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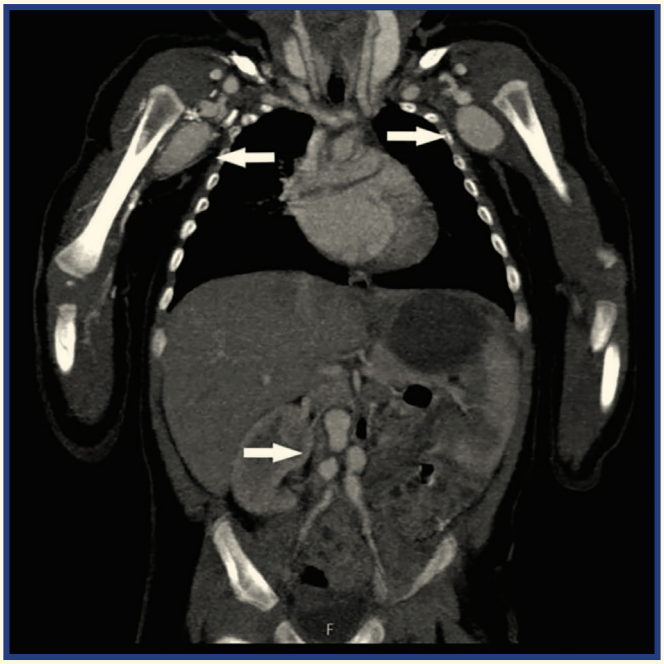

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EDITORIAL

Dear readers,

We are pleased to inform you that the new issue of The Journal of Pediatric Research has been published, which is indexed in Emerging Sources Citation Index, Embase, Directory of Open Access Journals, EBSCO, CINAHL Complete Database, ProQuest, CABI, Gale/Cengage Learning, Ulakbim TR Dizin, TurkMedline, J-GATE, IdealOnline, Hinari, GOALI, ARDI, OARE, AGORA, and the Türkiye Citation Index.

We present 8 articles, including five original research articles and three case reports from different disciplines. We hope our readers will find interest in the article entitled "Survival Predictors and Morbidity Risk Factors in Extremely Preterm Infants: A Clinical Cohort Study" from Türkiye, which aims to enhance knowledge regarding risk factors and outcomes of extremely preterm infants. Another interesting article is "Identification of Breastfeeding Problems and the Effect of Educational Breastfeeding Support on the Breastfeeding Success". Breastfeeding is the gold standard of infant feeding, as it is cheap, safe, and always readily available. Therefore, this article may enhance the awareness of pediatricians in overcome challenges in this critical period for both infants and mothers. Another article, "Cerebral and Mesenteric Perfusion Changes Due to Mydriatic Use for Retinopathy of Prematurity (ROP) by Near-infrared Spectroscopy,". Mydriatic agents mostly used ROP screening. During this procedure, near-infrared spectroscopy screening of cerebral and mesenteric tissue oxygenation may reduce the risk and contribute to safer ROP screening protocols in premature infants. Our readers can find information about "False Positive Peripheral Blood Cultures in Children with Leukaemia: A Descriptive Retrospective Prevalence Study' in this issue. These findings may help reduce the overuse of antibiotics and contribute to the development of antimicrobial stewardship programs. Also, this issue covers the article entitled "Determination of Biotinidase Enzyme Levels in Umbilical Cord Blood and Comparisons with Dried Blood Spot Testing in Newborns".

Two interesting case reports are included in this issue. The first describes a patient presenting with multiple giant coronary and systemic arterial aneurysms associated with Kawasaki Disease; the second details an acute soft head syndrome in a pediatric sickle cell anemia patient from India. The last is a letter to the editor about young children with β-thalassemia major who received ruxolitinib as a bridge therapy to avoid splenectomy.

As a pediatrician, I once again commemorate the children lost due to malnutrition in the ongoing war in Gaza and hope that this war will end as soon as possible so that all children around the world can grow up healthy and in peace.

We want to thank the authors, reviewers, editorial team, and Galenos Publishing House for their support in preparing this issue. We look forward to your scientific contributions to future editions.

Best wishes,

Assoc. Prof. Zümrüt Şahbudak Bal, MD

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Original Article

Survival Predictors and Morbidity Risk Factors in Extremely Preterm Infants: A Clinical Cohort Study

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ABSTRACT

Aim: Extremely preterm infants (born before 28 weeks of gestation) face substantial risks of mortality and severe morbidity. This study aimed to identify early clinical predictors of survival and major complications in this vulnerable population in order to guide individualized neonatal care strategies.

Materials and Methods: A retrospective cohort analysis was conducted on 102 infants born between 22+0 and 27+6 weeks of gestation and admitted to a tertiary neonatal intensive care unit from 2017 to 2020. Demographic, perinatal, and clinical variables were extracted from their medical records. Survival and morbidity outcomes were compared across gestational subgroups. Statistical analyses included chi-square, t-tests, and receiver operating characteristic (ROC) curve analysis.

Results: The overall survival rate was significantly influenced by gestational age, birth weight, and the type of respiratory support received. Infants born at 22-25 weeks exhibited lower survival rates and higher incidences of respiratory distress syndrome, invasive ventilation, and patent ductus arteriosus (PDA). Mortality was independently associated with lower birth weight ($p<0.0001$), invasive ventilation ($p=0.0014$), and the presence of hemodynamically significant PDA ($p=0.0243$). In contrast, longer durations of non-invasive ventilation correlated with improved survival ($p<0.0001$). ROC analysis demonstrated high predictive performance for birth weight [area under the curve (AUC)=0.82] and non-invasive ventilation duration (AUC=0.96).

Conclusion: Early postnatal respiratory parameters, birth weight, and cardiovascular status are critical determinants of survival in extremely preterm infants. Optimizing non-invasive ventilation strategies and timely PDA management may enhance outcomes. Notably, the rate of antenatal corticosteroid administration was markedly low in our cohort, which may have contributed to adverse respiratory and survival outcomes, underscoring the need for improved perinatal care strategies in extremely preterm births.

Keywords: Extremely preterm infants, neonatal survival, respiratory support, patent ductus arteriosus, morbidity

Introduction

Preterm birth, defined as delivery before 37 completed weeks of gestation, remains a major global public health concern, accounting for an estimated 13.4 million births annually and contributing to over one million deaths among children under the age of five each year (1,2). Among these, extremely preterm infants, those born before 28 weeks of gestation, represent the most vulnerable subgroup facing a substantially higher risk of mortality and severe long-term morbidities (3,4). The immaturity of vital organs such as the lungs, brain, and gastrointestinal system

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underlies many of the complications encountered by these infants. These include respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), and sepsis, all of which significantly contribute to neonatal morbidity and mortality (5-8). Survivors often face lifelong challenges including neurodevelopmental impairment, growth restriction, and chronic respiratory disease (9-12).

Despite global advances in neonatal care, survival outcomes for extremely preterm infants remain variable and are influenced not only by gestational age and birth weight, but also by postnatal factors such as the need for respiratory support, the presence of hemodynamically significant patent ductus arteriosus (PDA), and exposure to antenatal corticosteroids (ACS) (13-16). In this context, ACS therapy has been shown to enhance fetal lung maturation and improve neonatal outcomes when administered to mothers at risk of preterm delivery (17-19). Several maternal and perinatal conditions, including preeclampsia, maternal diabetes, oligohydramnios, and placental abruption, have also been implicated as risk factors for extreme prematurity and its associated complications (20-22).

Preterm birth continues to pose a significant clinical challenge worldwide, and extremely preterm infants remain at high risk of adverse outcomes, particularly in centers with varying levels of neonatal care capacity. Differences in perinatal management strategies and neonatal care practices contribute to heterogeneity in survival and morbidity rates across institutions.

This study aimed to evaluate the survival dynamics and early clinical predictors of mortality and morbidity among extremely preterm infants born before 28 weeks of gestation in a tertiary neonatal intensive care unit (NICU). By identifying critical early risk factors, we aimed to contribute to risk stratification models and support evidence-based, individualized care approaches which can enhance survival and reduce long-term complications in this vulnerable patient group.

Materials and Methods

Study Design

This retrospective cohort study was conducted with data from the tertiary NICU of MP İzmir Hospital. Medical records from January 2017 to December 2020 were retrospectively reviewed.

Study Population

A total of 102 extremely preterm infants born between 22+0/7 and 27+6/7 weeks of gestation and admitted to the NICU were included in this study.

Inclusion Criteria

- Infants born at gestational ages between 22+0/7 and 27+6/7 weeks
- The availability of complete medical records

Exclusion Criteria

- Infants born at or after 28+0 weeks of gestation
- Major congenital anomalies incompatible with life
- Missing or incomplete clinical data

Data Collection

The patient data were retrieved from the electronic medical records and included:

- Demographic characteristics: Gestational age, sex, birth weight, mode of delivery, Apgar scores
- Perinatal variables: ACS administration, maternal age, parity, preeclampsia, diabetes, preterm premature rupture of membranes (PPROM), oligohydramnios, and multiple gestation
- Neonatal outcomes: The need for resuscitation and intubation at birth, the presence of RDS, surfactant therapy, PDA, NEC, IVH, BPD, ROP requiring treatment, feeding intolerance, and mortality
- Respiratory support variables: The duration and mode of mechanical ventilation (invasive vs. non-invasive)
- Cardiovascular interventions: Medical or surgical PDA closure

Ethical Approval

This study protocol was reviewed and approved by the Buca Seyfi Demirsoy Training and Research Hospital Non-Interventional Research Ethics Committee (approval number: 2024/368, date: 25.12.2024). As this was a retrospective study using anonymized data, informed consent was waived. Its procedure complied with the Declaration of Helsinki guidelines.

Statistical Analysis

Descriptive statistics were calculated for all variables. Continuous variables are presented as mean ± standard deviation and compared using independent samples t-tests. Categorical variables are summarized as frequencies and percentages, and comparisons between groups were made

using Pearson's chi-square or Fisher's exact test where appropriate. Statistical significance was set at a two-tailed p-value of <0.05. All analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA).

Results

A total of 102 extremely preterm infants were included in this study. Among them, 59.8% were male and 40.2% were female, with a mean gestational age of 25.62±1.42 weeks. Cesarean section was the predominant mode of delivery (78.4%). Half of the mothers were aged between 26 and 35 years, and maternal risk factors such as PPROM (40.2%), multiple gestation (29.4%), and primiparity (47.1%) were frequently observed. Detailed demographic and maternal characteristics are presented in Table Ia.

Regarding neonatal outcomes, 40.2% of the infants required resuscitation at birth, and 32.4% underwent intubation in the delivery room. ACS therapy was administered to 37.3% of the cases. RDS was diagnosed in 91.2% of the infants, all of whom required surfactant therapy. Pneumothorax occurred in 3.9% of the cases, while culture-proven early- or late-onset sepsis were observed in 2.9% and 13.7% of the infants, respectively. Congenital anomalies were identified in 5.9% of the infants. The neonatal clinical findings are summarized in Table Ib.

A subgroup analysis comparing those infants born at 22+0–25+6 weeks and 26+0–27+6 weeks revealed significant differences in their clinical outcomes. The lower gestational age group had significantly higher rates of intubation at birth (p=0.042), invasive mechanical ventilation (p<0.001),

Table Ia. Demographic and maternal characteristics of extremely preterm infants (n=102)

	Category	**n**	**%**
Sex	Male	61	59.8
	Female	41	40.2
Gestational age	26+0 - 27+6 weeks	55	53.9
	22+0 - 25+6 weeks	47	46.1
Mode of delivery	Cesarean section	80	78.4
	Vaginal delivery	22	21.6
Maternal age	17-25 years	28	27.5
	26-35 years	51	50.0
	36-45 years	23	22.5
Maternal diabetes	Yes	4	3.9
	No	98	96.1
Maternal preeclampsia	Yes	5	4.9
	No	97	95.1
Preterm premature rupture of membranes	Yes	41	40.2
	No	61	59.8
Placental abruption	Yes	6	5.9
	No	96	94.1
Oligohydramnios	Yes	9	8.8
	No	93	91.2
Polyhydramnios	No	102	100.0
Maternal smoking	Yes	8	7.8
	No	94	92.2
Multiple pregnancy	Twins	30	29.4
	Singleton	72	70.6
Primiparity	Yes	48	47.1
	No	54	52.9

Values are presented as number and % unless otherwise stated

Table Ib. Neonatal clinical characteristics of extremely preterm infants (n=102)			
	Category	**n**	**%**
Apgar score (1st minute)	1-3	27	26.5
	4-6	53	52.0
	7-8	22	21.5
Apgar score (5th minute)	4-6	40	39.2
	7-9	62	60.8
Resuscitation at birth	Yes	41	40.2
	No	61	59.8
Intubation in delivery room	Yes	33	32.4
	No	69	67.6
Antenatal corticosteroids	Yes	38	37.3
	No	64	62.7
Respiratory distress syndrome	Yes	93	91.2
	No	9	8.8
Surfactant therapy	Yes	93	91.2
	No	9	8.8
Pneumothorax	Yes	4	3.9
	No	98	96.1
Early-onset sepsis (culture proven)	Yes	3	2.9
	No	99	97.1
Late-onset sepsis (culture proven)	Yes	14	13.7
	No	88	86.3
Congenital anomalies	Present	6	5.9
	Absent	96	94.1

and surfactant use (p=0.004). Additionally, all infants in this group were diagnosed with RDS (p=0.004). In contrast, non-invasive ventilation was more commonly used in the higher gestational age group (p<0.001). Mean birth weight was also significantly lower in the younger group (701.7±135.5 g vs. 955.8±211.1 g; p<0.001). These findings are summarized in Table II. A full comparison of all recorded variables is presented in Supplementary Table SI.

PDA was significantly more common in the lower gestational age group and was strongly associated with an increased risk of invasive ventilation (p=0.045) and hemodynamically significant ductal shunting (p<0.001). While associations with ROP and IVH were not statistically significant, higher frequencies were noted in those infants with PDA. Those infants with PDA also had significantly lower birth weights (701.7±135.5 g vs. 955.8±211.1 g; p<0.001). These findings are summarized in Table III.

Mortality was significantly associated with lower birth weight (p<0.0001), an increased need for invasive mechanical ventilation (p=0.0014), and the presence of hemodynamically significant PDA (p=0.0243). Survivors showed longer durations of non-invasive ventilation (p<0.0001) and a higher prevalence of bronchopulmonary dysplasia (BPD) (p<0.0001), likely reflecting a survival bias. No significant associations were found for small for gestational age (SGA) or ROP. SGA and treatment-requiring ROP were more frequently observed among non-survivors; however, these differences did not reach statistical significance (p=0.061 and p=0.165, respectively). These findings are summarized in Table IV.

Receiver operating characteristic (ROC) curve analyses were performed in order to assess the predictive value of clinical variables for neonatal mortality. The area under the curve for birth weight was 0.82, and for non-invasive ventilation duration, it was 0.96, indicating high

Table II. Comparison of significant clinical variables by gestational age group

	22+0 - 25+6 weeks (n=47)	26+0 - 27+6 weeks (n=55)	p-value
Intubation in delivery room	42.6%	23.6%	0.042
Invasive mechanical ventilation	59.7%	40.3%	<0.001
Non-invasive ventilation	36.7%	63.3%	<0.001
Respiratory distress syndrome	100.0%	83.6%	0.004
Surfactant therapy	100.0%	83.6%	0.004
Birth weight (mean ± SD)	701.7 ± 135.5 g	955.8 ± 211.1 g	<0.001

Data are presented as % or mean ± SD as appropriate. p-values were calculated using chi-square test or independent samples t-test
SD: Standard deviation

Table III. Clinical associations of PDA and gestational age

	PDA absent	PDA present	p-value
Treatment-requiring ROP	26.1%	73.9%	0.345
Intraventricular hemorrhage	26.7%	73.3%	0.499
Invasive ventilation required	8.0%	27.3%	0.045
Hemodynamically significant PDA	0.0%	100.0%	<0.001
Postnatal steroid use	30.0%	62.9%	0.461
Gestational age ≤ 25+6 weeks	29.8%	70.2%	0.373
Birth weight (mean ± SD)	955.8± 211.1 g	701.7±135.5 g	<0.001

Data are presented as % or mean ± SD. p-values were calculated using chi-square test or independent samples t-test as appropriate
ROP: Retinopathy of prematurity, SD: Standard deviation, PDA: Patent ductus arteriosus

Table IV. Clinical risk factors associated with mortality in extremely preterm infants

	Survivors (mean ± SD or %)	Deaths (mean ± SD or %)	p-value
Birth weight (g)	903.63±200.65	658.37±165.71	<0.0001
Duration of non-invasive ventilation (days)	36.40±16.55	2.56±8.24	<0.0001
Invasive ventilation required	67	100	0.0014
PDA	59	85	0.0243
BPD	77	15	<0.0001
SGA	7	22	0.0611
ROP requiring treatment	27	11	0.1645

Data are presented as % or mean ± SD. p-values were calculated using chi-square test or independent samples t-test as appropriate
SD: Standard deviation, PDA: Patent ductus arteriosus, BPD: Bronchopulmonary dysplasia, ROP: Retinopathy of prematurity, SGA: Small for gestational age

discriminatory ability (Figure 1). Additionally, the presence of invasive mechanical ventilation was significantly associated with mortality. All infants who died had received invasive ventilation, whereas a substantial proportion of survivors did not. This yielded an infinite (odds ratio=∞; 95% confidence interval: ∞-∞; p<0.0001), indicating a strong statistical and clinical association.

Discussion

This study investigated the early clinical characteristics and survival dynamics of extremely preterm infants in a tertiary NICU. The findings indicate that lower birth weight, the need for invasive mechanical ventilation, and the presence of hemodynamically significant PDA were associated with increased mortality, while prolonged use of non-invasive ventilation was linked to improved survival outcomes.

