B-vitamins, which decline further during septic shock. Widespread nature of significant subclinical deficiencies may have limited their ability to differentiate survivors from non-survivors. These findings highlight an opportunity to explore the adjunctive therapeutic role of B-vitamins in mitochondrial resuscitation for pediatric septic shock in LMICs with potential to improve relevant clinical outcomes.

Declarations

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Conflict of Interest:

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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ROLE OF CONTRIBUTORS

AK did literature review, prepared draft protocol, enrolled patients, collected and analysed data, and prepared the first draft of manuscript. AKB reviewed literature, developed hypothesis, conceptualized, planned and developed study protocol, supervised conduct of study and statistical analysis, finalised manuscript and will act as guarantor. SVA planned biochemical methods, did laboratory analysis, interpreted laboratory data and reviewed the final draft. PKM planned statistical methods, supervised statistical analysis and reviewed the final draft. AGS reviewed the final draft, and suggested valuable modifications. JM critically reviewed the final draft. All authors approved the final version and will be accountable for all aspects of the work.

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Figures

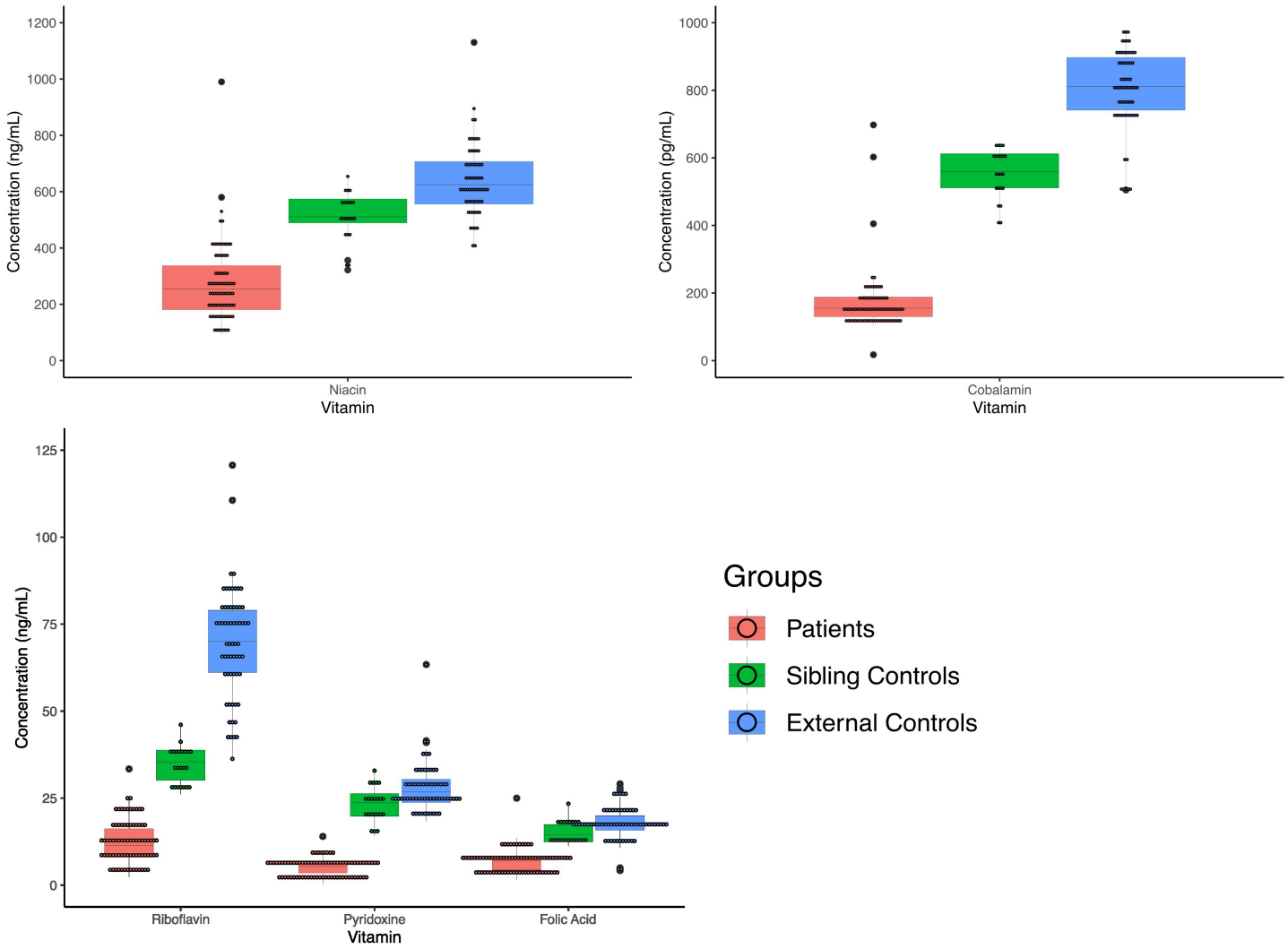


Figure 1

Boxplot (with dots) comparing distribution of plasma levels of Riboflavin, Niacin, Pyridoxine, Folic Acid and Cobalamin between septic shock patients (at enrolment), sibling controls and external controls *(refer to Table 1 for statistical comparisons)*.

