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Original Article

Paediatrics Section

Umbilical Cord Blood Culture as an Aid to the Diagnosis of Early Onset Neonatal Sepsis: A Cross-sectional Study

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ABSTRACT

Introduction: Neonatal sepsis is one of the leading causes of neonatal morbidity and mortality globally, accounting for an estimated neonatal mortality rate of 23.9 per 1000 live births. Due to overlapping signs and symptoms, a specific diagnosis of sepsis poses a diagnostic challenge. Blood collected from a peripheral vein for sepsis screening and blood culture remains the gold standard for diagnosing neonatal sepsis. The umbilical cord is still not routinely used as a site for collecting blood for sepsis screening and blood culture.

Aim: To determine the diagnostic efficacy of Umbilical Cord Blood Culture (UCBC) compared to Peripheral Venous Blood Culture (PVBC) in Early Onset Neonatal Sepsis (EONS).

Materials and Methods: This cross-sectional observational study was conducted at the Neonatology Unit, Department of Paediatrics, Assam Medical College and Hospital (AMCH), Dibrugarh, Assam, India, involving 110 neonates with two or more risk factors for EONS over a one-year period (August 2021-July 2022). Umbilical cord blood and peripheral venous blood were collected and cultured, and the neonates were monitored throughout their hospital stay. Statistical significance was determined using the Chi-square test for categorical variables and the t-test for continuous variables (with a p-value <0.05 considered statistically significant). The validity of UCBC for diagnosing early neonatal sepsis was assessed based on sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV).

Results: The mean gestational age was 34.95±3.314 weeks and mean birth weight was 2.08±0.790 grams. Of the 110 high-risk neonates, sepsis screening was positive in 67 (61%), while UCBC and PVBC were positive in 19 (17.3%) and 10 (9.09%), respectively. *Acinetobacter* was the most common organism found in both cultures. The sensitivity and specificity of sepsis screening were 100% and 47.25% compared to UCBC and 90% and 42% compared to PVBC. In comparison to PVBC, UCBC demonstrated a sensitivity and specificity of 70% and 88%, with a diagnostic accuracy of 86.36%.

Conclusion: The UCBC exhibits good diagnostic accuracy for diagnosing EONS and can be utilised due to it being a painless and technically less challenging method of blood sampling, with high sensitivity and specificity.

Keywords: Diagnostic accuracy, Efficacy, Peripheral venous blood culture, Sepsis screening

INTRODUCTION

Neonatal sepsis is a significant cause of neonatal morbidity and mortality. It has been categorised into two subtypes: early onset sepsis, typically presenting within the first 72 hours of life, often caused by vertically transmitted pathogens from the mother to the infant before or during delivery; and late-onset sepsis, which usually manifests after 72 hours of life. The mortality rate associated with Early Onset Neonatal Sepsis (EONS) is higher than that of late-onset sepsis [1].

Due to overlapping signs and symptoms that hinder a specific diagnosis of sepsis, early recognition of the condition is crucial for promptly starting antibiotics to prevent neonatal morbidity and mortality. While laboratory markers of sepsis aid in diagnosis, isolating organisms from the patient's blood remains the gold standard for diagnosing neonatal sepsis. The yield of blood culture is influenced by various factors, with blood volume being the most critical factor [2].

The variable sensitivity of blood culture is primarily attributed to inadequate sample volume, the use of intrapartum antibiotics, and the administration of antibiotics before sample collection [3,4]. Additionally, blood collection from a peripheral vein for culture is a painful procedure that necessitates skilled healthcare workers dedicating quality time for sampling; the quantity of blood obtained is often insufficient, making it challenging to isolate organisms from a small blood sample. In contrast, blood collection from the umbilical cord is a painless procedure that ensures an adequate blood volume for culture with minimal contamination.

The aim of the study was to determine the diagnostic efficacy between Umbilical Cord Blood Culture (UCBC) and Peripheral Venous Blood Culture (PVBC) in Early Onset Neonatal Sepsis (EONS). The objective was to investigate the association between UCBC and PVBC and compare the isolated organisms.