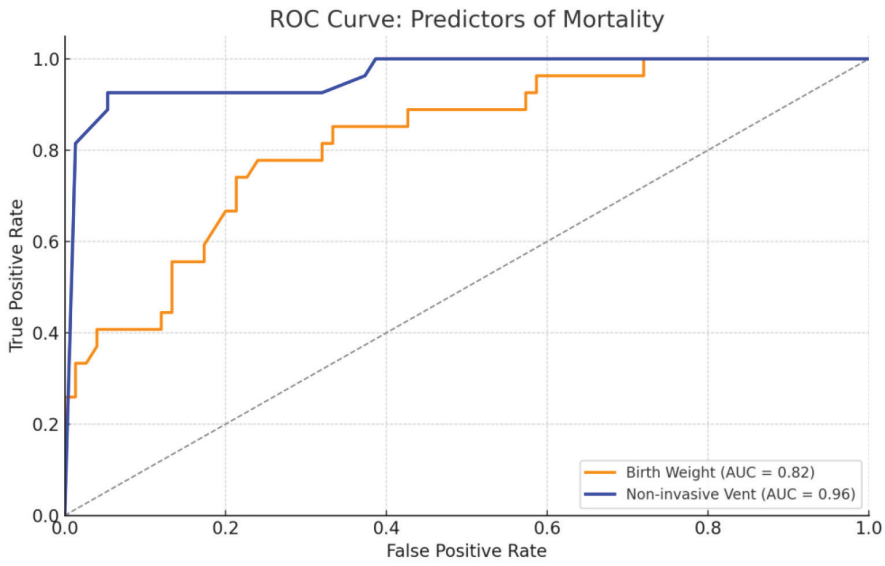

Figure 1. ROC curve analysis for predictors of mortality

ROC curve analysis of birth weight and non-invasive ventilation duration for predicting in-hospital mortality among extremely preterm infants. The area under the curve (AUC) was 0.82 for birth weight and 0.96 for non-invasive ventilation duration, indicating high discriminatory ability for both variables.
ROC: Receiver operating characteristic

Consistent with previous large-scale cohort studies, our results confirm that birth weight and gestational age remain key predictors of neonatal survival among extremely preterm infants (23,24). Infants born at 22-25 weeks of gestation had significantly lower survival rates, higher incidences of RDS, and greater reliance on invasive respiratory support compared to those born at 26-27 weeks. These findings highlight the importance of even slight increases in gestational maturity in improving neonatal outcomes, particularly for those on the threshold of viability (25).

The association between invasive mechanical ventilation and mortality observed in our cohort echoes findings from earlier reports, which suggest that prolonged or early invasive ventilation may contribute to lung injury and systemic inflammation (26,27). Conversely, the positive correlation between prolonged non-invasive ventilation and survival supports the growing preference for gentle ventilation strategies aimed at reducing iatrogenic harm in fragile preterm lungs.

In our study, both birth weight and the duration of non-invasive ventilation were found to be strong indicators of survival among extremely preterm infants. The high predictive performance observed in ROC analyses suggests that these variables can be effectively used in early clinical risk assessment. Additionally, all infants who did not survive required invasive mechanical ventilation, indicating a strong association between the need for advanced respiratory support and mortality. While this relationship is noteworthy, it likely reflects the severity of the infant's underlying condition. Infants with more severely critical illnesses are inherently more likely to require invasive ventilation, making it both a marker of disease severity and a potential contributor to adverse outcomes.

PDA was another critical factor influencing neonatal outcomes. Hemodynamically significant PDA was exclusively observed in the lower gestational age group and was significantly associated with increased mortality. These findings are in line with previous studies indicating that persistent PDA can lead to pulmonary overcirculation, systemic hypoperfusion, and an increased risk of IVH and BPD (28,29). Targeted echocardiographic screening and individualized medical or surgical closure strategies may be necessary in order to improve outcomes in this subgroup.

Our analysis also identified a potential association between invasive ventilation and the development of treatment-requiring ROP. Those infants requiring invasive respiratory support exhibited higher rates of severe ROP, which is in accordance with the prior literature linking excessive oxygen exposure and mechanical ventilation with retinal vascular proliferation (30). This finding emphasizes the need for precise oxygen targeting and careful respiratory management in this population.

Although feeding intolerance and NEC were more frequent among the most immature infants, no statistically significant associations were observed. Nonetheless, these complications remain clinically important in extremely preterm infants, where intestinal immaturity and altered microbial colonization may predispose to gastrointestinal injury (31,32). Strategies promoting enteral nutrition with breast-milk and cautious advancements of feeding volumes should remain central to NEC prevention protocols.

While the associations between SGA and ROP with mortality did not reach statistical significance in our cohort, both conditions were observed more frequently among non-survivors. The higher rate of SGA in the mortality group (22% vs. 7%) aligns with previous studies demonstrating increased morbidity and mortality among very preterm SGA infants (33). Similarly, those infants who died also had a higher proportion of treatment-requiring ROP (11% vs. 27%), consistent with known associations of ROP development with extreme prematurity, low birth weight, and oxygen-related retinal injury (34). While these trends did not achieve significance, likely due to limited sample size, they warrant further investigation in larger prospective cohorts.

An unexpected finding was that BPD was significantly more common among survivors. This may reflect a form of "survivor bias", wherein infants must live long enough in order to manifest chronic lung disease. While BPD is typically associated with long-term morbidity, its presence in survivors should not be interpreted as protective but rather as a marker of prolonged NICU stay and ventilation (35).

Maternal factors such as preeclampsia, PPROM, and multiple gestation were frequently observed in this cohort, consistent with known etiologies of spontaneous and indicated preterm birth (36). Despite the recognized benefits of ACS in promoting fetal lung maturation and improving survival outcomes (37), only 37.3% of mothers in this cohort received ACS. This unexpectedly low rate of ACS administration may be attributed to factors such as delayed maternal admission, lack of timely prenatal care, or emergent deliveries which precluded the opportunity for full steroid course administration. Future quality improvement efforts should focus on ensuring that eligible mothers receive timely and complete antenatal steroid therapy in order to optimize neonatal outcomes.

Study Limitations

This study contributes meaningful insights into the clinical trajectories of extremely preterm infants, particularly in relation to early survival and morbidity; nonetheless, certain limitations warrant consideration. Although our analysis is based on systematically documented clinical data, its retrospective nature may introduce biases related to data accuracy and completeness. The single-center design, shaped by institution-specific practices and resource availability, may also limit the broader applicability of our findings. Additionally, the absence of long-term neurodevelopmental follow-up data restricts conclusions regarding the translation of early survival into later functional, cognitive, or behavioral outcomes. In order to strengthen external validity and inform long-term care strategies, future studies incorporating prospective, multicenter designs with extended follow-ups are needed.

Conclusion

This study highlights the critical importance of early respiratory support modalities, birth weight, and cardiovascular status in determining survival outcomes among extremely preterm infants born before 28 weeks of gestation. Those infants with lower birth weight, increased need for invasive mechanical ventilation and hemodynamically, significant PDA, exhibited markedly higher mortality rates. In contrast, prolonged use of non-invasive ventilation was associated with improved survival, supporting the implementation of lung-protective, individualized respiratory strategies in the early postnatal period. ACS therapy, a well-established intervention known to reduce respiratory morbidity and mortality, was notably underutilized in our cohort (administered in only 37.3% of cases). This finding underscores a significant gap in perinatal care which may have adversely impacted neonatal outcomes and represents an actionable target for quality improvement in delivery room management. To the best of our knowledge, this is one of the few clinical cohort studies from a tertiary NICU in a middle-income setting which rigorously evaluates the combined predictive value of non-invasive ventilation duration, PDA status, and birth weight on survival rates in extremely preterm infants. These findings may contribute to enhanced risk stratification models and inform the development of targeted care protocols aimed at improving early survival. Future multicenter studies incorporating long-term neurodevelopmental outcomes are warranted in order to validate these results and ensure that early survival gains translate into meaningful long-term health benefits in this highly vulnerable population.

Ethics

Ethics Committee Approval: This study protocol was reviewed and approved by the Buca Seyfi Demirsoy Training and Research Hospital Non-Interventional Research Ethics Committee (approval number: 2024/368, date: 25.12.2024).

Informed Consent: As this was a retrospective study using anonymized data, informed consent was waived.

Footnotes

Authorship Contributions

Concept: M.T.A., S.G., A.A.S., B.C., S.Ş., Design: M.T.A., S.G., A.A.S., B.C., S.Ş., Data Collection or Processing: S.G., A.A.S., B.C., Analysis or Interpretation: M.T.A., Literature Search: M.T.A., S.G., S.Ş., Writing: M.T.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support

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Supplementary Table SI. Full comparison of perinatal and clinical characteristics in extremely preterm ınfants stratified by gestational age group

	Categories	Premature babies between 26+0 and 27+6 weeks		Premature babies between 22+0 and 25+6 weeks		P-value
		N	%	N	%	
Gender	Female	26	47.30	15	31.90	0.115
	Male	29	52.70	32	68.10	
Birth type	Cesarean Section	47	85.50	33	70.20	0.062
	Vaginal delivery	8	14.50	14	29.80	
Apgar score (1st minute)	1	2	3.60	2	4.30	0.502
	2	2	3.60	4	8.50	
	3	9	16.40	8	17.00	
	4	6	10.90	3	6.40	
	5	7	12.70	7	14.90	
	6	16	29.10	14	29.80	
	7	8	14.50	9	19.10	
	8	5	9.10	0	0.00	
Apgar score (5th minute)	4	0	0.00	1	2.10	0.288
	5	3	5.50	3	6.40	
	6	15	27.30	18	38.30	
	7	24	43.60	20	42.60	
	8	9	16.40	5	10.60	
	9	4	7.30	0	0.00	
Resuscitation needed at birth	No	35	63.60	26	55.30	0.393
	Yes	20	36.40	21	44.70	
Intubation needed in delivery room	No	42	76.40	27	57.40	0.042
	Yes	13	23.60	20	42.60	
Antenatal steroid administration	No	30	54.50	34	72.30	0.064
	Yes	25	45.50	13	27.70	
Need for admission to the neonatal intensive care unit	Yes	55	100.00	47	100.00	
Respiratory distress syndrome (RDS)	No	9	16.40	0	0.00	0.004
	Yes	46	83.60	47	100.00	
Surfactant requirement	No	9	16.40	0	0.00	0.004
	Yes	46	83.60	47	100.00	
Pneumothorax	No	52	94.50	46	97.90	0.388
	Yes	3	5.50	1	2.10	
Early-onset neonatal sepsis (culture-proven)	No	54	98.20	45	95.70	0.468
	Yes	1	1.80	2	4.30	
Late-onset neonatal sepsis (culture-proven)	No	50	90.90	38	80.90	0.141
	Yes	5	9.10	9	19.10	

Supplementary Table SI. Continued

	Categories	Premature babies between 26+0 and 27+6 weeks		Premature babies between 22+0 and 25+6 weeks		P-value
		N	%	N	%	
Congenital anomaly	Cleft palate	1	1.80	0	0.00	0.374
	Congenital cataract	0	0.00	1	2.10	
	Hydrops fetalis	1	1.80	0	0.00	
	No	53	96.40	43	91.50	
	Omphalocele	0	0.00	1	2.10	
	Polydactyly	0	0.00	1	2.10	
	Tracheoesophageal fistula	0	0.00	1	2.10	
Feeding intolerance during hospitalization	No	24	0.649	13	0.351	0.094
	Yes	31	0.477	34	0.523	
Patent ductus arteriosus (PDA)	No	21	0.6	14	0.4	0.373
	Yes	34	0.507	33	0.493	
Small for gestational age (SGA)	No	47	0.516	44	0.484	0.185
	Yes	8	0.727	3	0.273	
Large for gestational age (LGA)	No	54	0.535	47	0.465	0.353
	Yes	1	1	0	0	
Necrotizing enterocolitis (NEC)	No	44	0.53	39	0.47	
	Yes	11	0.579	8	0.421	
Bronchopulmonary dysplasia (BPD)	No	20	0.5	20	0.5	0.700
	Yes	35	0.565	27	0.435	
Invasive Mechanical Ventilation Required	No	24	0.96	1	0.04	0.000
	Yes	31	0.403	46	0.597	
Non-Invasive Mechanical Ventilation Required	No	5	0.217	18	0.783	0.000
	Yes	50	0.633	29	0.367	

Data are presented as % or mean ± SD. P-values were calculated using chi-square test or independent samples t-test where appropriate

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Original Article

Identification of Breastfeeding Problems and the Effect of Educational Breastfeeding Support on the Breastfeeding Success

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ABSTRACT

Aim: The identification of breastfeeding problems in the postpartum period and the effect of interventions on these problems.

Materials and Methods: This was a retrospective and cross-sectional study. This study included mothers who had an infant and were admitted to the breastfeeding and lactation unit (BLU) during the study period. Individualized educational breastfeeding support (EBS) was provided to the study mothers at the BLU. The demographic data and breastfeeding problems of the included infants and their mothers were obtained from files of the BLU and they were retrospectively analyzed. Breastfeeding duration was evaluated after the infants in this study group reached 6 months of age.

Results: During the study period, 163 infants of 158 mothers were enrolled. The most common complaint on admission to the BLU was breast and nipple problems (60.1%). Sub-complaints included poor grasping of the nipple (30.1%), breast engorgement (12.3%), breast refusal (8.1%), the use of nipple shields (6.1%), and cracked nipples (2.5%). With the exception of cases involving breast and nipple problems, the most prevalent reason for presentation to the BLU was the early initiation of formula feeding (13.5%). Among the 114 infants whose mothers received EBS and who were older than 6 months at the time of follow-up, 75% were exclusively breastfed, and the median breastfeeding duration was 10 months (range: 6-20).

Conclusion: Breast and nipple problems, which constitute the majority of the reasons for admission to the BLU, can be resolved with EBS. Consequently, EBS has the potential to enhance the rate of exclusive breastfeeding for a period of at least six months, thereby surpassing the national average for breastfeeding.

Keywords: Breastfeeding, educational breastfeeding support, breast problems, cracked nipple, formula feeding, lactation training

Introduction

Breastfeeding has an important role in infant and maternal health as well as in public health. Therefore, both national and global policies to promote breastfeeding need to be supported (1,2). The World Health Organization recommends exclusive breastfeeding for all infants up to 6 months and continued breastfeeding until at least 2 years of age, depending on the desires of the mother and child (3). It has been reported that around 820,000 children's lives

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could be saved annually through breastfeeding alone, and around 87% of these children are infants under 6 months of age (4). It is also known that in low- and middle-income countries, similar to our country, mortality due to diseases such as pneumonia or diarrheal decreases 11 to 15 times as a result of breastfeeding (5). The benefits of breastfeeding are not only limited to the breastfeeding period, but also have significant positive effects on health in adulthood. Therefore, the importance of breastfeeding in establishing the fundamentals of a healthy life cannot be denied (6).

According to the Türkiye Demographic and Health Survey (TDHS) 2018, the rate of exclusive breastfeeding was 41% of children younger than 6 months of age in our country. TDHS data reported that exclusive breastfeeding was 59% in the neonatal period, but this rate decreased to 45% in infants aged 2-3 months and to 14% in infants aged 4-5 months. The breastfeeding behavior of mothers is influenced by many factors and may require support. These factors may be due to socio-demographic, obstetric, maternal or infant-related reasons. These factors may be due to breastfeeding failure and reluctance may occur in the early period or during follow-up (7,8).

The main aim of this study was to determine the causes of breastfeeding failure in mothers and their babies who presented with breastfeeding problems. The secondary aim was to determine the effects of educational breastfeeding support (EBS) on breastfeeding behavior and its duration.

Materials and Methods

Study Design

This retrospective and cross-sectional study included mother-baby pairs who were admitted to the "breastfeeding and lactation unit" (BLU) of Kırıkkale University Faculty of Medicine between 01.11.2022 and 01.12.2023 for breastfeeding difficulties and problems and whose retrospective records were available for review. The study protocol was approved by the Non-Interventional Clinical Research Ethics Board of Kırıkkale University Faculty of Medicine (approval no.: 2024.02.07, date: 14.02.2024). The authors have confirmed in writing that they complied with the World Medical Association Declaration of Helsinki regarding the ethical conduct of research involving human subjects and/or animals.

Settings and Relevant Context

The mothers in this study received individualized EBS from a certificated nurse practitioner (Ç.A). The EBS targeted the mother and/or family members such as parents. This support included increasing the mother's motivation to breastfeed, whether that be via providing information about the health outcomes of breastfeeding, providing women with the skills and confidence to commence breastfeeding, or using more structured approaches such as motivational interviewing which sought to "increase an individual's belief that they can achieve a desired outcome" (8).

Sample

Educational Breastfeeding Support (EBS)

• The benefits of breast milk and breastfeeding were explained to mothers.

• Mothers were briefed on breastfeeding positions, the stomach capacity of the newborn, signs of the newborn being full with breast milk and assessing the adequacy of breast milk, expressing and storing breast milk, breast care, and breast rejection.

• When the nipple was inverted, mothers underwent Hoffman exercises to help them initiate and maintain breastfeeding. Hoffman exercises were performed by taking the nipple between the index finger and thumb and gently pulling it into the baby's mouth and using the same technique to stretch the nipple and make it visible (9).

• When the mother suffered breast engorgement, warm gauze pad application to the breast, taking a hot shower, massaging the breast softly, massaging the shoulder and back area, and hand milking were recommended and practiced.

• Management of mastitis included increased fluid intake, rest, antibiotic treatment and drainage of excess milk from the breast. General surgery consultation was indicated for drainage in mastitis when complicated with abscesses.

• Crying and screaming while on the mother's breast or stopping breastfeeding after feeding for a very short period of time were considered as breast refusal. The rejection of the breast may arise from either the mother or the infant. Some conditions of the infant, such as thrush, pharyngitis, clavicle fracture or diseases of the mother, the smell of perfume, or a lack of knowledge and experience, can cause breast rejection. Breast refusal is resolved by finding the underlying cause of the infants' breast refusal and providing an appropriate approach.

• If the baby's nipple latching problem was caused by the mother, the mother-baby pair was encouraged to provide support for each breastfeeding at 2 to 3 hours intervals all day long, which was called "breastfeeding camp". At each breastfeeding period during the day, the mother was shown

how to breastfeed appropriately with an individualized approach and given the opportunity to practice exercises.

• Counseling on breastfeeding and how to continue breastfeeding was provided for those mothers who had returned to their jobs.

• Re-lactation means re-establishing (restarting) breastfeeding. Various methods such as nipple stimulation, the breastfeeding support system, the drop and drip technique and skin-to-skin contact were used to achieve lactation. Lactogogues are rarely necessary for re-lactation.

Routinely, all mothers who received EBS by the BLU nurse (Ç.A.) were contacted by phone in order to obtain information regarding breastfeeding durations when their babies had reached 6 months of age.

Statistical Collection

The data of the included infants and their mothers were obtained from files of the BLU. Demographic data including gender, gestational age, birth weight, mode of delivery, co-morbidities, maternal age, maternal education and occupation, duration of breastfeeding, risk factors, and breastfeeding problems were retrospectively analyzed. When their children were six months old, the families were contacted again and their breastfeeding information was recorded.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 28 (SPSS, Chicago, IL, USA) was used for the statistical analysis. The data are expressed as mean ± standard deviation, medians (minimum-maximum values), percentages and ratios. The variables were analyzed for normal distribution using the Shapiro-Wilk test. Mean and standard deviation values were calculated for the data which matched the normal distribution, and median and minimum-maximum values were calculated for the data which did not match the normal distribution.

Results

During the study period, 166 infants were admitted. Sixteen of these babies were twins. Three of these infants who were twin pairs were not included in this study because they did not have breastfeeding problems. The remaining 163 infants from 158 mothers were enrolled (Figure 1). The rate of caesarean section (CS) was 88% (n=144) among the mothers. The demographic data on the infants and mothers are shown in Table I. The rate of initiation of breastfeeding in the first 1 hour after birth was 73% (n=116).