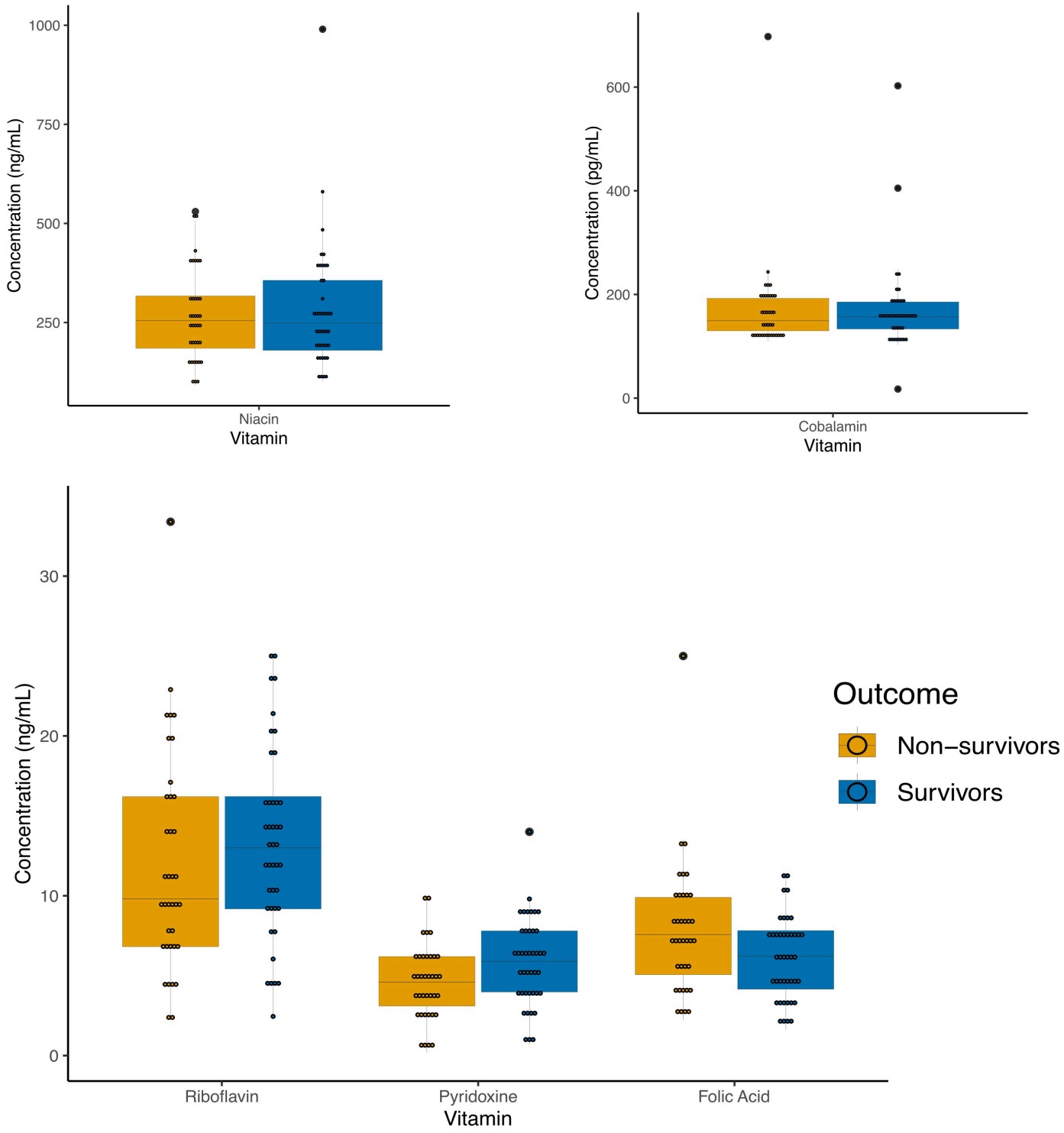


Figure 2

Boxplot (with dots) comparing distribution of plasma levels of Riboflavin, Niacin, Pyridoxine, Folic Acid and Cobalamin at enrolment among survivors and non-survivors of septic shock patients (*refer to Table 3 for statistical comparisons*