MATERIALS AND METHODS

This cross-sectional observational study was conducted in the Neonatology Unit, Department of Paediatrics, and the Department of Obstetrics and Gynaecology at Assam Medical College and Hospital (AMCH), Dibrugarh, Assam, India over a period of one year (August 2021-July 2022). Ethical clearance (AMC/EC/PG/5534) was obtained, and informed consent was obtained from the parents for the study.

Sample size calculation: The sample size was calculated based on the following formula.

$$N=\frac{Z^2\times p\times p}{d^2}$$

Considering a 95% confidence interval with an absolute precision of 5% and based on the study conducted by Mandot S Gandhi JS, where 7.5% (p=7.5%) of newborns had positive UCBC [5], the sample size for the study was calculated and rounded-off to 110.

Inclusion criteria: Inborn neonates with a gestational age ≥28 weeks and two or more risk factors for sepsis were included. Risk factors considered for the inclusion of cases were as follows [6,7].

- Prematurity (<34 weeks)
- Premature Rupture Of Membrane (PROM)
- Preterm Premature Rupture Of Membrane (pPROM)
- Prolonged rupture of membrane (>18 h)
- Maternal fever >100.4°F
- Prolonged labour >24 h
- Chorioamnionitis

Exclusion criteria: Newborns with congenital anomalies and those with ≤700 grams were excluded from the study.

Study Procedure

Newborns were assessed at birth for the presence of risk factors for developing sepsis. The umbilical cord was clamped at both the placental side and the neonatal side, and then the cord was cut. Initially, the placental end was rinsed with 70% isopropyl alcohol, then with betadine, and again with 70% isopropyl alcohol using aseptic measures. Approximately 2 mL of blood was collected using an 18-gauge syringe from the placental end of the umbilical artery/vein, of which 1 mL was transferred to the conventional blood culture bottle.

Neonates who met the inclusion criteria were further evaluated for clinical signs of sepsis. Similarly, within six hours of birth, under strict aseptic measures, 1 mL of blood was drawn from a peripheral vein for blood culture, and another 1 mL was collected for sepsis screening.

Both culture samples were sent to the Microbiology laboratory. The conventional samples were plated on Maconkey agar and blood agar media, and preliminary results were provided within 48 hours based on growth or colour changes, with the final report issued after five days.

For the sepsis screen, several investigations were conducted. The presence of any two of the following criteria indicated a positive sepsis screen:

Sepsis screen criteria [8]:

1. Absolute Neutrophil Count (ANC) <1800 cells/mm^3.
2. Immature/Total (I/T) ratio >0.2.
3. ANC plotted on Monroe's chart for term neonates and Mouzinho's chart for preterm neonates [9,10].
4. C-Reactive Protein (CRP) >1 mg/dL (according to laboratory standards).
5. Micro Erythrocyte Sedimentation Rate (μESR) value (in mm^3 in the first hour) >3 + age in days in the first week of life, or >15 thereafter is considered positive.

In all cases, the first-line antibiotics (ciprofloxacin and gentamicin) were empirically administered as per our unit's antibiogram and subsequently adjusted based on the sepsis screen and culture analysis.

STATISTICAL ANALYSIS

All the data were compiled in an Microsoft excel spreadsheet. Qualitative variables were presented as frequency and percentage, while quantitative variables were expressed in terms of mean and standard deviation. Statistical significance was determined using the Chi-square test for categorical variables and the t-test for continuous variables. A p-value less than 0.05 was considered statistically significant. The validity of UCBC for the diagnosis of early neonatal sepsis was assessed through sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV).

RESULTS

Among the 110 neonates [Table/Fig-1], the mean gestational age was 34.95±3.314 weeks, with a male to female ratio of 1.8:1. The mean birth weight was 2.08±0.790 kg.

Variables	Frequency n (%)
Gender	
Male	73 (66.3%)
Female	37 (33.6%)
Gestational age	
28-<34 weeks	46 (42%)
34-36 weeks	23 (21%)
>37 weeks	41 (37%)
Birth weight	
700 g-1 kg	8 (7%)
1-1.49 kg	24 (22%)
1.5-2.49 kg	37 (34%)
>2.5 kg	41 (37%)
Mode of delivery	
Normal vaginal delivery	79 (72%)
Lower segment caesarean section	31 (28%)

[Table/Fig-1]: Distribution of cases.