The most common complaint reported by mothers on admission to the BLU was breast and nipple problems (60.1%). Among those mothers admitted to BLU with breast and nipple problems, the most common sub-complaint was poor grasping of the nipples (30.1%). Early initiation of formula feeding (13.5%) was the most common complaint in the "other reasons" group. Table II shows the reasons for applying to the BLU for other breastfeeding problems.

Seventy-two (44%) of the infants used formula at some point in their lives. Table III summarizes when and for what reasons the infants were started on formula and who recommended it.

Thirty (18.4%) of the infants who participated in this study were hospitalized within the first 15 days of life. The reasons for hospitalization were indirect hyperbilirubinemia (n=18), prematurity (n=5), early-onset sepsis (n=5), prematurity (n=2) and hyperbilirubinemia (n=2).

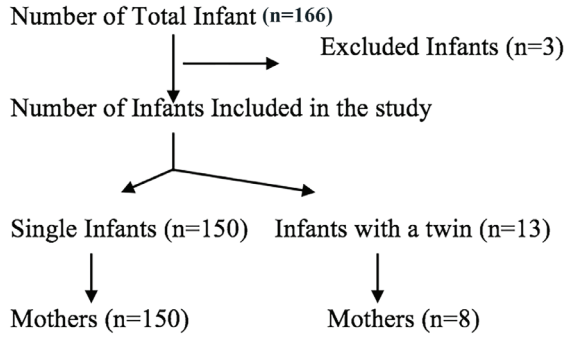

Figure 1. The mother-infant pair in the study

Table I. Demographic data on the infants and mothers

		Infants (n=163)	Mothers (n=158)
Gestational age (week)*		38 (31-42)	
Birth weight (gr)**		3,069±526	
Caesarean section (CS) (n, %)		144 (88)	
Singleton birth (n, %)		153 (94)	
Female (n, %)		84 (52)	
Day of admission*		21 (1-270)	
Mothers' age*			28 (18-42)
Mother's education (n, %)	University		67 (43)
	High school		75 (48)
	Elementary		16 (10)
Employed mother (n, %)			4 (3)

*Median (minimum-maximum), **Mean ± standard deviation

Mastitis or nipple problems were detected on breast examination in 57 (36%) of the mothers presenting to the BLU. The most common maternal nipple problem was found to be inverted nipple (49.1%) (Table IV).

Table II. The reasons of breastfeeding problems expressed by mothers, n (%)	
Breast and nipple problems	97 (60.1)
Poor grasping of the nipple	49 (30.1)
Breast engorgement	20 (12.3)
Breast refusal	14 (8.6)
Use of nipple shields	10 (6.1)
Cracked nipples	4 (2.5)
Other reasons	66 (39.9)
Early initiation of formula	22 (13.5)
Psychological problems of mothers	19 (11.7)
Reduction or cessation of breast milk	10 (6.1)
Failure to gain weight	8 (4.9)
Delayed lactation	6 (3.7)
Adoption	1 (0.6)

Table III. Feeding with formula (n=72)	
Reason for giving formula, n (%)	
Maternal anxiety	28 (39)
Failure to gain weight	22 (30)
Lack or delay of mother's milk	16 (22)
Indirect hyperbilirubinemia	5 (7)
Excessive weight loss during exclusively breastfeeding	1 (2)
When was formula started? n (%) 72 (44)	
<7 days	42 (58)
7-14 days	23 (32)
14-30 days	3 (4)
>30 days	4 (6)
Who recommended giving formula to the baby? n (%)	
Mother	41 (57)
Health professional	31 (43)

Table IV. Maternal nipple problems and mastitis	
	n: 57, 36%
Cracked nipples	13 (22.8)
Inverted nipple	28 (49.1)
Breast engorgement without mastitis	15 (26.3)
Mastitis	1 (1.8)

The rate of initiation of exclusive breastfeeding was 52.1% (n=85/163) among those infants whose mothers received EBS. Exclusive breastfeeding was documented in 52% (n=75/144) of those babies born by CS. Exclusive breastfeeding was documented in 10 (52.6%) of the 19 babies born by normal spontaneous vaginal delivery. There was no difference in breastfeeding success between vaginal delivery and CS (p=0.083).

After a single visit to the BLU, 22 (14%) mothers achieved breastfeeding success. The remaining 136 (86%) mothers achieved breastfeeding success after more than one BLU visit. The median number of readmissions to the BLU was 1 (1-4).

During the study period, 114 (75%) of the 152 mothers with infants aged six months or over were contacted via telephone. Of these infants, 85 (75%) were exclusively breastfed, 13 (11%) received both formula and breastmilk, and 16 (14%) received only formula. The median duration of breastfeeding in the exclusively breastfed infants was 10 months (range: 6-20).

Discussion

In this study, we investigated factors affecting breastfeeding success, and then examined long-term breastfeeding rates after breastfeeding interventions. We found that the main reasons for admission to the BLU were poor latching and breast refusal due to breast and nipple problems (60.1%). The most common sub-complaint of the mothers was poor grasping of the nipples (30.1%). Similarly, in a Danish study, 40% of the admissions for breastfeeding failure were attributed to the baby's inability to latch onto the breast. The next reason was reported as sore, wounded or cracked nipple (10). In line with our results, Gerd et al. (11) showed the same breastfeeding problems in their study.

In our study, the most common reason for formula initiation was maternal anxiety with a rate of 39%. Similarly, in a Canadian cohort study of 306 women, maternal anxiety was associated with an 11% reduction in the odds of exclusive breastfeeding at 6 months (12). However, the study by Arifunhera et al. (13) showed that maternal anxiety had a weak effect on breastfeeding. A Turkish cohort study of 60 mothers measured state and trait anxiety, and they found no significant differences between levels of anxiety and exclusive breastfeeding status at 4 months postpartum (14).

In the present study, the decision to start formula feeding was made by the mother herself in 57% of the cases, while 43% were influenced by health professionals. In an Australian study, it was reported that the person who

started formula was the mother, and the most common reason was that she thought her milk decreased in the second week after discharge (15). Furthermore, it was found that the total duration of breastfeeding was shorter in those who started formula in the first days in line with the recommendations of health professionals (16). In order to prevent this situation in our hospital, it is planned to increase the frequency of training for health professionals, as recommended by the Turkish Ministry of Health (17).

The present study found that 36% of breastfeeding problems in mothers were due to nipple problems. Feenstra et al. (10) reported that 38% of mothers had nipple cracks, 7% had mastitis, 40% had breast rejection due to various factors, and 4% had sucking failure due to breast engorgement and hyperlactation. The prevalence of nipple-related issues, including sore, wounded, or cracked nipples, was found to be 22.8% in the present study, which is consistent with the findings of Swedish mothers who reported experiencing sore nipples (25%) (11).

The rate of initiation of breastfeeding within the first 1 hour after birth was 73% in the present study. This might have contributed to the high success rate of breastfeeding. Many studies have shown that breastfeeding within the first hour after birth has an impact on breastfeeding success. They suggested that the value of breastfeeding and skin-to-skin contact within the first hour should be emphasized (18-20). Similarly, in a study conducted in Spain on 151 mother-infant pairs, it was found that the early breastfeeding group (within the first hour of life) had a significantly higher rate of exclusive breastfeeding at hospital discharge or within 15 days after delivery (21).

It is known that the rate of breastfeeding decreases and formula feeding increases after CS due to maternal pain, the effect of anesthetic agents and surgical complications. In this context, the negative effects of CS on breastfeeding cannot be underestimated (22,23). In the present study, 87% of deliveries were by CS, and 60.2% of them were able to breastfeed exclusively.

In the literature, the primary expectations of mothers from health workers are that breastfeeding support should be tailored to the specific needs of each woman, grounded in evidence-based practices, and provide practical assistance (24,25). It has been observed that our BLU has positive contributions to ensure that the mother can breastfeed correctly and effectively. After a single visit to the BLU, 22 (14%) mothers achieved breastfeeding success. The remaining 136 (86%) mothers achieved breastfeeding success after more than one BLU visit.

According to the TDHS 2018 data, the rate of exclusive breastfeeding of infants in our country decreases from 45% at 3 months to 14% at 5 months (7). In our study, it was shown that 75% of infants with a 6-month median age (6-20) were exclusively breastfed. This demonstrated that EBS had significant positive effects on exclusive breastfeeding success, especially in mother-infant pairs with breastfeeding problems. Similarly, it was reported in the literature that interactive, applied breastfeeding trainings given to mothers have positive contributions towards the development of breastfeeding behavior (25-28). Also, a Canadian study of vulnerable mothers in need of breastfeeding counselling found that breastfeeding rates increased after counselling (29).

Study Limitations

There were some limitations in the present study. Firstly, this study had a retrospective design. Secondly, this study had a small sample size. The third limitation was that some of the patients could not be reached when they were called by phone during the follow-up.

Conclusion

Breast and nipple problems, which constitute the majority of the reasons for admission to our BLU, can be resolved with EBS. Consequently, EBS has the potential to enhance the rate of exclusive breastfeeding for a period of at least six months, thereby surpassing the national average for breastfeeding. Motivating the mother during follow-up should be an important task of healthcare professionals. Furthermore, BLUs should be established more widely in order to enhance support for breastfeeding mothers. Future research should focus on evaluating the long-term outcomes of EBS interventions, ideally through prospective and multi-center studies.

Ethics

Ethics Committee Approval: The study protocol was approved by the Non-Interventional Clinical Researches Ethics Board of Kırıkkale University Faculty of Medicine (approval no.: 2024.02.07, date: 14.02.2024).

Informed Consent: This is a retrospective and cross-sectional study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ü.A.T., Ç.A., S.A., H.F.G., Concept: Ü.A.T., Ç.A., S.A., H.F.G., Design: Ü.A.T., Ç.A., S.A., H.F.G., Data Collection or Processing: Ü.A.T., Ç.A., Analysis or Interpretation: Ü.A.T., S.A., H.F.G., Ü.A.T., Ç.A., S.A.,

H.F.G., Literature Search: Ü.A.T., Ç.A., S.A., Writing: Ü.A.T., Ç.A., S.A., H.F.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

Cerebral and Mesenteric Perfusion Changes Due to Mydriatic Use for Retinopathy of Prematurity by Near-Infrared Spectroscopy

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ABSTRACT

Aim: Retinopathy of prematurity (ROP) screening requires pharmacologic pupil dilation, which may be associated with systemic side effects in preterm infants. This study aimed to evaluate the impact of low-dose mydriatic eye drops on cerebral and mesenteric tissue oxygenation using near-infrared spectroscopy (NIRS).

Materials and Methods: In this single-center prospective observational study, 30 preterm infants gestational age ≤32 weeks and/or birth weight ≤1,500 g) underwent 61 ROP examinations. A low-dose mydriatic protocol (0.625% phenylephrine and 0.25% tropicamide) was administered in two cycles. Regional oxygen saturation (rSO_2) was measured at multiple time points (from 15 minutes before to 48 hours after administration) at three sites: (rSO_2- cerebral right), (rSO_2-cerebral left), and mesenteric (rSO_2-M). Splanchnic-cerebral oxygenation ratios (SCOR-R and SCOR-L) were calculated. Clinical complications were recorded.

Results: Complications occurred in 13% of examinations, including apnea (6.5%) and feeding intolerance (9.8%), predominantly in infants with lower gestational age and primarily after the first examination. Overall temporal changes in NIRS parameters were minimal. However, in infants who developed complications, rSO_2-M and SCOR-R values were significantly lower during the first examination compared to the second (p=0.043 and p=0.044, respectively). rSO_2-M values below 70% and SCOR below 0.75 were noted within the first 6 hours post-medication, followed by recovery within 36-48 hours.

Conclusion: Low-dose mydriatic regimens appear to be safe in preterm infants, with minimal disturbances in cerebral and mesenteric oxygenation. Transient reductions in mesenteric perfusion parameters may help identify those infants at risk of gastrointestinal intolerance, supporting delayed refeeding after ROP screening. These findings support the use of NIRS for monitoring post-mydriatic effects and may contribute to safer ROP screening protocols in premature infants.

Keywords: Retinopathy of prematurity, mydriatics, near-infrared spectroscopy, premature infant, oxygen saturation

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Introduction

Retinopathy of prematurity (ROP) remains one of the leading causes of preventable visual impairment in children (1,2). Initial screening, specifically a retinopathy of prematurity eye examination (ROPEE), must be conducted before discharge from a neonatal intensive care unit (NICU) with pre-examination local analgesia under close monitoring (3). The recommended medications for pupil dilation include 0.5-1% tropicamide or 0.5% cyclopentolate, followed by 2.5% phenylephrine, administered two or three times at five-minute intervals (4-6). Optimal pupil dilation is typically achieved 45-60 minutes after medication.

Mydriatic drops are associated with various systemic side effects including gastrointestinal and neurological disturbances (7-9). Additionally, necrotizing enterocolitis (NEC) may develop due to the systemic effects of mydriatic drops, potentially caused by disrupted intestinal perfusion and tissue hypoxia (10). The risk of systemic adverse effects is heightened with repeated administration due to increased dosage volume (11). A systematic review recently recommended the lowest effective regimen: Phenylephrine 1% combined with cyclopentolate 0.2% (12). However, we further reduced the dosage to 0.625% phenylephrine and 0.25% tropicamide following a previous incident of NEC in one of our patients who was treated at the recommended dosage (13).

Near-infrared spectroscopy (NIRS) is a non-invasive technique which measures regional oxygen saturation (rSO_2) to assess tissue blood flow. Studies suggest that NIRS can detect early-stage intestinal perfusion disorders, which are challenging to identify in the initial stages of NEC (14-18). This study aimed to investigate cerebral and mesenteric perfusion changes in premature infants receiving mydriatic drops for ROP screening using NIRS.

Materials and Methods

This single-center prospective observational study included 30 premature infants followed up in an NICU. Eligible participants had a gestational age (GA) ≤32 weeks and/or a birth weight (BW) ≤1,500 grams. NIRS measurements were performed during routine ROPEE. Demographic data were recorded for all cases. Ethical approval was obtained from the Ege University Faculty of Medicine, Clinical Research Ethics Committee (approval number: 15-11/15, date: 18.12.2015), and written informed consent was provided by the parents or legal guardians.

To minimize the risk of vomiting and aspiration, patients fasted for three hours prior to medication and received intravenous fluid replacement. Mydriatic drops (0.625% phenylephrine and 0.25% tropicamide) were administered to both eyes at one drop per cycle, five minutes apart. The first cycle was termed "Medication-one", and the second cycle, administered 15 minutes later, was termed "Medication-two". The eye examination, using 0.5% proparacaine for local anesthesia, was conducted 45 minutes after "Medication-two".

NIRS measurements were obtained using the Equanox 7600 device (Nonin Medical Inc., MN, USA). Probes were placed on the right and left frontal regions to record cerebral oxygenation [rSO_2-cerebral right (rSO_2-CR) and rSO2- cerebral left (rSO_2-CL)] and on the periumbilical region to measure mesenteric oxygenation [rSO_2-mesentery (rSO_2-M)]. Stabilization was ensured with five minutes of monitoring before data collection. Measurements were taken at predefined intervals: "Premed" (15 minutes before medication), "Med" (between "Medication-one" and "Medication-two"), "Postmed" (15 minutes after "Medication-two"), "Postexam" (15 minutes after the examination), and at the 3rd, 6th, 12th, 24th, 36th, and 48th hours after medication. The timeline of measurements and examinations is shown in Figure 1.

The splanchnic-cerebral oxygenation ratio (SCOR) was calculated as the ratio of mesenteric rSO_2 to cerebral rSO_2 (SCOR-R=rSO_2-M / rSO_2-CR; SCOR-L=rSO_2-M / rSO_2-CL). During NIRS measurements, heart rate (HR), blood pressure, oxygen saturation, and perfusion index were also recorded.

Statistical Analysis

Data analysis was performed using IBM SPSS 22.0 (IBM Corp., Armonk, NY, USA). NIRS measurements, SCOR values, and vital signs were evaluated for temporal changes at each interval. Repeated-measures ANOVA was used to analyze normally distributed variables, including rSO_2-M, SCOR-R, SCOR-L, HR, and blood pressure. Non-parametric variables, such as rSO_2-CR, rSO_2-CL, perfusion index, and oxygen saturation, were analyzed using the Brunner-Langer model (F1-LD-F1 design, R3.1.3, nparLD package). Post-hoc tests included the t-test for parametric data and the Mann-Whitney U test for non-parametric data, with Bonferroni correction applied to p-values from two-way comparisons. The Brunner-Langer model (F1-LD-F1 design) was selected because it accommodates repeated-measures data with non-normal distributions and allows robust analysis even when normality is inconsistent across time points.

Analyses were conducted separately for "ROPEEs with and without complications" and for "first versus repeated

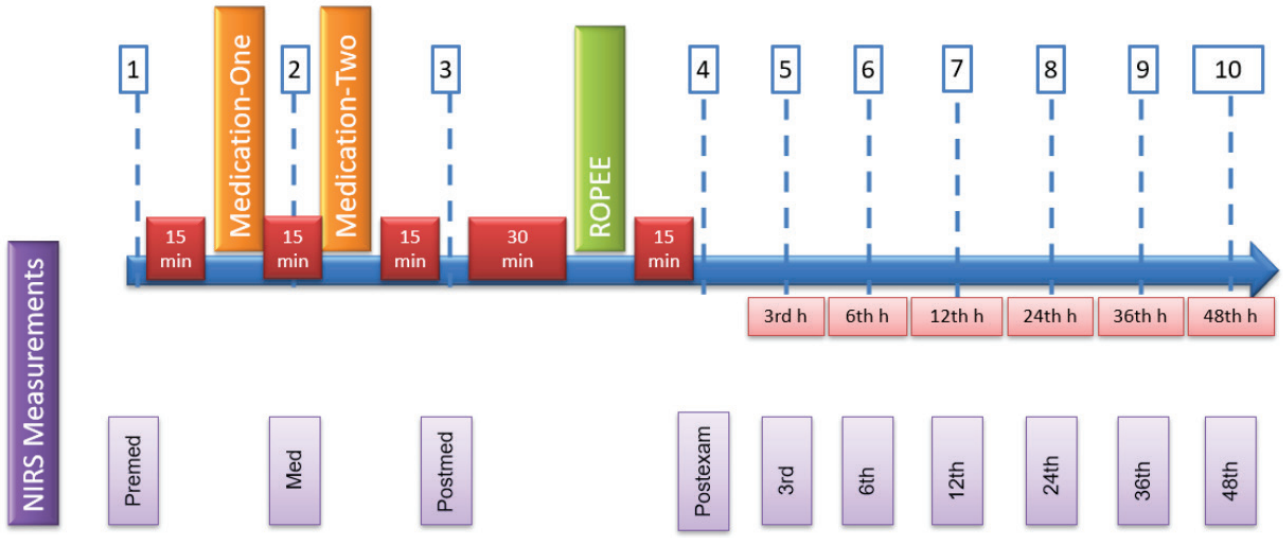

Figure 1. The timeline of NIRS (near-infrared spectroscopy) measurements and examination
ROPEE: Retinopathy of prematurity eye examination

ROPEEs". The Brunner-Langer model was used to assess temporal changes, as normality was not consistent across time points. Interactions between groups and temporal changes were also examined. Similar temporal patterns across groups were indicated by the absence of interaction effects. Finally, BW and GA differences between groups with and without complications were analyzed using Student's t-test. Statistical significance was defined as $p<0.05$.

Results

A total of 610 measurements were analyzed from 61 ROPEEs conducted on 30 premature infants. The median number of ROPEEs per patient was two (range: 1-6). The mean BW of the patients was 1,346.23±496.00 grams, and the mean GA was 29.40±2.52 weeks.

Complications occurred in six patients (20%, 6/30) following eight ROPEEs (13%, 8/61), all within the first 24 hours. Two patients experienced complications in two separate ROPEEs, and one patient had complications only during the second ROPEE. Apnea was observed in 6.5% (4/61) of ROPEEs, while feeding intolerance occurred in 9.8% (6/61). Complications began as early as the 6th hour post-ROPEE. In four of the eight cases, complications resolved within 24 hours, while in the remaining 4 cases, they persisted up to 48 hours. No complications extended beyond 48 hours.

Those infants with complications had a significantly lower GA (27.50±2.16 weeks) and lower BW (1,180.00±366.76 grams) compared to those without complications (GA 29.87±2.41 weeks, BW 1,387.79±521.48 grams). The difference in GA was statistically significant ($p=0.037$), but the difference in BW was not ($p=0.368$). Complications were more frequent during the first ROPEE (18.5%) than during repeated ROPEEs (8.8%), but this difference was not significant ($p=0.447$).

Vital signs, including HR, mean arterial pressure, perfusion index, and oxygen saturation, showed no significant temporal changes across all measurements ($p=0.471$, $p=0.413$, $p=0.095$, and $p=0.135$, respectively).

When assessing temporal changes in NIRS measurements (rSO_2-CR, rSO_2-CL, rSO_2-M, SCOR-R, and SCOR-L), a significant difference was identified only for rSO_2-CL ($p=0.007$) as shown in Figure 2 and Table I. Post-hoc analysis revealed significant differences between "Postmed" and "3rd hour" and between "Postmed" and "6th hour" measurements ($p=0.014$ and $p=0.002$, respectively) (Figure 2b). Median values and temporal change curves indicated an insignificant decline in rSO_2-CL between "Med" and "Postmed" ($p=0.112$).

Comparing patients with and without complications, HR values were significantly higher in the group with complications across all time points ($p=0.013$). However, no

temporal changes in HR were observed (p=0.644), as shown in Figure 3a. Temporal changes in rSO_2-CL were significant overall (p=0.012), with similar patterns in both groups, indicating no interaction (p=0.119). In the complications group, an insignificant decline in rSO_2-CL was observed between "Premed" and "Med" (median values: 76.00 vs. 74.50; p=0.081). Significant improvements in rSO_2-CL were identified when comparing "Med" vs. "12th hour", "Postmed"

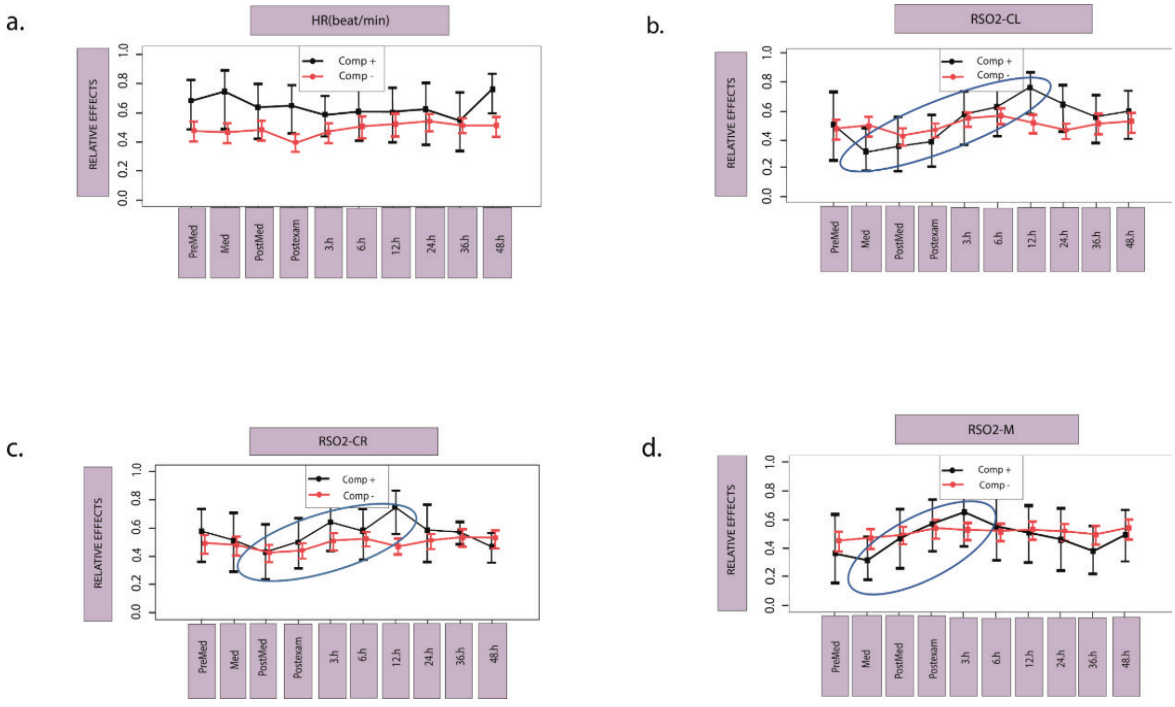

Figure 2. Temporal changes in rSO_2 (regional tissue saturation) values during the 48-hour follow-up: (a) rSO_2-CR (cerebral right), (b) rSO_2-CL (cerebral left), (c) rSO_2-M (mesenteric), (d) SCOR-R (splanchnic-cerebral oxygenation ratio using rSO2-CR), (e) SCOR-L (using rSO_2-CL)

Table I. Median, minimum, and maximum values of rSO_2-CR, rSO_2-CL, and mean value rSO_2-M, SCOR-R, and SCOR-L measurements

	rSO_2-CR	**rSO_2-CL**	**rSO_2-M**	**SCOR-R**	**SCOR-L**
Measurement time	**Median (Min-Max)**	**Median (Min-Max)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Premed	78.00 (24-91)	77.00 (63-93)	63.59 (±2.95)	0.84 (±0.21)	0.83 (±0.17)
Med	76.00 (30-100)	77.00 (52-90)	64.93 (±11.08)	0.85 (±0.18)	0.85 (±0.15)
Postmed	76.00 (28-93)	76.00 (54-90)	65.86 (±10.03)	0.88 (±0.17)	0.88 (±0.14)
Postexam	77.00 (57-86)	77.00 (54-88)	67.83 (±10.56)	0.89 (±0.14)	0.90 (±0.14)
3rd hour	78.00 (55-100)	78.00 (52-100)	67.85 (±11.03)	0.87 (±0.16)	0.87 (±0.15)
6th hour	78.00 (60-92)	79.00 (67-93)	67.33 (±9.89)	0.86 (±0.14)	0.86 (±0.13)
12th hour	78.00 (60-90)	77.00 (66-100)	66.96 (±9.62)	0.86 (±0.13)	0.85 (±0.13)
24th hour	78.00 (59-89)	77.00 (60-89)	66.36 (±9.97)	0.85 (±0.14)	0.87 (±0.14)
36th hour	79.00 (60-97)	77.00 (61-100)	65.70 (±8.93)	0.84 (±0.11)	0.84 (±0.12)
48th hour	78.00 (48-92)	78.00 (62-92)	67.22 (±10.17)	0.87 (±0.16)	0.87 (±0.14)
p-value	0.071	**0.007***	0.197	0.465	0.219

rSO_2-CR: Regional tissue saturation-cerebral right, rSO_2-CL: Regional tissue saturation-cerebral left, rSO_2-M: Regional tissue saturation-mesenteric, SCOR-R: Splanchnic-cerebral oxygenation rate-right, SCOR-L: Splanchnic-cerebral oxygenation rate-left, SD: Standard deviation
*p<0.05

vs. "6th hour", "Postmed" vs. "12th hour", and "Postexam" vs. "12th hour" (p=0.007, p=0.028, p=0.036, and p=0.021, respectively). Temporal change patterns for rSO_2-CR and rSO_2-M followed a similar trend, showing an initial decline followed by improvement, though these changes were not statistically significant (p=0.105 and p=0.240, respectively) (Figures 3c-d).

When comparing the first and second ROPEEs in patients with complications, five of the six patients with complications experienced them during the first ROPEE, while three patients experienced complications during the second ROPEE. Although the difference between first (18.5%) and repeated ROPEEs (8.8%) was not statistically significant, this higher frequency in the initial exams likely reflects lower GA at the time of screening. rSO_2-M, SCOR-R, and SCOR-L showed notable drops after the 6th hour during the first ROPEE, with recovery observed between the 24th and 36th hours as shown in Figure 4. These temporal changes were not statistically significant (p=0.338, p=0.397, and p=0.278, respectively). However, when comparing the first and second ROPEEs in infants with complications, significant between-group differences were observed for rSO_2-M and SCOR-R values (p=0.043 and p=0.044, respectively), as shown in Figure 4. No significant differences or temporal changes were found in rSO_2-CR and rSO_2-CL values between the first and second ROPEEs (p=0.314 and p=0.964 for group differences; p=0.628 and p=0.225 for temporal changes, respectively).

Discussion

This study was the first to evaluate the effects of mydriatic drops on mesenteric and cerebral oxygenation during ROPEE and 48 hours after ROPEE using NIRS and their associations with cerebral and gastrointestinal complications. Our findings suggest that monitoring cerebral and mesenteric oxygenation with NIRS in the early period after mydriatic drop administration may be beneficial in promptly detecting potential complications. Furthermore, the use of low-dose mydriatics, compared to the recommended doses, appears to result in a lower incidence of systemic side effects. These findings highlight the need to explore lower-dose protocols to enhance safety, particularly in preterm infants, and suggest that routine monitoring with NIRS after ROPEE could be incorporated into clinical practice.

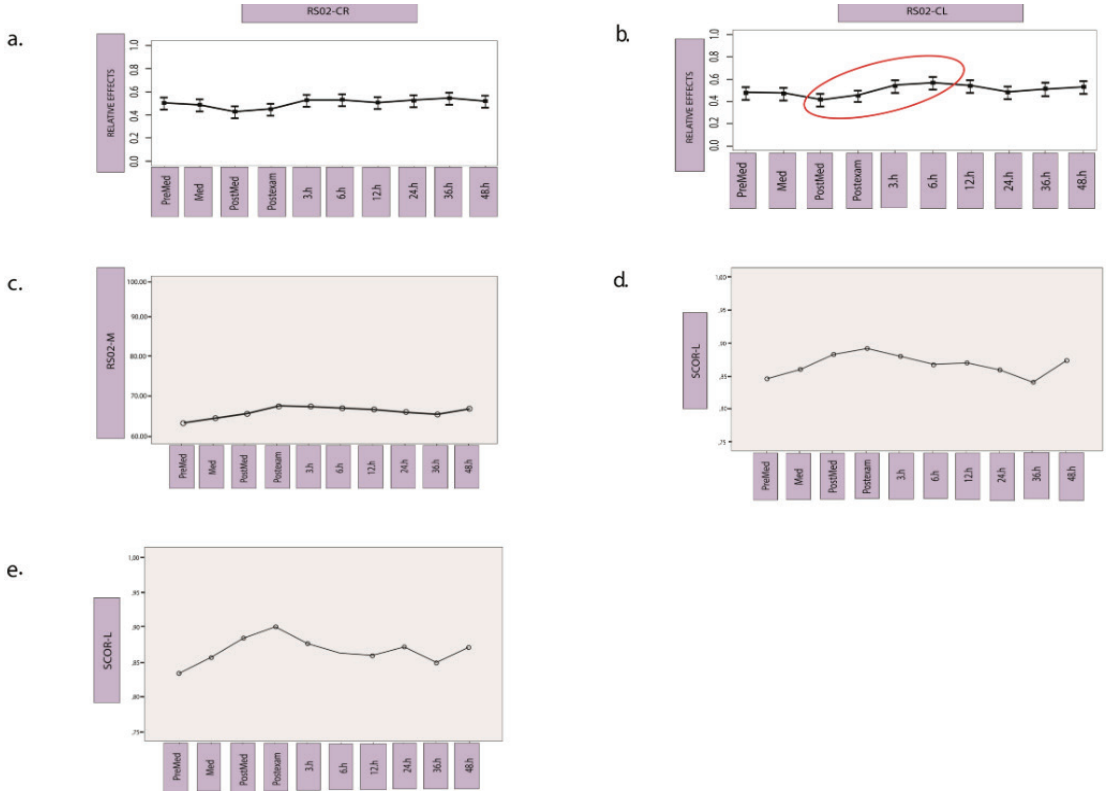

Figure 3. Comparison of patients with and without complications: (a) heart rate (HR), (b) rSO_2-CL, (c) rSO_2-CR, (d) rSO_2-M over time

Previous studies have identified apnea as a common adverse effect following ROPEE (19-21). Among non-mechanically ventilated patients, the incidence of apnea was reported as being 11.7% within the first 24 hours and 23.5% between 24 to 48 hours after ROPEE (22). In contrast, the rates observed in this study were notably lower, at 6.5% (4/61) and 4.9% (3/61), respectively. This reduction in apnea incidence may be attributed to the use of mydriatic drops at lower concentrations in the present study.

Another set of complications identified following ROPEE involves the gastrointestinal system, including temporary paralytic ileus and feeding intolerance (23-25). Hermansen and Sullivan (26) reported abdominal distention in 12% of cases and increased gastric residual volume in 16% after using 2.5% phenylephrine and 0.5% cyclopentolate. In our observations, feeding intolerance was the most frequently noted gastrointestinal adverse effect, occurring in 9.8% of cases. Notably, feeding intolerance is considered a precursor to NEC, one of the most severe complications associated with ROPEE (13,27). However, none of our patients developed NEC.

Siu et al. (28) reported on cases of NEC in two out of three 27-week triplets who had received 2.5% phenylephrine and 0.5% cyclopentolate for pupil dilation. While both initially improved with supportive care, one was seen to have developed severe bradycardia and apnea at a follow-up examination and subsequently died due to NEC. This case raised concerns regarding systemic risks associated due to repeated mydriatic use. In contrast, our study observed more complications during the first ROPEEs, likely due to the lower gestational ages at the time of screening.

A previous report suggested that adverse effects following ROPEE may be mediated via the oculocardiac reflex (29). However, in our study, we considered the complications unlikely to be attributable to the ROPEE itself, as the earliest events occurred six hours after mydriatic administration (i.e., five hours post-examination).

Increases in cerebral blood flow are typically associated with elevated cerebral oxygenation values, whereas disruptions in cerebral perfusion, such as those caused by hypotension or apnea, are reflected by decreases in cerebral rSO_2 values (30-32). In our analysis of 61 ROPEEs, significant temporal changes were observed only in rSO_2-CL values. Specifically, the median rSO_2-CL values showed a non-significant decline between the "Med" and "Postmed" time points, potentially indicating a transient reduction in cerebral perfusion due to medication. This was followed by a significant increase between "Postmed" and both the 3rd and 6th hour measurements. This trend may suggest a compensatory cerebrovascular autoregulatory response. While these changes did not reach pathological thresholds, they likely reflect intact cerebrovascular autoregulation. These compensatory increases highlight the resilience of cerebral perfusion following pharmacologic pupil dilation, and reinforce the importance of interpreting statistically significant changes in the context of their clinical impact.

The association between NEC and mesenteric NIRS measurements indicative of intestinal ischemia has been previously reported on (14,33). A 2014 study demonstrated that mesenteric rSO_2 values in infants with NEC (70.7% ±19.1) were significantly lower than in those without NEC, and that rSO_2 values below 56% were associated with a 14-fold increased risk of developing NEC (34). In contrast, in our study, rSO_2-M values remained stable in both patients with and without complications, which may be attributable to the use of a lower-dose mydriatic regimen.

During hypoxic-ischemic events, blood is redistributed from the mesenteric circulation to the brain. Accordingly, the SCOR, calculated by dividing mesenteric NIRS values by cerebral NIRS values, serves as a useful indicator of mesenteric ischemia (35). Stapleton et al. (33) observed a decrease in both mesenteric NIRS and SCOR values during NEC with recovery-associated increases in a term infant with congenital heart disease. Similarly, Fortune et al. (15) reported a significantly lower median SCOR value of 0.66 in those infants with acute abdominal pathology compared to 0.96 in healthy controls. A SCOR value below 0.75 was considered indicative of intestinal ischemia, while values above 0.96 were regarded as safe. That study also reported a 90% sensitivity for SCOR in detecting intestinal ischemia.