In the 110 neonates [Table/Fig-2], the most common risk factor for sepsis was prolonged rupture of the membrane, present in 55 (50%) of the study participants. The second most common risk factor was pPROM, observed in 48 (43.6%) neonates, followed by prematurity in 45 (40.9%) neonates. It was noted that risk factors like prolonged rupture of the membrane had a higher incidence of UCBC positivity. However, this association was not statistically significant. On the other hand, a history of maternal fever and PROM was found to be statistically significant (p-value<0.05).

Risk factors	Number (%)	UCBC		p-value
		Positive	Negative	
Prolong labour (>24 hours)	32 (29%)	6	26	0.79
Prematurity	45 (40.9%)	7	38	0.71
Prolonged rupture of membrane	55 (50%)	11	44	0.68
Preterm Premature Rupture Of Membrane (pPROM)	48 (43.6%)	9	39	0.80
H/o maternal fever	16 (14.5%)	3	13	0.04
Foul smelling liquor	21 (19%)	6	15	0.31
Chorioamniotis	8 (7.3%)	1	7	0.91
Premature Rupture Of Membrane (PROM)	16 (14.5%)	3	13	0.04

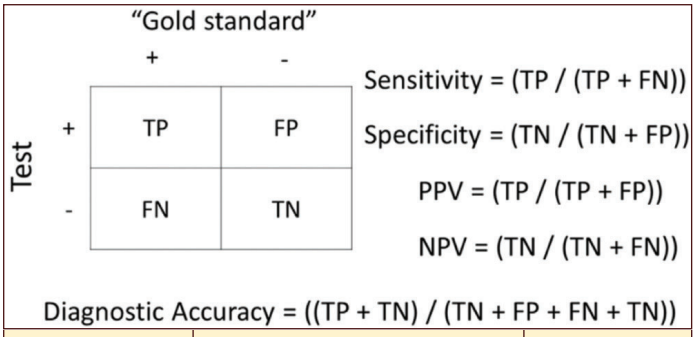
[Table/Fig-2]: Distribution according to presence of risk factor and bivariate analysis with UCBC result.
(p-value calculated by Chi-square test) UCBC-Umbilical cord blood culture)

Out of the 110 neonates [Table/Fig-3], 67 (61%) had a positive sepsis screen, 19 (17.3%) had growth in UCBC, and 10 (9.09%) had PVBC growth, with clinical sepsis present in 74 (67%).

Variables	Sepsis screen	UCBC	PVBC	Clinical sepsis
Positive	67 (61%)	19 (17.3%)	10 (9.09%)	74 (67%)
Negative	43 (39%)	91 (82.7%)	100 (90.9%)	36 (33%)

[Table/Fig-3]: Distribution of cases according to sepsis screen.
(UCBC: Umbilical cord blood culture, PVBC: Peripheral venous blood culture)

Among the 67 neonates with a positive sepsis screen [Table/Fig-4], 19 (28.4%) were found to be positive for UCBC. Comparing sepsis screen results with UCBC, the sensitivity of sepsis screen was 100% and specificity was 47.25%. The PPV and NPV were 28.36% and 100%, respectively. The diagnostic accuracy was found to be 56%.



Sepsis screening	UCBC		Total
	Positive	Negative	
Positive	19 (28.4%)	48 (71.6%)	67 (60.9%)
Negative	0 (0%)	43 (100%)	43 (39.09%)
Total	19 (17.27%)	91 (82.72%)	110

[Table/Fig-4]: Diagnostic efficacy of sepsis screening to UCBC.
(UCBC: Umbilical cord blood culture)

In the group of 67 neonates with a positive sepsis screen [Table/Fig-5], 9 (13.4%) were found to be positive for PVBC, and 1 (10%) with a negative sepsis screen had a positive PVBC result. Comparing sepsis screen results with PVBC, the sensitivity of sepsis screen was 90%, and specificity was 42%. The PPV and NPV were 13.43% and 97.67%, respectively. The diagnostic accuracy was found to be 46.8%.