In the temporal changes analysis of NIRS data from the first and second ROPEEs in the six patients who experienced complications, statistically significant differences were observed in rSO_2-M and SCOR-R values. Although SCOR-L followed a similar trend, the difference was not statistically significant. These findings indicate that rSO_2-M, SCOR-R, and SCOR-L values were lower during the first ROPEE. Notably, during the first ROPEE, the decline in rSO_2-M and SCOR values began after the "Postexam" time point and became more pronounced at the 6th hour measurement, with values falling below 70% and 0.75, respectively (Table II and Figure 4). Recovery in these parameters was observed after the 36th hour measurement. Although overall temporal changes in rSO_2-M and SCOR values were minimal across the entire cohort, the transient reductions below 70% and 0.75 within the first 6 hours in those infants who

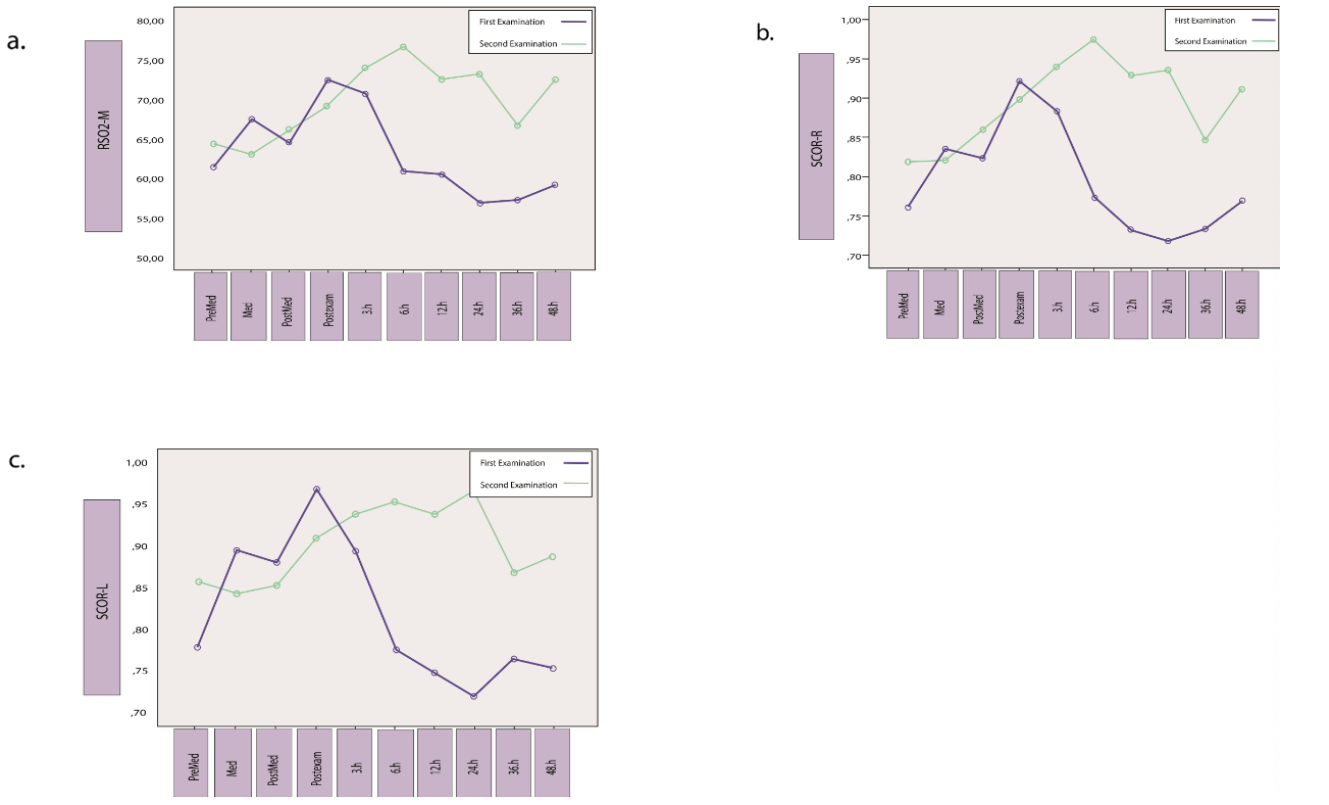

Figure 4. Temporal changes in mesenteric perfusion parameters in the six patients with complications, comparing first and second ROPEEs: (a) rSO_2-M, (b) SCOR-R, (c) SCOR-L
ROPEE: Retinopathy of prematurity eye examination, rSO_2-M: Regional tissue saturation-mesenteric, SCOR-R: Splanchnic-cerebral oxygenation rate-right, SCOR-L: Splanchnic-cerebral oxygenation rate-left

Table II. Mean (SD) values of rSO_2-M, SCOR-R, and SCOR-L from first and second ROPEEs for the six patients with complications

	rSO_2-M Mean (SD)		**SCOR-R Mean (SD)**		**SCOR-L Mean (SD)**	
Measurement time	**First examination**	**Second examination**	**First examination**	**Second examination**	**First examination**	**Second examination**
Premed	61.33 (±10.72)	64.33 (±5.52)	0.76 (±0.12)	0.81 (±0.07)	0.77 (±0.13)	0.85 (±0.07)
Med	67.50 (±5.89)	63.00 (±3.86)	0.83 (±0.05)	0.82 (±0.05)	0.89 (±0.07)	0.84 (±0.04)
Postmed	64.50 (±7.56)	66.16 (±2.40)	0.82 (±0.09)	0.86 (±0.02)	0.88 (±0.09)	0.85 (±0.02)
Postexam	72.50 (±5.09)	69.16 (±3.24)	0.92 (±0.07)	0.89 (±0.04)	0.96 (±0.07)	0.90 (±0.03)
3rd hour	70.66 (±5.17)	74.00 (±3.33)	0.88 (±0.08)	0.94 (±0.05)	0.89 (±0.08)	0.93 (±0.02)
6th hour	60.83 (±2.58)	76.66 (±4.57)	0.77 (±0.05)	0.97 (±0.05)	0.77 (±0.05)	0.95 (±0.05)
12th hour	60.50 (±2.69)	72.50 (±3.41)	0.73 (±0.02)	0.92 (±0.04)	0.74 (±0.03)	0.93 (±0.06)
24th hour	56.83 (±3.42)	73.16 (±3.40)	0.71 (±0.03)	0.93 (±0.05)	0.71 (±0.03)	0.96 (±0.07)
36th hour	57.16 (±3.98)	66.66 (±4.02)	0.73 (±0.04)	0.84 (±0.05)	0.76 (±0.05)	0.86 (±0.06)
48th hour	59.16 (±4.65)	72.50 (±2.14)	0.77 (±0.05)	0.91 (±0.03)	0.75 (±0.05)	0.88 (±0.02)
p-value	**0.043***		**0.044***		0.114	

rSO_2-M: Regional tissue saturation-mesenteric, SCOR-R: Splanchnic-cerebral oxygenation rate-right, SCOR-L: Splanchnic-cerebral oxygenation rate-left, SD: Standard deviation
*p<0.05

developed complications may represent clinically relevant early warning indicators of gastrointestinal compromise. These results support the notion that SCOR values below 0.75 and rSO_2-M values below 70% may guide clinicians to delay enteral feeding after ROP screening, in line with the prior findings by Fortune et al. (15) and Patel et al. (34).

Few studies have investigated the effects of mydriatic eye drops used during ROP screening on neonatal tissue oxygenation using NIRS. In one study involving 26 infants, two measurements were taken before and after mydriatic administration in order to assess early hemodynamic effects, with no significant changes reported in vital signs or NIRS parameters (36). Another study, which included 62 infants, performed four cerebral NIRS measurements at baseline, 15-30 minutes, 30-60 minutes, and beyond, revealing a significant decline in oxygenation during the latter two-time intervals (37). In contrast, our study is the first to extend NIRS monitoring up to 48 hours post-administration, enabling the evaluation of both the immediate and delayed effects of mydriatic drops on cerebral and mesenteric perfusion.

This study's strengths include all NIRS measurements being performed by the same investigator and the consistent execution of all ROPEEs by the same experienced ophthalmologist, which enhanced measurement reliability. Additionally, this study had extended post-examination monitoring for up to 48 hours following mydriatic administration, offering valuable insight into the short-term temporal course of perfusion changes. The single-center nature and the relatively small sample size of this study limit the statistical power and external validity of our findings. A small cohort increased the risk of type II error, meaning that clinically relevant effects may not have reached statistical significance. Furthermore, rare adverse events such as NEC might not have been detected due to the limited sample size. While the observed trends were clinically relevant, multicenter studies with larger cohorts are warranted in order to validate and generalize these results.

Another limitation was the absence of a control group (e.g., infants receiving standard or higher-dose mydriatic regimens). Our observational design precluded definitive causal inference regarding the safety and efficacy of the low-dose protocol compared to standard practice.

Conclusion

Our findings suggest that low-dose mydriatic protocols cause minimal disruption to cerebral and mesenteric oxygenation in preterm infants. However, transient reductions in rSO_2-M and SCOR observed within the first 6 hours post-administration may serve as early indicators of gastrointestinal intolerance. These results support the implementation of extended NIRS monitoring and cautious refeeding strategies after ROP screening, particularly in those infants with lower gestational ages.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ege University Faculty of Medicine, Clinical Research Ethics Committee (approval number: 15-11/15, date: 18.12.2015).

Informed Consent: Written informed consent was provided by the parents or legal guardians of the participants.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: B.E.D., F.E., S.N., N.K., Concept: B.E.D., S.N., Ö.A., D.T., M.Y., M.A., N.K., Design: B.E.D., Ö.A., D.T., M.Y., M.A., N.K., Data Collection or Processing: B.E.D., F.E., Ö.A., D.T., Analysis or Interpretation: B.E.D., F.E., Ö.A., D.T., N.K., Literature Search: B.E.D., F.E., S.N., Ö.A., D.T., M.Y., M.A., N.K., Writing: B.E.D., F.E., S.N., Ö.A., D.T., M.Y., M.A., N.K.

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Original Article

False Positive Peripheral Blood Cultures in Children with Leukaemia: A Descriptive Retrospective Prevalence Study

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ABSTRACT

Aim: False positive blood cultures are one of the critical quality indicators in healthcare services and high rates cause severe quality problems. This study aimed to determine the rate of false positive peripheral blood cultures (FPPBC) and possible associated factors in children with leukaemia.

Materials and Methods: A descriptive observational study was conducted with data from children hospitalised in the haematology oncology clinic of a children's hospital between March 2021 and March 2024. The results of those children who underwent peripheral blood cultures in routine care were collected using the "case report form" and "peripheral blood culture evaluation form-children" by analysing the electronic medical records.

Results: In this study, 1,003 peripheral blood culture results from 100 patients were followed up. Of these, 90 (8.9%) were defined as positive blood cultures. Of these, 27 (2.69%) were FPPBC The most common contaminants were *Staphylococcus epidermidis* (n=31) and *Staphylococcus hominis* (n=6). The highest seasonal contamination rates were observed in winter (29.6%), and during the daytime shift (48.1%).

Conclusion: The false positive peripheral blood culture rate was found to be within the optimal range in this study. It may be effective in reducing the contamination rates when infection controllers and educator nurses make the right interventions and provide training prepared in line with the guidelines. It should be highlighted that false positivity in peripheral blood culture collection is an important health and quality problem, and therefore, awareness-raising and training activities should be continued among nurses performing these collections.

Keywords: Children, false-positive, leukaemia, peripheral blood culture

Introduction

Leukaemias are the most common malignancy of childhood. The main treatment for childhood leukaemia is chemotherapy (1,2). Leukemic children frequently encounter bloodstream infection (BSI) due to the immunosuppressive side effects of chemotherapy treatment (3). BSIs are defined as the primary cause of morbidity and mortality in children with leukaemia. Therefore, early diagnosis of BSI ***via*** blood culture and the initiation of appropriate treatment are of vital importance (4). Blood culture obtained by peripheral venipuncture is essential in diagnosing the causative agent of the infection, especially in immunosuppressed patients.

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It is especially used for the diagnosis of invasive infections characterized by fever and/or other signs of sepsis (5). The growth of a microorganism in one blood culture set (aerobic and anaerobic) may mean that the patient has an infection. It is important to determine the microorganisms present as quickly as possible in order to reveal whether they are causative or contaminant, to perform antibiotic susceptibility tests of the microorganism accepted as causative and to direct the treatment correctly so as to reduce mortality and morbidity (6).

Despite today's advanced technologies, blood culture has remained the gold standard for the detection of BSIs for many years. Failure to follow the necessary techniques during culture collection may result in false-positive blood cultures in some cases. A positive peripheral blood culture a positive peripheral blood culture result may also be seen in the event of contamination with the skin flora of the person performing the blood culture collection (7). False positive peripheral blood cultures (FPPBC) are a single-culture bottle containing microorganisms belonging to the skin flora (8). False positive results can lead to many unfavourable situations (9). FPPBC may cause prolonged hospital stays, additional tests, unnecessary exposure of the patient to antimicrobial agents, the development of antibiotic resistance, increased risk of other infections, and increased hospital costs (10-12).

FPPBC infection rates may vary according to clinical settings. Most studies in the literature are conducted in emergency departments and are not associated with any underlying disease, and FPPBC rates vary between 1% and 10% in different studies (13). However, as recommended by the Clinical Laboratory Standards Institute (CLSI), the rate of blood culture contamination should not exceed 3% (6,14). However, some studies have reported that this rate may be around 10% (15,16). Since approximately 20% of microorganisms can persist despite skin antisepsis, it is not possible to eliminate this rate (17). Many internationally accredited clinical laboratories routinely calculate and report the institution's blood culture contamination rate as a quality assurance indicator. Rates are regularly reported to infection prevention, antibiotic stewardship and nurse/phlebotomy teams in order to target improvements in this pre-analytic quality indicator (18,19).

Purpose of this Study

In this context, this study aimed to determine the prevalence of FPPBC by retrospectively examining the peripheral blood culture results obtained during routine follow-ups from children hospitalized with leukaemia in a pediatric haematology/oncology (PHO) in-patient setting.

Materials and Methods

Study Design, Setting, and Sampling

This descriptive, observational, single-centred retrospective chart study was conducted using data from pediatric patients treated for leukaemia as inpatients in the PHO clinic of a tertiary university hospital between March 2021 and March 2024. The Strengthening the Reporting of Observational Studies in Epidemiology (20) guideline was adhered to throughout this research process. The PHO clinic consists of 17 beds.

Participants

No sample selection was made in this study, and all children who were newly diagnosed within three years were included (n=106). Those who had never had a peripheral blood culture during hospitalisation (n=5) and those who had a disease other than leukaemia (n=1) were excluded from this study. As a result, a total of 100 patients were included in the final analysis.

The inclusion criteria were that the children were between 0-18 years of age, did not have any disease other than leukaemia, received inpatient chemotherapy treatment at the PHO within the three-year period, had a peripheral blood culture test, and the culture results were accessible through the electronic medical records of the hospital. The exclusion criteria included those patients who had never had a peripheral blood culture taken and those diagnosed with a disease other than leukaemia.

Definitions

After analysing the electronic medical records of the hospital, the investigators in the research team decided together with the infection control team whether all peripheral blood culture results reported as positive were true positive (true BSI) or false positive (contamination). According to previous studies in this field (21,22), we defined the criteria for true BSI as follows:

• *Staphylococcus aureus,* gram-negative bacilli or Candida species were considered true positive if isolated from any bottle; skin contaminants [coagulase negative *Staphylococcus* (*Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus haemolyticus*), *Viridans streptococci, Aerococcus, Micrococcus, Propionibacterium* spp., *Bacillus* spp., (*B. Anthracis*), *Corynebacterium* spp., and alpha-gamma haemolytic streptococci, *Dermabacter* spp, *Rothia* spp., and *Kocuria* spp.,] were isolated from at least two bottles collected from different sites and were considered true positive if the

patient had a high body temperature (>38.3 °C), chills or hypotension (systolic blood pressure <90 mmHg) and any of these microorganisms were also present in the patient's port catheter culture (all children in the sample also had a port catheter). The results of those children who had the same microorganism grow in both the peripheral culture and the catheter culture taken at the same time were considered true positives. Skin contaminants were defined according to the Centers for Disease Control and Prevention/National Healthcare Safety Network commensal list (21). When these criteria were insufficient, the assessors reviewed the clinical information to judge the results.

Peripheral Blood Culture Collection Practices at the Study Institution

Preparation: In Ege University Children's Hospital PHO Clinic, the necessary materials are prepared after a physician's order to perform this procedure. The procedure is explained to the child and parents. It was observed that the nurse who performed the culture collection generally washed her hands.

Skin antisepsis: In the institution, skin antisepsis is provided with the available antiseptic agent. This agent can be 70% isopropyl alcohol, 10% povidone-iodine or 2% chlorhexidine gluconate +70% isopropyl alcohol. In the establishment of skin antisepsis, the technique of rubbing back and forth or cleaning from the centre outwards was generally not used, and the area to be cultured was wiped from top to bottom several times. It was observed that not all nurses waited for the appropriate drying time after skin antisepsis. Non-sterile gloves were mostly used during culture collection.

Time and number of cultures taken: In the clinic, peripheral blood cultures are taken, especially when patients show signs of fever and tremor, when signs of sepsis are present and also before antimicrobial therapy is started. If the patient already has ongoing antibiotherapy, the rule that it should be taken immediately before the next dose may be ignored.

Blood volume: Especially in children receiving chemotherapy, intravenous interventions may be more difficult due to a deterioration of vascular structures, the presence of subcutaneous tissue around the vessels and/or problems in communicating with children. Therefore, in some cases, the required blood volume of 3-5 mL may not be reached.

Culture collection technique: In the clinic, blood cultures were taken by clinical nurses. The clinic did not have a special phlebotomy team. In cases where palpation could not be performed properly, it was observed that the person taking the culture touched the skin again after skin antisepsis. The rubber caps of the culture bottles were wiped with 70% isopropyl alcohol before occlusion of the blood samples. No special peripheral culture collection technique e.g., the aseptic non-touch technique) was applied.

Data Collection Procedure

In this study, we assessed all peripheral blood culture results of those children who met the inclusion criteria. The relevant data was obtained by accessing the medical records of the hospital. The protocol numbers of the eligible children were determined, and their relevant records were assessed and recorded in the data collection forms.

Data Collection Forms

Case report form: This form consists of different questions determining the socio-demographic characteristics (sex, age, diagnosis, risk group, relapse status) of the children.

Peripheral blood culture evaluation form-children: This form was developed by the researchers in line with the literature (22,23). It included the dates of the peripheral blood culture of the child, the exact time of the culture, the growth status of the peripheral blood culture, the name of the microorganism which grew, and the contamination status.

Ethics

This study was conducted following the Declaration of Helsinki and approved by the Ege University Medical Research Ethics Board on (approval no.: 24-3T/48, date: 07.03.2024) and study permission was obtained from the hospital where this study was conducted. As the study had a retrospective design and the data were accessed through the hospital's electronic medical records, the requirement for written informed consent was waived. The study protocol was registered at ClinicalTrials.gov: NCT06336837. https://clinicaltrials.gov/study/NCT06336837.

Statistical Analysis

All the statistical analysis was performed using the IBM Statistical Package for Social Sciences program version 25.0. A descriptive analysis was conducted for demographic, clinical characteristics, and microbial organisms. Categorical variables are presented as frequencies and percentages. Mean ± standard deviation was computed for continuous data. Statistical analyses were performed using the proportions of FPPBC with respect to the shifts and

dates when they were carried out using cross tabulations. The chi-square test was used to compare these proportions. The FPPBC rate was calculated by dividing the total number of contaminated blood cultures by the total number of cultures and multiplying by 100 (6). In this study, logistic regression (LR) analysis was also performed in order to evaluate the factors affecting FPPBC.

Results

Characteristics of the Participants

The mean age of the children in this study was 7.65±4.74 years. Most of the children were male (62%) and most of them were diagnosed with acute lymphoblastic leukaemia (ALL) (85%). More than half of the children were receiving chemotherapy in accordance with the high-risk group protocol (55%) and 75% were receiving leukaemia treatment for the first time (Table I).

Characteristics of the Blood Cultures

Of the 1,003 peripheral blood cultures, 90 (8.9%) were defined as positive blood cultures showing microbial growth. Of these, 43 were blood culture contaminations, representing 2.69% of the total blood cultures and 30% of the positive blood culture samples. The most prevalent contaminant species were ***Staphylococcus epidermidis*** (66.7%) and ***Staphylococcus hominis*** (14.8%). The other species which were identified are shown in Table II.

Possible Related Factors

When the distribution of FPPBC was analysed in the results, it was found that FPPBC occurred most frequently during the daytime shift (48.1%). When the seasonal difference was investigated, it was found that FPPBC was most frequently seen in the winter months (29.6%). However, neither difference was statistically significant (p=0.236 and p=0.939, respectivel) (Table III).

The enter method was used to determine the final model factors affecting FPPBC. According to this model, neither risk group nor relapse status had a statistically significant effect on the development of FPPBC. On the other hand, the sex and the diagnosis of the participants were found to be effective on FPPBC. According to the model, the risk of FPPBC increased approximately 3-fold if the child had been diagnosed with ALL and was male (Table IV).

Table I. Descriptive characteristics of the participants

Descriptive characteristics of the children	**n**	**%**
Children's age mean (SD)	7.65 (4.74)	Min=1 Max=18
Children's sex		
Female	38	38
Male	62	62
Children's diagnosis		
Acute lymphoblastic leukaemia	85	85
Acute myeloblastic leukaemia	15	15
Risk group of children		
Standard risk group	26	26
Middle risk group	19	19
High risk group	55	55
Relapse status of children		
Yes	25	25
No	75	75

SD: Standard deviation

Table II. Microorganisms in positive and false-positive cultures

	n	**%**
Number of peripheral blood cultures	1,003	100
PPBC microorganisms		
Klebsiella pneumoniae	13	27.7
Escherichia coli	10	21.3
Enterococcus faecium	7	14.9
Stenotrophomonas maltophilia	6	12.8
Number of positive peripheral blood cultures	90	8.97
Number of false-positive peripheral blood cultures	27	2.69[a]
FPPBC microorganisms		
Staphylococcus epidermidis	18	66.7
Staphylococcus hominis	4	14.8
Streptococcus mitis/oralis	3	11.1
Streptococcus sanguinis	1	3.7
Staphylococcus haemolyticus	1	3.7

[a]: FPPBC rate was calculated by dividing the total number of contaminated blood cultures by the total number of cultures and multiplying by 100
FPPBC: False-positive peripheral blood cultures, PPBC: Positive peripheral blood cultures

Table III. Distribution of false-positive peripheral blood culture rates

	n	**%**	**Chi-square**	**p**
Shift of FPPBC collection				
08.00-15.59 (Day-shift)	13	48.1		
16.00-23.59 (Evening-shift)	8	26.9	2.889	0.236
00.00-07.59 (Night-shift)	6	22.2		
Date of FPPBC collection				
Winter	8	29.6		
Spring	6	22.2	0.407	0.939
Summer	6	22.2		
Autumn	7	25.9		

Chi-square test was used p<0.05
FPPBC: False-positive peripheral blood cultures

Table IV. Evaluation of factors affecting false-positive peripheral blood cultures

Regression coefficients								
	B	**SE**	**Wald**	**Df**	**p**	**Exp(β)**	**90% CI for Exp (β)**	
							Lower	**Upper**
Constant	1.682	0.970	3.005	1	0.083	5.375	-	-
Sex								
Male[a]								
Female	1.171	0.653	3.215	1	0.073[b]	0.310	0.106	0.908
Diagnosis								
AML[a]								
ALL	1.118	0.571	3.837	1	0.050[b]	3.057	1.196	7.815
Risk group								
SRG[a]			2.689	2	0.261			
HRG	0.338	0.920	0.135	1	0.713	0.713	0.157	3.241
MRG	1.231	0.991	1.544	1	0.214	0.292	0.057	1.490
Relapse status								
Yes[a]								
No	0.403	0.773	0.272	1	0.602	0.668	0.187	2.383

Logistic Regression was used
[a]Reference, [b]p<0.10
ALL: Acute lymphoblastic leukaemia, AML: Acute myeloblastic leukaemia, HRG: High-risk group, MRG: Middle-risk group, SRG: Standard-risk group, SE: Standard error, Df: Degrees of freedom, CI: Confidence interval

Discussion

In this study, peripheral blood culture results obtained from 100 different children with leukaemia over a three-year period were retrospectively analysed. In our study, 62% of the participants were male and 85% were diagnosed with ALL. Out of the 1,003 peripheral blood cultures analysed in this study, 90 showed growth, and 27 of these were FPPBC (2.69%). ***Staphylococcus epidermidis*** (72.1%) and ***Staphylococcus hominis*** (14%) were the most commonly cultured microorganisms. This study found that FPPBCs were most commonly observed in cultures taken during the day shift during the winter months.

Although medical technologies are improving day by day, blood culture remains the most reliable method for the diagnosis for BSI. Even in such a reliable test, errors made during culture collection may result in inaccurate results (24). False results may have negative consequences on both the patient and the health care service. Therefore, the management of FPPBC, which is accepted as one of the quality indicators in health care in many developed countries, is a clinically important issue. This study aimed to retrospectively examine the results of peripheral blood cultures obtained from children hospitalised with leukaemia in the PHO clinic of a tertiary university hospital.

The fact that the majority of the participants of our study were male (62%) and their mean age was 7.65 (4.74) years was consistent with the literature. According to the American National Cancer Institute SEER data, the incidence of acute leukaemia in boys is 5.4/100.000, while it is 4.3/100.000 in girls and the median age is 6 years (2).

In this study, 1,003 peripheral blood culture results obtained between March 2021 and March 2024 were analysed through the electronic medical records of the hospital. Of the 1,003 peripheral blood cultures, 90 were found to have growth. True positive and false positive culture results were determined in accordance with the criteria specified in the definitions section of this study. FPPBC growth was detected in 27 of all cultures and this rate was calculated as being 2.69%, which is below the threshold rate as proposed by CLSI (<3%) (14). In a study conducted by Gorfinkel et al. (4) and colleagues with pediatric cancer patients, the FPPBC rate was reported to be 3.7%. Similarly, in the prevalence study conducted by Mullan et al. (25) in a pediatric emergency department, the rate of FPPBC was reported as being 3.17%. Likewise, the rate of FPPBC was reported to be 4.17% in the study by Aiesh et al. (26) in which the results of peripheral blood cultures obtained from all patients in a tertiary healthcare institution were analysed.

Staphylococcus epidermidis (72.1%) and ***Staphylococcus hominis*** (14%) were the most commonly cultured microorganisms. Similarly, ***Staphylococcus epidermidis*** (49.2%) was the most commonly grown microorganism in the study by Aiesh et al. (26). The reason for the

isolation of ***Staphylococcus epidermidis*** in many studies is thought to be that this microorganism is the most common staphylococcal species on the skin (27). In recent years, the widespread use of medical devices and the excessive, incorrect or prolonged use of antibiotics and the ability of ***Staphylococcus epidermidis*** to adhere to smooth surfaces of different structures have caused this microorganism to emerge as an important nosocomial pathogen (28).

Also, in this current trial, FPPBC was most frequently seen in the peripheral blood cultures taken during the winter months (29.6%) and during the day shift (48.1%). Similarly, de Ponfilly et al. (29) indicated that the season in which peripheral blood cultures were most frequently obtained was winter. Although not statistically significant, it is thought that this situation is related to the fact that the clinical workload is more intense, especially in the winter months, and also that nurses work more heavily on the day shift. It is also thought that factors such as environmental conditions, mental workload, distraction and excessive stress, which are more intense during the day shift, may have led to an increase in implementation errors.

The method of obtaining the blood culture, the quality of the antiseptic solution, the gloves used in the culture collection, needle changes before inoculation, the training and experience of the health professional who perform the collection, the clinic's patient density, having nurses working under appropriate conditions, and regular notifications of peripheral blood culture contamination rates may support the clinic in keeping contamination levels low. For instance, in a study by He (30), the contamination rate was 4.96% (144/2903) in blood cultures collected by newly graduated nurses and 3.52% in blood cultures collected by senior nurses with more than five years of work experience. In our study, since the data were collected ***via*** an electronic medical record, it did not include data on the characteristics of the nurse who performed the blood culture collection. However, the fact that the majority of clinical nurses had more than five years of experience and that these clinical nurses were permanent were thought to be factors which reduced the rate of FPPBC.

In this study, factors which may affect the development of FPPBC were also evaluated by LR analysis. In the demographic data of our study, FPPBC was most common in the high risk group, with a rate of 55%. However, in LR analysis, it was found that the group most affected by the development of FPPBC was the standard risk group. This is contrary to the logic that the child with the highest neutropenia is exposed to more contamination. However, since FPPBC is one of the most important quality indicators and is basically a nursing care fault, a statistically significant difference between the false positive culture result and the diagnosis, risk group, and relapse status of the participants is not expected. LR analysis confirmed this expectation and showed that FPPBC was independent of risk group and relapse status.

In this study, the process steps of the nurses performing peripheral blood culture collection were also observed. Some problems were detected in these observations. The first of these was that not all nurses performed hand hygiene before culture collection. Other factors were the lack of proper technique in the application of the skin antiseptic and not waiting for the antiseptic to dry due to workload. Due to the difficulty of peripheral intervention in pediatric patients, it was also observed that the culture was taken with an insufficient amount of blood in some cases. Palpation after antisepsis and the lack of appropriate peripheral culture collection techniques were other root causes.

Study Limitations

This study addressed a critical issue by focusing on FPPBC and contamination obtained from a vulnerable population of leukemic children receiving chemotherapy in a PHO during routine care. The retrospective nature of this study allowed researchers to examine this subject as it occured in routine clinical care. Such patterned studies usually provide large study populations and longer observation periods, allowing for the examination of specific populations. In this study, researchers collected data over 3 years and examined over a thousand culture results. The findings provide information on a subject that is relatively less covered in national and international literature, especially with regards to revealing the frequency of FPPBC in pediatric patients with neutropenia. On the whole, this study can raise awareness of the false-positive blood culture rate, which is one of the most important health care indicators, and by extension, nursing care techniques.

While our study has the potential to be the first study to define and measure the rate of FPPBC in pediatric leukaemia patients in our country, it had some limitations, such as being a descriptive and single-centre study. The retrospective design of this study, the lack of data such as demographic data (education level, work experience) of the nurses who performed the culture collections which resulted in false positives, and the appropriateness of the techniques/materials used in the culture collection, etc. are the other limitations of this study. Further comparative studies with larger samples including multiple centres are recommended.

In addition to the results provided by this study, further retrospective and prospective studies are needed in order to study the rates of FPPBC and related factors in pediatric patients, especially in neutropenic and vulnerable populations. Performing larger, multi-centre studies will greatly improve the generalisability of these results, enable a more varied patient population to be included, and capture differences in care practices between different children's hospitals. In addition, the training of nurses who perform this collection process, regardless of whether it is peripheral or catheter culture, and the effect of other related factors on reducing contamination rates should also be investigated. Understanding how the knowledge, skills, and experience of nurses or other factors in the environment they work in (work shift, patient density, workload, etc.) affect contamination rates can lead to improved patient outcomes and higher healthcare quality. Finally, it is extremely important to use and validate a risk assessment tool specifically designed for pediatric populations. In particular, with the integration of policies and government organizations, the quality of healthcare service can be increased by taking into account the research recommendations listed above.

Conclusion

In this study, the most frequently observed microorganisms in the PHO clinic were found to be *Staphylococcus epidermidis* and *Staphylococcus hominis*. In addition, FPPBC was found to be higher during the winter months and on day shifts. In the analysis of the factors affecting the development of FPPBC, it was found that factors other than diagnosis and gender did not create a statistically significant difference.

The FPPBC rate was found to be lower than the maximum level recommended by CLSI. Although the FPPBC rate was within acceptable limits, appropriate and routine peripheral blood culture collection methods should be integrated into all patient care environments in order to prevent contamination and further reduce this rate.

In addition, within the scope of the education role, which is one of the most important professional roles of nurses, the awareness, knowledge and skill levels of nurses should be enhanced by using educational materials prepared in line with current guidelines and appropriate techniques. The main purpose of these trainings should be to improve the quality of nursing care so as to reduce or prevent contamination.

Ethics

Ethics Committee Approval: The study was conducted following the Declaration of Helsinki and approved by the ethics committee of Ege University Medical Research Ethics Board on (approval no.: 24-3T/48, date: 07.03.2024) and study permission was obtained from the hospital where the study was conducted.

Informed Consent: As the study had a retrospective design and data were accessed through the hospital's electronic medical records, the requirement for written informed consent was waived.

Footnotes

Authorship Contributions

Concept: S.A.S, F.Y., Design: S.A.S, F.Y., D.Y.K., Data Collection or Processing: S.A.S., N.K., Analysis or Interpretation: S.A.S., N.K., Literature Search: S.A.S., Writing: S.A.S, F.Y., D.Y.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

Determination of Biotinidase Enzyme Levels in Umbilical Cord Blood and Comparisons with Dried Blood Spot Testing in Newborns

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ABSTRACT

Aim: Biotinidase deficiency (BD) is an autosomal recessive inherited metabolic disorder caused by enzymatic deficiency. Untreated patients may develop neurologic symptoms, hearing loss, optic atrophy, skin eruptions and/or alopecia. Although cord blood has been explored as a possible sampling type for various newborn screening tests, there is limited data on its use to measure biotinidase activity. In particular, there are no standard reference ranges or established cut-off values for BD detection in cord blood. In this prospective cohort study, we aimed to determine biotinidase activity levels in cord blood samples from newborns and to investigate their comparability with dried blood spot (DBS) measurements.

Materials and Methods: This prospective cohort study was conducted between October 2020 and December 2021. Biotinidase activity was measured in umbilical cord blood samples at birth and in DBS samples collected as part of the national screening program. In addition, venous blood samples were taken from 20 newborns who agreed to have their biotinidase activity remeasured from venous blood after six months.

Results: This study included 97 newborns, 53 girls (54.6%) and 44 boys (45.4%). Measurements of biotinidase activity in umbilical cord blood revealed an enzymatic deficiency in twenty patients (20%). These 20 patients had normal biotinidase enzyme activities according to their DBS samples. None of the 97 patients had a biotinidase enzyme activity of less than 65 U in their DBS samples. The sensitivity of the cord blood measurement, which was defined as the reference standard compared to the DBS test results, was calculated to be 79.38%.

Conclusion: The measurement of biotinidase activity in umbilical cord blood may not be suitable for routine screening due to its high rate of false-positive results and uncertain specificity.

Keywords: Biotinidase deficiency, newborn screening, cord blood

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Introduction

Biotinidase deficiency (BD) is an inherited metabolic disease caused by enzymatic deficiency in releasing and recycling endogenous biotin. Biotin is an essential water-soluble vitamin and a cofactor of carboxylase enzymes. In this context, progressive biotin depletion in BD leads to a deterioration in amino acid catabolism, fatty acid synthesis and gluconeogenesis as a consequence of the effects on carboxylase reactions in which biotin acts as a cofactor (1,2). Patients with a residual enzyme activity of less than 10% are classified as "severe BD" and patients with an activity between 10-30% as "partial BD" (2). The clinical severity of the disease depends mainly on the amount of free biotin in the diet and the residual activity of the enzyme. Untreated patients may develop refractory seizures, hypotonia, lethargy, ataxia, developmental delay, hearing loss, optic atrophy, skin eruptions and/or alopecia. Although the clinical phenotype of severe BD is often characterized by neurologic and cutaneous symptoms which can progress to coma and even death, other manifestations can also be observed. Myopathy, peripheral neuropathy and symptoms mimicking neuromyelitis optica can occur in older individuals with partial deficiency (3).

The incidence of BD is reported in the literature to be 1:40,000-60,000 live births. It is known that the incidence of this disease varies according to region and can increase up to 1:9,000 (4). Recent studies have found a higher incidence in some countries such as Brazil and Italy, which are similar to Türkiye (5-7). The incidence of BD in Türkiye, which has been investigated since 2008 as part of the "National Newborn Screening Program" in our country, is reported in the literature as being 1:7,116 (8-10).

There are various methods for measuring biotinidase activity, such as the fluorometric method, the spectrophotometric method and measurement by high-performance liquid chromatography (8-11). However, the most commonly used method is the colorimetric (spectrophotometric) measurement of enzyme activity in plasma or serum samples (12,13). After the development of a colorimetric method for the determination of biotinidase activity in dried blood spots (DBS'), BD has been included in the screening program of many countries around the world (2,14) and BD patients are diagnosed at an early stage and life without sequelae has become possible (15).

Newborn screening using umbilical cord blood sampling for various diseases has been discussed in the literature as a possible option, but it is not without controversy (16-18). There is a lack of information on the utility of measuring biotinidase activity in cord blood samples. Furthermore, there are currently no established reference ranges or cut-off values for biotinidase activity in cord blood. In this preliminary study, we aimed to present the biotinidase activity levels in cord blood samples from newborns and to investigate their comparability with DBS measurements.

Materials and Methods

Study Design and Participants

This prospective study was conducted with babies born between October 2020 and December 2021 in three tertiary hospitals in İstanbul.

The inclusion criteria were: those babies whose mothers were monitored regularly throughout their pregnancy, those whose mothers did not have any chronic/pregnancy-related diseases and/or drug treatments, and those whose birth history did not include perinatal asphyxia or difficult labor. Additionally, the babies included had to have reached at least 37 weeks gestation.

The exclusion criteria were: those babies who were born before 37 weeks gestation, those who were small for their gestational age, and those who had intrauterine growth retardation, or were treated in a neonatal intensive care unit.

Those babies who met the inclusion criteria and whose families had given their consent were enrolled into this study. Ethical approval was obtained from the İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee (approval number: E-83045809-604.01.02-3183, date: 05.01.2021).

Sample Collection

Two milliliters of umbilical cord blood were collected after clamping the umbilical cord immediately after birth. The collected cord blood was centrifuged at 3,000 rpm for 5 minutes and then the serum was separated. It was stored at -80 °C. The following demographic and clinical data were collected from those patients from whom samples were taken as part of national newborn screening prior to discharge: the identity of the baby and mother, contact details, maternal age, maternal medical history, birth method, gestational age and birth weight, and time of DBS sampling.

Preparation of the Samples

The collected samples were transferred via a cold chain to the pediatric metabolism research laboratory. After transfer, biotinidase activity was measured immediately after thawing

the samples using a colorimetric spectrophotometric method. Those patients who experienced pre-analytical/analytical problems during the study period, such as inappropriate samples, incorrect transfers and/or missing data, were excluded from this study.