Sepsis screening	PVBC		Total
	Positive	Negative	
Positive	9 (13.4%)	58 (86.6%)	67 (60.9%)
Negative	1 (2.3%)	42 (97.6%)	43 (39.09%)
Total	10 (9.09%)	100 (90.9%)	110

[Table/Fig-5]: Diagnostic efficacy of sepsis screening to PVBC.
(PVBC: Peripheral venous blood culture)

Compared to PVBC, the sensitivity of UCBC [Table/Fig-6] was 70%, and specificity was 88%. The NPV was 96.7%, the PPV was 36.84%, and the diagnostic accuracy was found to be 86.36%.

UCBC	PVBC		Total
	Positive	Negative	
Positive	7 (36.8%)	12 (63.1%)	19 (17.27%)
Negative	3 (3.3%)	88 (96.7%)	91 (82.72%)
Total	10 (9.09%)	100 (90.9%)	110

[Table/Fig-6]: Comparison of Umbilical Cord Blood Culture (UCBC) and Peripheral Venous Blood Culture (PVBC).
(UCBC: Umbilical cord blood culture, PVBC: Peripheral venous blood culture)

The most common organisms [Table/Fig-7] found in the cultures obtained from both umbilical cord blood and peripheral venous blood were *Acinetobacter baumannii*, followed by *Escherichia coli*. *Enterococcus faecalis* was only present in the UCBC samples but absent in the PVBC.

Organism	PVBC	UCBC
Acinectobacter baumannii	7 (70%)	8 (42%)
Escherichia coli	2 (20%)	5 (26%)
Enterococcus cloacae	1 (10%)	3 (16%)
Enterococcus faecalis	0	3 (16%)
Total	10 (100%)	19 (100%)

[Table/Fig-7]: Comparison of the organisms between the UCBC and PVBC.
(UCBC: Umbilical cord blood culture; PVBC: Peripheral venous blood culture)

Most of the study participants with positive clinical features [Table/Fig-8] were discharged 66 (89%), but no statistically significant association was observed.

Clinical features	Outcome		Total	p-value
	Died	Discharged		
Positive	8 (11%)	66 (89%)	74 (67.27%)	0.67
Negative	4 (11%)	32 (89%)	36 (32.72%)	
Total	12 (11%)	98 (89%)	110 (100%)	

[Table/Fig-8]: Association between clinical features and the outcome.
(p-value is calculated using Chi-square test)

DISCUSSION

The present study was conducted on 110 neonates at risk of developing sepsis. The study found that the mean birth weight was 2.08±0.790 kg, the mean gestational age was 34.95±3.314 weeks, and the male-to-female ratio was 1.8:1. No significant association was found between the demographic profile and the development of probable sepsis. The demographic profile was similar to studies conducted by Aundhakar C et al., where the mean gestational age was 36.6±0.7 weeks and the mean birth weight was 1.956±0.667 kg. Mandot S and Gandhi JS and Aundhakar C found the mean gestational age to be 35 weeks and the mean birth weight to be 2.35 kg [5,11].

The most common risk factor among the study participants was prolonged rupture of the membrane, present in 55 (50%) of the participants, followed by preterm PROM in 48 (43.6%) and prematurity in 45 (40.9%). These observations were consistent with other studies [11-13] that identified prolonged rupture of the membrane and prematurity as significant risk factors. This could be due to the association of maternal genital tract infection, frequent vaginal examinations, the link between ruptured membranes and spontaneous prematurity, and the underdeveloped immune system in preterm infants. Meena J et al., found that prolonged rupture of the membrane was present in 45.4% of cases [12]. A similar risk factor was also reported in studies by Aundhakar C et al., and Ojha M et al., [11,13].

The incidence of probable sepsis was higher in the current study, with 67 (61%) cases compared to Shukla G et al., who found 35% of cases were sepsis screen positive [14]. Mandot S and Gandhi JS reported 23 positive cases out of 80 babies [5]. This difference may be attributed to the increased risk of EONS when membranes are ruptured ≥18 hours before delivery [15,16], and nearly half of the study participants had prolonged rupture of the membrane as one of the risk factors.

When compared to UCBC, the sensitivity of the sepsis screen was 100%, with a specificity of 47.25%. The PPV was 28.36%, and the NPV was 100%. The sensitivity of the sepsis screen compared to PVBC was 90%, with a specificity of 42%. The PPV was 13.43%, and the NPV was 97.67%. In a study by Menaka P and Vani S, the sensitivity and specificity of the sepsis screen were reported as 76.6% and 74.8%, respectively, when compared to UCBC. The PPV and NPV were 19.6% and 97.5%, respectively. In comparison to PVBC, the sensitivity of the sepsis screen was 57.6%, with a specificity of 72%. The PPV was 12%, and the NPV was 96% [17].