Spectrophotometric Enzyme Measurement

This study was carried out using the Secomam S.750 spectrophotometer. Biotinidase enzyme activity was measured spectrophotometrically using the method of Wolf et al. (19). In this method, the enzyme activity of biotinidase was determined by measuring the hydrolysis of the substrate N-biotinyl-p-aminobenzoate (20). Buffer A pH 6.0 solution was prepared with a total volume of 1 mL [0.0067 mol potassium dihydrogen phosphate, 0.0067 mol potassium phosphate, 0.0003 mol ethylenediaminotetraacetic acid (EDTA), 26.3 mg serum albumin, 1.64 µmol biotinidase substrate (N-biotinyl-p-aminobenzoate)]. Buffer B solution was prepared to contain a pH 6.0 mixture (0.0067 mol potassium dihydrogen phosphate, 0.0067 mol potassium phosphate, 0.0003 mol EDTA, 26.3 mg serum albumin) so that the total volume was 1 mL. To assay biotinidase enzyme activity, the samples were prepared as described in Table I and incubated at 37 °C for 30 minutes. Then, 100 µL of 30% trichloroacetic acid was added to each tube and the reaction was stopped. Centrifugation was carried out at 10,000 rpm for 5 minutes. The supernatant was removed and put into separate tubes. During the reaction, p-aminobenzoic acid (PABA), released by the activity of the biotinidase enzyme in the patient's serum, was diazotized with 100 µL of fresh sodium nitrite. The tubes were vortexed and left for 3 minutes. Following this, 100 µL of ammonium sulfate was added to remove excess nitrite. The tubes were vortexed and allowed to stand for 3 minutes. Finally, diazotized PABA was reacted with 100 µL of N-1-naphthyl ethylenediamine dihydrochloride to form a colored product. The resulting product was measured spectrophotometrically at a wavelength of 546 nm. Net absorbance was measured in relation to the amount of PABA released and thus the biotinidase enzyme activity in the sample was determined. Enzyme activity was expressed as µmol/min of PABA released into the serum (19). In accordance with the literature, BD is typically defined as enzyme activity below 30% of the normal reference range. Cut-off values should be set individually by each laboratory, taking into account their own reference values for enzyme activity (2). Based on our reference range, a value below 3.5 IU/L corresponds to an activity of less than 30% and was therefore considered as an indicator of BD in this study.

The national newborn screening program standards were used to define BD in the DBS samples. Accordingly, it was recommended that those babies with an enzyme activity of less than 65 U, as measured by a fluorometric method, be screened for BD (21).

Evaluation of the Measurements of the Biotinidase Activity of Umbilical Cord Blood in Comparison to DBS

After the cord blood sample measurements were completed, the results were compared with the DBS results obtained from the national newborn screening in the same participant. After this procedure, all patients in this study were called back for further sampling and the measurements were repeated in new peripheral blood samples. Peripheral blood samples were collected from those participants who agreed to provide new samples within six months of birth. As there may be a temporary decrease in enzyme activity in early infancy, repeated measurements are recommended for a more accurate diagnosis (22).

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences Version 25.0 (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as arithmetic means ± standard deviations; categorical variables are presented as frequencies or percentages. The normal distribution of the data was assessed using the Kolmogorov-Smirnov test. The repeated measures analysis of variance test was used to determine the relationship between measurements. Normally distributed categorical variables were analyzed using the simple Paired Samples t-test. A p value ≤0.05 was set to determine statistical significance.

Table I. Measurement of biotinidase activity in serum samples using the spectrophotometric method

Tubes	Buffer A solution	Buffer A solution	PABA (standard)	Serum	Distilled water
Standard blind	-	950 µL	-		50 µL
Standard	950 µL	-	50 µL		-
Sample	950 µL	-	-	50 µL	-

PABA: P-aminobenzoic acid

Results

This study included 97 newborns, 53 girls (54.6%) and 44 boys (45.4%). The initial testing of the cord blood samples from all participants was performed at birth. DBS analyses were performed at different time points: in the first 24 hours of life in 77 (79.3%) and between 24-48 hours of life in 9 (19.5%) babies, in accordance with the national newborn screening protocol. All participants were called back to the hospitals six months after birth and invited to provide a new peripheral blood sample for the measurement of biotinidase enzyme activity. New peripheral blood samples were analyzed from 21 patients who accepted the invitation.

Comparison of Umbilical Cord Blood and DBS with Regard to the Measurement of Biotinidase Activity

The measurement of biotinidase activity in umbilical cord blood revealed an enzymatic deficiency in twenty patients (20%). These 20 patients had normal biotinidase enzyme activities according to their DBS samples. None of the 97 patients had a biotinidase enzyme activity of less than 65 U in their DBS samples. Based on these results, the sensitivity rate of cord blood measurement was estimated to be 79.38%. Due to the insufficient number of samples, the specificity rate could not be determined and was assumed to be uncertain. The accuracy rate of cord blood sampling was also determined to be 79.38%; however, the false positive rate of the test was 20.6%.

Based on the DBS results, which are considered the reference standard, the diagnostic performance of biotinidase activity in cord blood was further evaluated. Since no true positive cases were detected among the 97 participants, the positive predictive value was calculated as 0% (0/19). The negative predictive value was 99% (77/78), indicating a high rate of correctly identified unaffected individuals. The overall diagnostic accuracy, defined as the proportion of true results (true positive and true negative) out of the total number of cases, was 79% (77/97).

Comparison of Umbilical Cord Blood and Peripheral Blood when Measuring Biotinidase Activity Six Months Apart

Biotinidase activity was measured in the peripheral blood samples of 21 patients (21%) at six months of age. Three patients (14%) had BD when their cord blood was measured. However, all 21 patients had normal biotinidase activity on the second measurement in their peripheral blood samples. A statistically significant difference between the two measurements was found in all individuals ($p<0.001$) (Table II).

Discussion

In this study, we aimed to evaluate biotinidase activity in umbilical cord blood samples from newborns and to compare it with DBS measurements. We found that the sensitivity of the cord blood samples was 79.38%, but the specificity could not be determined due to the limited number of samples. The false positive rate of the test was 20.6%. The overall diagnostic accuracy was 79% (77/97). Based on these preliminary results of this study, the measurement of biotinidase activity in cord blood may not be suitable for routine screening due to its high rate of false-positive results, uncertain specificity and limited detection of true positives.

Several factors can influence the measurement of biotinidase enzyme activity and these must be carefully considered when interpreting results. Pre-analytical variables, such as sample collection and storage conditions, are particularly critical. Blood spots from newborn screening should be thoroughly dried before delivery to the laboratory in order to prevent the loss of enzyme activity due to moisture. For serum or plasma samples, freezing is essential in order to preserve enzyme activity. However, storage at -20 °C can lead to a decrease in enzyme activity over time. For quality control, long-term storage should therefore be at -80 °C (23). Apart from pre-analytical conditions, reduced enzyme activity in the first weeks of life should be taken into consideration, especially in preterm infants and those individuals with liver disease (24,25). In our

Table II. Comparison of umbilical cord blood and peripheral blood biotinidase activity measurements performed 6 months apart

	Mean ± SD	Median (min.-max.)	p value
Biotinidase activity in umbilical cord blood at birth*	4.67±1.57	4.70 (0.60-10.10)	**<0.001**
Biotinidase activity in peripheral blood at 6 months of age**	7.45±1.10	7.50 (5.70-9.50)	

*n: 97 patients **n: 21 patients
SD: Standard deviation, min.-max.: Minimum-maximum

study, prematurity was an exclusion criterion. Additionally, there was no evidence of liver disease in any of the babies. The storage procedures and transfer periods followed the guidelines appropriately. For these reasons, the results of the analyses can be considered to be reliable.

The use of umbilical cord blood for newborn screening for inherited metabolic diseases has been reported in a large-scale study involving 24,983 newborns. In that study, cord blood testing was limited to the analysis of acylcarnitine and amino acids and its effectiveness in detecting inherited metabolic diseases was found to be limited (26). To our knowledge, only one study has reported the measurement of biotinidase activity in cord blood samples. In 2022, in addition to congenital hypothyroidism, cystic fibrosis and glucose-6-phosphate dehydrogenase deficiency, BD was assessed in the cord blood samples from 26 newborns. None of the babies were found to have profound BD. As that study was performed on a very limited number of patients, the enzyme measurement was not repeated and the cord blood sample results were not compared with other samples, which was considered a limitation by its authors, Singh et al. (16). In contrast, our prospective study included a larger sample size and it directly compared the biotinidase activity measured in cord blood with DBS and peripheral venous samples, allowing for a more comprehensive assessment of diagnostic performance. Therefore, this study provides the first comparative data from Türkiye on biotinidase activity in cord blood and it can serve as a reference for future studies aiming to establish standardized cut-off values and screening protocols.

Various recommendations and examples of specific target values for the sensitivity and specificity of screening tests can be found in the literature. Although there are no defined target values, it is generally expected that screening tests should have a high sensitivity and specificity (27,28). In a study of Turkish patients in whom BD was confirmed by *BTD* gene analysis, spectrophotometric measurements of enzyme activity in serum samples showed a diagnostic sensitivity and specificity of 93.1% and 95.1%, respectively (29). In addition to these findings, Göksoy (30) evaluated the results of newborn screening for BD in 211 infants from southeastern Türkiye in a recent large-scale study. Their study reported that 48.3% of patients were ultimately diagnosed with BD, while 51.7% were classified as false-positive cases. Notably, molecular analysis confirmed BD in 26.8% of those patients who had normal quantitative enzyme activity, emphasizing the limitations of enzymatic testing alone and the additional value of genetic confirmation. These results highlight important diagnostic challenges, including increased false-positive rates and genetic diversity in different regions, and they support the need for region-specific screening strategies. Although our study is preliminary, it provides an initial insight into the use of cord blood for measuring biotinidase activity and it may contribute to future studies aimed at refining and optimizing BD screening approaches.

Study Limitations

Our study had some limitations. The most important limitation was the small cohort of the study. Another important limitation was the fact that not all individuals underwent follow-up at six months, which significantly limited the power of this study, especially for comparisons between the subgroups.

Conclusion

In conclusion, the measurement of biotinidase activity in umbilical cord blood may not be suitable for routine screening due to its high rate of false-positive results and uncertain specificity. Further studies with larger sample sizes are needed in order to determine the normal reference ranges of biotinidase activity in cord blood samples for future screening strategies.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee (approval number: E-83045809-604.01.02-3183, date: 05.01.2021).

Informed Consent: Those babies who met the inclusion criteria and whose families had given their consent were enrolled into this study.

Authorship Contributions

Concept: G.U., T.Z., B.T., E.K., A.Ç.A.Z., Design: G.U., T.Z., E.İ., M.Ş.C., B.T., E.K., A.Ç.A.Z., Data Collection and/or Processing: G.U., E.İ., M.Ş.C., E.D., A.U.Z., G.K., Analysis and/or Interpretation: G.U., E.İ., M.Ş.C., E.D., A.U.Z., G.K., Literature Search: G.U., T.Z., A.Ç.A.Z., Writing: G.U., T.Z., B.T., E.K., A.Ç.A.Z.

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Case Report

Multiple Giant Coronary and Multiple Systemic Arterial Aneurysms in an Infant with Kawasaki Disease

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ABSTRACT

We present the case of a 4-month-old infant diagnosed with Kawasaki disease (KD), who developed giant coronary artery aneurysms and multiple systemic artery aneurysms due to delayed diagnosis and treatment. Early recognition and timely management of KD are crucial in order to prevent life-threatening complications, particularly in young infants with atypical presentations.

Keywords: Systemic aneurysm, children, Kawasaki disease

Introduction

Kawasaki disease (KD) is one of the most common vasculitis in childhood and is typically self-limiting. However, delayed or missed diagnoses may result in severe cardiovascular complications, including coronary artery aneurysm, myocardial infarction, heart failure, and, rarely, systemic arterial aneurysms (1). In untreated patients, the incidence of coronary artery aneurysms is approximately 15% to 25%, but timely administration of intravenous immunoglobulin (IVIG) markedly reduces this risk. These aneurysms are most frequently observed in children under 12 months of age and may develop even in the absence of the classical clinical features of KD, particularly in infants (2). Although systemic arterial aneurysms are rare, they are often associated with giant coronary aneurysms and younger age at diagnosis (3). Early diagnosis in infants is especially challenging due to incomplete or atypical clinical presentations. Here, we report a rare case of KD in a 4-month-old infant who developed multiple giant coronary and systemic arterial aneurysms as a result of a delayed diagnosis.

Case Report

A four-month-old female infant was admitted to our hospital with persistent fever. One month prior, she had been hospitalized at another facility with a two-day history of fever. Laboratory tests at that time revealed

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pyuria, leukocytosis, and elevated C-reactive protein (CRP) levels. She was diagnosed with a urinary tract infection and started on third-generation cephalosporin therapy. During treatment, she developed a rash and conjunctival injection, which were interpreted as a drug reaction. As the fever persisted, repeated laboratory evaluations showed leukocytosis, normocytic normochromic anemia, thrombocytosis, elevated CRP levels, and hypoalbuminemia. An echocardiogram was performed with a preliminary diagnosis of KD; however, it revealed only a patent foramen ovale and minimal pericardial effusion, with normal coronary artery findings. Based on these results, KD was considered unlikely, and treatment was changed to carbapenem and glycopeptide antibiotics. The patient underwent evaluation for other causes of prolonged fever. A peripheral blood smear was normal, and no growth was observed in her urine, blood, or cerebrospinal fluid cultures. Brucella agglutination and *Mycoplasma pneumoniae* enzyme-linked immunosorbent assay tests were negative. Radiologic investigations, including chest X-ray, abdominal ultrasonography, and cranial magnetic resonance imaging, were unremarkable. Despite extensive evaluation over a total illness duration of 28 days, the etiology of the fever remained unclear. The patient was subsequently referred to our hospital for further assessment.

The patient's personal and family history were unremarkable. She was the only child of healthy, non-consanguineous parents. On physical examination, weight was 5.9 kg (25th percentile), height 62 cm (25th percentile), heart rate 155 beats per minute, respiratory rate 42 breaths per minute, blood pressure 90/47 mmHg, and body temperature 36.4 °C. A grade 3/6 systolic murmur was auscultated at the cardiac apex, accompanied by approximately 2×2 cm palpable masses with thrills in both axillary regions. Peripheral pulses were palpable, and circulation was normal. No findings suggestive of connective tissue disease were identified on examination.

Her laboratory results were as follows: white blood cell 13.77×10^3/μL, absolute neutrophil count 8.39×10^3/μL (61%), absolute lymphocyte count 3.2×10^3/μL (24%), hemoglobin 10.2 g/dL, platelet count 355×10^3/μL, albumin 32.7 g/L, CRP 138.4 mg/L, erythrocyte sedimentation rate 42 mm/hour, procalcitonin 0.38 μg/L, and troponin T 254 ng/L (normal range <14). Electrocardiogram demonstrated sinus tachycardia without ST-segment or T-wave abnormalities. Echocardiography revealed normal ventricular function, moderate mitral regurgitation, and a 5-mm pericardial effusion. A giant coronary artery aneurysm was identified in the right coronary artery (RCA), measuring 7.8 mm (Z-score +21.8), and in the left anterior descending artery (LAD), measuring 4.9 mm (Z-score +16.4) (4). No thrombus was detected within the aneurysms (Figure 1).

Computed tomography angiography revealed aneurysmal dilatations in the LAD and RCA, with multiple aneurysms along the RCA. Additional aneurysmal dilatations were observed in the subclavian arteries and their branches, as well as in the axillary, brachial, intercostal, lumbar, main iliac, and internal iliac arteries. No evidence of thrombosis or luminal obstruction was detected within the aneurysms (Figure 2).

The patient was diagnosed with KD, and treatment was initiated with IVIG (2 g/kg), acetylsalicylic acid

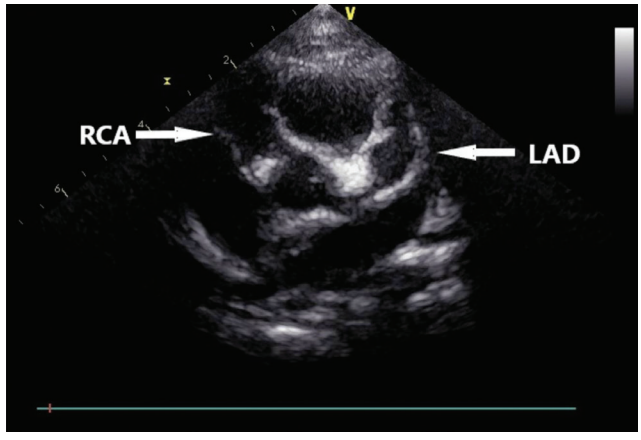

Figure 1. Giant coronary artery aneurysms showed from parasternal short-axis view
RCA: Right coronary artery, LAD: Left anterior descending artery

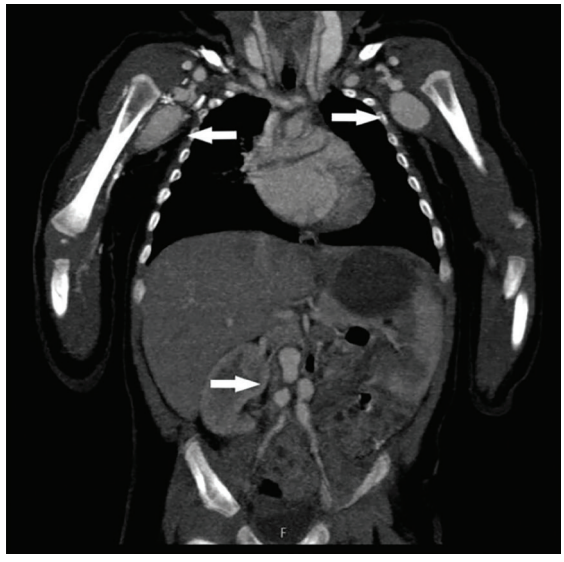

Figure 2. Computed tomography angiography showing multiple aneurysmal dilatations in systemic arteries

(80 mg/kg), clopidogrel (0.2 mg/kg), and enoxaparin (1 mg/kg). Within five days of IVIG administration, laboratory parameters normalized, and the patient remained afebrile. She was discharged on acetylsalicylic acid, clopidogrel, and enoxaparin, and continued to be followed up in our outpatient clinic.

Discussion

KD is a systemic vasculitis mostly affecting medium-sized arteries. It can lead to cardiovascular complications, primarily coronary artery aneurysms. Coronary artery aneurysms can result in serious morbidity and mortality (5). Administration of IVIG therapy within 10 days is recommended in order to prevent coronary artery aneurysms (2). Although IVIG treatment significantly reduces coronary artery aneurysms, they occur in 4% to 6% of cases and approximately 1% of cases develop giant coronary artery aneurysms (6). In this case report, we present a 4-month-old infant with a persistent fever lasting approximately one month, who was ultimately diagnosed with KD at a late stage. KD diagnosis is based on clinical criteria, and these criteria do not include echocardiographic findings.

Giant coronary artery aneurysms are associated with the highest risk of morbidity and mortality, and in these patients, shock, myocardial infarction or sudden death may occur (7). In the previous healthcare facility, the patient had a fever persisting for 17 days during her 28-day hospital stay. IVIG treatment was administered at our hospital on the 29th day of her illness. There were certain risk factors for coronary artery aneurysm such as her prolonged fever, her age being younger than 12 months, anemia, leukocytosis, hypoalbuminemia, and delayed treatment (4,8). Despite the presence of giant coronary artery aneurysm, complications such as thrombosis or myocardial infarction were not observed.