Among the 110 neonates with risk factors, 91 (82.7%) did not show any growth, while the remaining 19 (17.3%) showed growth of organisms in UCBC. Similarly, 10 (9.1%) of the 110 neonates showed growth in peripheral vein blood cultures. Out of the 19 UCBC positive neonates, six exhibited the same bacterial isolates in both UCBC and PVBC, with *Acinetobacter* being the most common, followed by *Escherichia coli*. Of the remaining 13 positive UCBC cases, all were associated with probable sepsis, as indicated by a positive sepsis screen. However, nine UCBC-positive patients had no growth in PVBC, though this was not statistically significant (p=0.07). Comparing to PVBC, the sensitivity, specificity, PPV, and NPV were found to be 70%, 88%, 96.7%, and 36.84%, respectively, with UCBC demonstrating a diagnostic accuracy of 86.84%. Aundhakar C et al., in their study, reported a sensitivity of UCBC as 75% and a specificity of 85.92% [11]. The PPV and NPV were 23.08% and 98.39%, respectively, which were consistent with the findings of present study. Jain P and Gosai M observed a significant association between UCBC and PVBC outcomes, with sensitivities of 96.15% and 95.2%, respectively [18].

Among the 19 positive UCBC cases, the predominant bacterial isolates were gram-negative bacteria, specifically *Acinetobacter baumannii*. Other common organisms detected in positive UCBC included *Escherichia coli* and *Enterococcus*. Similarly, in the 10 positive PVBC cases, the predominant bacterial isolates were also gram-negative, with *Acinetobacter* being the most common. Other organisms detected in positive PVBC were *Escherichia coli* and *Enterococcus*. While the sensitivity and specificity were comparable to other studies, the most common organism in this study was *Acinetobacter*, both in UCBC and PVBC. According to the centres for Disease Control and Prevention, *Acinetobacter* is mainly associated with healthcare-acquired infections. Therefore, the most probable cause for the increased presence of *Acinetobacter* in UCBC cases was likely due to frequent unclean vaginal examinations after membrane rupture, leading to ascending infections. This was particularly observed in patients with risk factors such as prolonged rupture of the membrane and prolonged labour, or it could have been due to contamination during sample collection [19].

In contrast, Mandot S and Gandhi JS found that gram-negative organisms were predominant in their study, but the most common organism was *Klebsiella* (32.5%), followed by *Staphylococcus* (13.6%), *Escherichia coli* (10.6%), and *Acinetobacter* (2.7%) [5]. Similarly, Shukla G et al., identified *Klebsiella* followed by *Staphylococcus aureus* as the most common organisms [14]. Pramana KP et al., and Dutta R et al., reported *Klebsiella pneumoniae* and *Acinetobacter baumannii* as the most common organisms [20,21].

Limitation(s)

The UCBC has good diagnostic validity for the etiological diagnosis of bacterial sepsis. However, there is a higher risk of contamination from maternal vaginal and skin floras, so proper aseptic preparation of the umbilical cord is required to eliminate the chance of contamination.

CONCLUSION(S)

The present study concluded that, although peripheral blood culture is considered the gold standard for the diagnosis of neonatal early onset sepsis, its value for this specific diagnosis can be questioned. On the other hand, UCBC has higher sensitivity and comparable specificity for the diagnosis of neonatal early onset sepsis compared to peripheral blood culture, thus avoiding the risk of iatrogenic anaemia and potentially serving as a diagnostic tool for early onset sepsis. Umbilical cord sampling is technically less challenging and ensures an adequate volume of blood for culture, especially from low birth weight newborns. It can be obtained at the earliest possible time, facilitating the rapid initiation of antibiotics, and allowing normal appearing newborns to be monitored in the postnatal ward, thereby reducing the risk of complications related to sepsis. Umbilical cord blood is obtained from the placental end, reducing the likelihood of introducing iatrogenic infections compared to routine peripheral venous sampling.

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