The prevalence of systemic arterial aneurysms in untreated KD has been reported to be approximately 2% (9). Aneurysms can occur in many systemic arteries, most commonly in the axillary, iliac, and brachial arteries. It has been reported that systemic arterial aneurysms are seen in 38.6% of patients with giant coronary artery aneurysms, and patients with multiple systemic arterial aneurysms are observed to be at a younger age when compared to other patients (3). Multiple aneurysms were observed in the subclavian, axillary, brachial, intercostal, lumbar, main iliac, and internal iliac arteries in our patient. There were no signs of thrombus or obstruction in the coronary artery or systemic arterial aneurysms. In order to prevent potential complications, aspirin, clopidogrel, and enoxaparin treatments were initiated.

Long-term management of patients with giant coronary and systemic arterial aneurysms is critical due to the ongoing risks of thrombosis, stenosis, and myocardial ischemia. In such cases, combination antithrombotic therapy, typically low-dose aspirin and/or clopidogrel alongside warfarin or low-molecular-weight heparin, is recommended. Regular imaging with echocardiography and computed tomography or magnetic resonance angiography is essential to monitor aneurysmal changes (10). An individualized treatment approach and coordinated multidisciplinary follow-up are vital in order to reducing life-threatening complications and optimizing long-term outcomes.

Early diagnosis and treatment of KD are crucial in order to prevent the development of coronary and systemic arterial aneurysms. This case was presented to highlight the occurrence of systemic arterial aneurysms in patients diagnosed with KD at an early age and found to have giant coronary artery aneurysms, as well as to emphasize the importance of recognizing atypical presentations.

Ethics

Informed Consent: A written informed consent form was received from the patient's family giving permission for the publication of this case.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.B.B., E.D., F.E., G.K.K., Ş.Ş.Ö., M.Y., B.B.A., B.K.B., Z.Ü.T., Concept: E.D., Z.Ü.T., Design: E.D., Z.Ü.T., Data Collection or Processing: M.B.B., F.E., G.K.K., Ş.Ş.Ö., S.B., Analysis or Interpretation: M.B.B., E.D., Z.Ü.T., Literature Search: M.B.B., M.Y., B.B.A., B.K.B., Writing: M.M.B., Z.Ü.T.

Conflict of Interest: One author of this article, Zülal Ülger Tutar, is a member of the Editorial Board of the Journal of Pediatric Research. However, she did not involved in any stage of the editorial decision of the manuscript. The other authors declared no conflict of interest.

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Case Report

Beyond the Usual Crises: Acute Soft Head Syndrome in Paediatric Sickle Cell Anaemia

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ABSTRACT

There is very little information on the global occurrence and clinical features of acute soft head syndrome, as it is an incredibly rare condition. Subgaleal hematoma (SGH) represents an uncommon, yet significant complication in adolescents with sickle cell anaemia (SCA).

Here, we present a case of a 16-year-old with sickle cell disease who developed a spontaneous SGH. This report highlights the diagnostic challenges and management strategies associated with this rare occurrence and reviews recent literature in order to enhance understanding of SGH within the context of SCA.

Keywords: Acute soft head syndrome, subgaleal haematoma, sickle cell anaemia, periorbital swelling

Introduction

Subgaleal hematoma (SGH) represents a rare, yet significant complication in adolescents with sickle cell anaemia (SCA). This condition is marked by bleeding into the subgaleal space between the galea aponeurotica and the periosteum of the skull (1). Adolescents with SCA are particularly susceptible due to their frequent vaso-occlusive crises and bone infarctions, predisposing them to spontaneous bleeding events (2).

There is very little information on the global occurrence and clinical features of acute soft head syndrome (ASHS), as it is an incredibly rare condition. The lack of literature and the co-occurrence or overlap with other sickle cell disease (SCD)-related diseases, such as extramedullary haematopoiesis, make diagnosis especially difficult (3).

Here, we present the case of a 16-year-old with SCA who developed spontaneous SGH. This report highlights the diagnostic challenges and management strategies associated with this rare occurrence and reviews the recent literature in order to enhance understanding of SGH within the context of SCA.

Case Report

A 16-year-old male, the fifth child of a non-consanguineous marriage, presented with intermittent fever spikes, and a yellowish discoloration of the eyes lasting for 15 days. He also had had intermittent pain in his knee joint for a week (pain scale 4/10). Low grade fever was present accompanied with inter-febrile normal periods. The alarming symptom was the sudden onset of a painless swelling on the left side of his scalp over the

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temporoparietal area noticed by the child over 3-4 days which then progressed to right-sided periorbital swelling 2 days later. There was no history of loss or blurring of vision. There were no complaints of redness, pain in the eyes, floaters or flashes of light. There was no history of recent trauma to the head.

He had been taking hydroxyurea and folic acid for the management of SCA. His past medical history included a single episode of vaso-occlusive crisis requiring admission to the hospital. He had received erythrocyte transfusion only once 8 prior. The patient had poor compliance and had not gone for any regular follow-ups.

On examination he was conscious and well-oriented to time, place and person with a Glasgow Coma Scale of 15/15. He had severe pallor and deep icterus, vitally, a fever of 39 °C, tachycardia (112 beats/min.), and blood pressure of 104/64 mm Hg with an oxygen saturation of 99% in room air. His bilateral pupils were normal size and reacting to light normally. There were no signs of increased intracranial pressure or meningeal irritation.

Systemic examination revealed a mild splenomegaly (soft and non-tender) and a hemic murmur (a sign of severe anaemia). Central nervous system examination was within the normal limits. Local examination revealed a boggy swelling on the left parietotemporal region of the scalp, with ill-defined margins, fluctuant, transillumination negative, non-tender with normal overlying skin. There was also right-sided periorbital swelling with no signs of inflammation.

The initial differential diagnoses were retinal haemorrhage, central retinal arterial occlusion, or carotid artery infarcts/stroke.

Laboratory investigations revealed anaemia [haemoglobin (Hb): 7.00 g/dL, mean corpuscular volume 98 femtolitre with thrombocytopenia 73,000 x109/L, absolute neutrophil count: 3,600 x109/L with bilirubin (total bilirubin: 5.1 mg/dL; indirect bilirubin: 3.6 mg/dL with serum glutamic oxaloacetic transaminase: 190 IU/L, serum glutamic pyruvic transaminase: 76 IU/L). The Gruber-Widal test was positive. Blood culture was negative. Serum lactate dehydrogenase was 2,487 U/L, serum homocysteine was 48.74 µmol/L, erythrocyte sedimentation rate was 38 mm/hour, and coagulation profile was normal. Hydroxyurea was discontinued due to pancytopenia.

An ophthalmology opinion was taken regarding the periorbital and scalp swelling in order to rule out central retinal arterial occlusion. Fundus examination, bilateral pupillary reflexes and extraocular movements were all normal. A carotid Doppler was performed to rule out any atherosclerotic plaque disrupting the flow to the retinal vessels which were revealed to be normal. Two-dimensional echocardiography was carried out in order to check pulmonary pressures and thrombus, and these revealed normal findings.

Magnetic resonance imaging (MRI) brain with contrast (Figure 1) revealed skull bone infarcts in the bilateral parietal bone, haematoma in the right orbital (extraconal) along with a temporal area measuring 1.4x0.5 cm and right-sided subperiosteal haematoma (Figure 2). The haematoma had displaced the superior rectus muscle inferomedially with a size of 6.5x1.1 cm in the right parietal region.

A neurosurgery opinion was taken and conservative management of the scalp and periorbital swelling including cold fomentation, compression, and anti-inflammatory medications were recommended by the neurosurgeon in order to provide relief without the need for invasive interventions.

During hospitalization, the patient received symptomatic treatment for fever and joint pain along with ceftriaxone. He became symptomatically better over a week and there was a remarkable reduction in the periorbital swelling but the scalp swelling showed only mild reduction. The neurosurgeon suggested that it would take time to decrease in size. The patient was discharged and called for follow-up

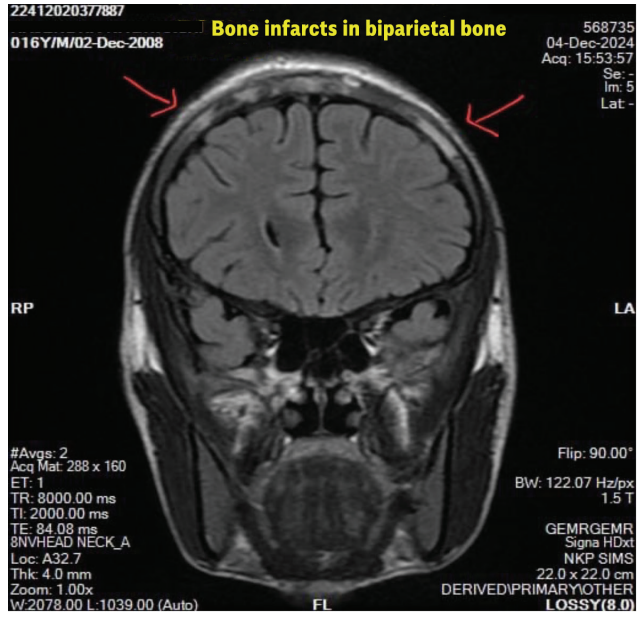

Figure 1. MRI brain with contrast showing bone infarcts in bilateral parietal bone
MRI: Magnetic resonance imaging

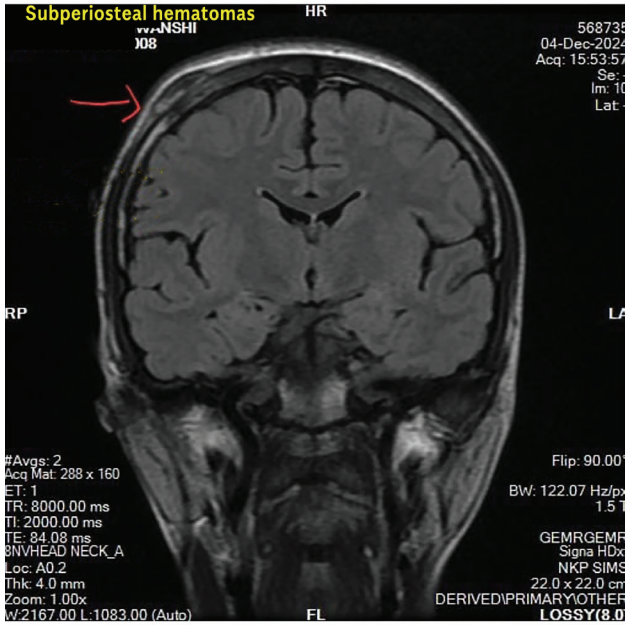

Figure 2. MRI brain with contrast showing right sided subperiosteal haematoma
MRI: Magnetic resonance imaging

after one week, where it was seen that the scalp swelling was significantly reduced.

This case underscores the challenges of managing SCD and the importance of a tailored, holistic approach. With appropriate treatment and regular monitoring, such patients are able to achieve better health outcomes and improved quality of life.

Discussion

SGH is a rare but serious complication in adolescents with SCD. The pathophysiology of SGH in SCD is thought to be related to bone infarctions and vaso-occlusive crises, which can lead to spontaneous bleeding into the subgaleal space.

An uncommon side effect of SCA is a ASHS, which presents as a widespread or localized swelling of the head due to edema and hematoma formation beneath the scalp's galea aponeurotica layer. Epidural hematoma frequently coexists with ASHS in most individuals with a number of hypotheses. The pathophysiology of ASHS is still only partially understood.

Firstly, hypoxia response in SCA is thought to cause prolonged extramedullary hematopoiesis in the skull bones, which weakens and thins the cortical matrices and increases their vulnerability (2). Secondly, angiogenic reactions brought on by hypoxia lead to the development of delicate local vascular beds. These beds work in concert with elevated cardiac output to cause bone fracture and blood leakage into the subgaleal area (3). Thirdly, repeated veno-occlusive crises (VOCs) may cause multiple, mild micro infarctions which, over time, cause bone thinning, local artery wall necrosis, and changed bone and periosteal structures, which can culminate in non-traumatic blood extravasation into subgaleal and epidural areas (4).

The patient's presentation with joint pain, fever spikes, and jaundice is indicative of an acute sickle cell crisis, which can predispose to complications such as SGH. Recent studies have highlighted the importance of recognizing SGH in SCD patients. Over a decade, there have been cases reported from Africa, Arabia, the USA. and recently India (2,5). There was a male preponderance which is similar to our report.

Alqurashi et al. (1) reported a case of a 17-year-old male with SCD who developed a spontaneous SGH, emphasizing the need for prompt medical intervention. In addition to these studies, Foula et al. (2) reported a case of spontaneous SGH in a patient with SCD, highlighting the need for comprehensive care and close follow-up in order to prevent further complications. The presence of jaundice and systemic symptoms suggests the need for evaluation in order to rule out additional complications such as infection or hematologic crisis (2). Recently, a systematic review carried out by Perez et al. (6) stated that headache was the most prevalent complaint at onset (88%). Imaging results frequently showed parietal bone involvement (82%) and bilateral skull infarction (50%) as well. In 65% of instances, an epidural hematoma formed with drainage necessary in 30% of cases and exchange infusion was noted in 18% of cases (6). There were no reported fatalities.

Pathognomonic radiologic characteristics are absent in ASHS. The most sensitive diagnostic method, MRI, usually shows several non-enhancing calvarial lesions which show up as hyperintense on T2-weighted imaging and hypointense on T1-weighted imaging. These lesions may show variable degrees of cerebral expansion without a noticeable mass effect, and they frequently accompany surrounding edema (6). Additionally, MRI is crucial in order to identify related intracranial abnormalities such as extramedullary haematopoiesis and extra-axial collections. Conservative therapy was associated with a prolonged hematoma resolution time of one to two weeks, according to earlier publications (2,4,7)

Drew et al. (8) screened 786 reports of ASHS in paediatric patients, and among these, there were descriptions of

epidural hematomas, subdural hematomas, or SGH. Any of the cranial bones may be affected by these issues (2). A VOC is frequently the setting for reported cases which was similar to our case. Frontal bone infarction in a patient with Hb sickle cell beta thalassemia genotype was reported in just one case (2). Extracranial or intracranial extensions have been reported in the majority of instances in the literature (8). There are many complications of SCD with the following guidelines and strategies for practitioners recommended by Drew et al. (8,9).

1. Cranial MRI is the preferred imaging modality for diagnosing ASHS detecting intracranial involvement and silent cerebral infarct areas.

2. Episodes of ASHS can include intracranial bleeding and thus require urgent neurological assessment in order to detect neurological emergencies.

3. Initial steps in managing ASHS should follow those strategies for VOC including hydration, pain control, and simple transfusion. In addition, exchange transfusion can rapidly and effectively resolve symptoms of ASHS, decreasing the risk of intracranial progression.

4. All patients with episodes of ASHS should have their chronic SCA management escalated (i.e., an increase in hydroxyurea dosing, and a consideration of an exchange transfusion program).

Conclusion

Skull infarction is a potentially serious side effect of SCD which poses particular clinical difficulties. However, although ASHS is usually treated conservatively, a precise diagnosis is essential, as a misdiagnosis could result in unnecessary surgical procedure. Comprehensive care and close follow-up are essential in managing such patients in order to prevent further complications and ensure optimal outcomes. The patient's SGH was managed conservatively with cold compression and anti-inflammatory drugs.

Ethics

Informed Consent: Informed written consent was obtained from one of parent.

Footnotes

Authorship Contributions

Concept: G.C.R., R.A.S., G.N., Design: G.C.R., R.A.S., Data Collection or Processing: G.C.R., R.A.S., D.A., Analysis or Interpretation: G.C.R., R.A.S., D.A., G.N., Literature Search: G.C.R., R.A.S., Writing: G.C.R., R.A.S.

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Letter to the Editor

Ruxolitinib as a Bridge to Avoid Splenectomy in Young Children with β-Thalassemia Major

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To the Editor,

β-thalassemia major remains one of the most challenging inherited hemoglobinopathies in pediatric hematology. Despite advances in transfusion protocols and chelation therapy, massive splenomegaly continues to represent a critical clinical problem in young children. Splenectomy, while effective in alleviating hypersplenism, carries significant lifelong risks and is generally deferred until after the age of five due to the danger of overwhelming post-splenectomy infection (1,2). In this context, the use of targeted pharmacologic approaches to reduce splenic extramedullary hematopoiesis and preserve splenic function has become the subject of increasing clinical interest.

The Janus kinase 2 (JAK2) pathway plays a central role in erythropoietin-driven erythroid proliferation in the spleen and liver. Ruxolitinib, a JAK1/2 inhibitor originally developed for myeloproliferative disorders, has been shown in experimental models to reduce splenomegaly by attenuating this signaling cascade (3,4). Early-phase clinical studies have suggested that ruxolitinib may be a safe and effective therapeutic option in transfusion-dependent thalassemia, though its use in pediatrics remains off-label (5).

We recently managed two young children with transfusion-dependent β-thalassemia major who presented with massive splenomegaly unresponsive to conventional management. Both patients were girls under five years of age, receiving regular transfusions and iron chelation, yet continued to demonstrate progressive spleen enlargement. Multidisciplinary consensus strongly recommended avoiding splenectomy at this age, prompting the initiation of low-dose ruxolitinib (5 mg twice daily).

Clinical response was rapid and favorable. Within the first month, both patients demonstrated significant reductions in spleen and liver size on imaging, accompanied by decreased transfusion requirements. For the first case, annual transfusion needs decreased from 185 mL/kg to 95 mL/kg, while in the second, from 190 mL/kg to 105 mL/kg. Importantly, treatment was well tolerated, with no observed adverse events including hypoglycemia, cytopenias, or infectious complications. Over one year of follow-up, both children maintained improved transfusion intervals (every 3-4 weeks), and splenectomy was successfully deferred.

Our clinical experience adds to the growing evidence that ruxolitinib may serve as a feasible bridge therapy for young children with refractory splenomegaly secondary to β-thalassemia major. Notably, the effective use of a lower dose than reported in phase 2a studies (5) highlights the potential for tailored dosing in younger populations, balancing efficacy with safety. While these results are encouraging, they must be interpreted cautiously given the limited number of patients and retrospective nature of the observation.

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Nevertheless, our findings raise important considerations for pediatric hematologists. Firstly, ruxolitinib may provide a therapeutic window in cases where splenectomy is indicated but contraindicated due to young age. Secondly, it demonstrates that even lower doses may confer significant clinical benefit. Finally, this report underscores the urgent need for prospective pediatric trials in order to establish standardized dosing regimens, long-term safety profiles, and validated clinical endpoints in this vulnerable population.

In conclusion, ruxolitinib represents a promising therapeutic option in deferring splenectomy in children with transfusion-dependent β-thalassemia major and massive splenomegaly. Larger multicenter studies are warranted in order to confirm these preliminary observations and to define the role of ruxolitinib in routine pediatric practice.

Footnotes

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