Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Pediatric Cardiology

Umbilical Cord Blood for the Screening of Early Onset Neonatal Sepsis among Those at Risk of Infection

Dr. Mohammad Shakhawat Alam[1*], Prof. M.A. Mannan[2], Dr. Sanjoy Kumer Dey[3], Dr. A Z M Raihanur Rahman[4], Prof. Mohammod Shahidullah[5]

[1]Assistant Professor, Department of Pediatric Cardiology, Bangabandhu Sheikh Mujib Medical University Hospital (BSMMU), Dhaka, Bangladesh
[2]Ex-Chairman & Professor, Department of Neonatology, Bangabandhu Sheikh Mujib Medical University Hospital (BSMMU), Dhaka, Bangladesh
[3]Professor, Department of Neonatology, Bangabandhu Sheikh Mujib Medical University Hospital (BSMMU), Dhaka, Bangladesh
[4]Junior Consultant, Department of Pediatric Gastroenterology, Bangabandhu Sheikh Mujib Medical University Hospital (BSMMU), Dhaka, Bangladesh
[5]Chairman & Professor, Department of Neonatology, Bangabandhu Sheikh Mujib Medical University Hospital (BSMMU), Dhaka, Bangladesh

DOI: 10.36347/sjams.2021.v09i03.032 | **Received:** 04.03.2021 | **Accepted:** 23.03.2021 | **Published:** 28.03.2021

***Corresponding author:** Dr. Mohammad Shakhawat Alam

Abstract **Original Research Article**

Background: Neonatal sepsis contributed to approximately 30 to 40% of neonatal deaths in the low-income countries. Maternofetal bacterial infection is one of the most common causes of neonatal sepsis. The most common risk factor is PROM occurs in 3% of pregnancies. Other risk factors include chorioamnionitis, untreated UTI etc. Many approaches are described to identify sepsis in newborns with initial risk of infection. Previously many studies have been performed by measuring inflammatory markers of sepsis in venous blood usually 12-24 hours after birth predicting severe infections in neonates. Very few studies aimed at testing of these parameters of CBC, I/T ratio, CRP & Blood culture in newborns with PROM and other risk of infection by sampling umbilical cord blood at birth in predicting early onset neonatal sepsis. This study was designed to see the usefulness of screening test to identify early onset neonatal sepsis measured in umbilical cord blood among those at risk of infection. Objectives: To see and compare the routine laboratory values of CBC, I/T ratio, CRP and blood culture yields in umbilical cord blood as well in the venous blood among those at risk of infection and to see the association between the risk factors of early onset neonatal sepsis with the values of routine laboratory parameters measured in umbilical cord blood. ***Methods:*** A prospective study was done in labor ward, post-natal ward and NICU, Bangabandhu Sheikh Mujib Medical University from October 2010 to September 2011.Total 147 newborns were enrolled in two group – 76 infants in case group having at risk of infection and the remaining 71 newborns were control group with similar demographic features having not at risk of infection. The Septic newborns were categorized again into 3 (Three) groups – proven sepsis, probable sepsis and no sepsis based on clinical features observed in the first 72 hours and laboratory values of umbilical cord blood at birth and neonatal blood at 24 hours of age and compared with control group. ***Results:*** Of the 147-study newborn there were 22 (28.9%) cases found to developed early onset sepsis in case group and only 2 (2.81%) were found in the control group. The differences of which were statistically significant (P value <0.05). In the case group, the septic newborns were categorized into three group- proven sepsis 6(7.89%) and probable sepsis 16 (21.0%) and the remaining 54 (71.1%) were no sepsis. In the control group only 2 (2.81%) were found to have probable sepsis. Unlike the blood culture yields and total WBC findings, CRP values and I: T ratio were raised in the umbilical cord blood as well in the venous blood among those at risk of infection. The differences were statistically significant (P value <0.05) compared with the control group having not at risk of infection. In the cord blood CRP had sensitivity, specificity, PPV and NPV of 100%, 89.3%, 43.9% and 100% and in the venous blood at 24 hours of age, CRP had sensitivity, specificity, PPV and NPV of 100%, 77.1%, 27.3% and 100% respectively. The ROC curve for the IT ratios unlike the WBC count differed significantly from the line of discrimination. The odd ratio (OR) revealed the IT ratio and CRP values unlike the CBC values and blood culture yields in the umbilical cord blood as well in the venous blood were more likely to be associated with the risk factors of infection. ***Conclusion*****:** IT ratio and CRP values were found raised in the umbilical cord blood at birth as well in the neonatal venous blood at 24 hrs among those with risk of infection in comparison to those without risk of infection. Negative CRP values were found to be more useful in excluding infection. Cultures obtained in umbilical cord blood at birth are not a good way for etiological diagnosis of early onset sepsis.

Keywords: Neonatal sepsis / CBC / CRP / Blood culture/ Umbilical cord blood.

INTRODUCTION

Neonatal sepsis is high-risk disease (approximately 13-25% mortality) with a low incidence (approximately 1-8cases/1000 live birth and 15-19 cases / 1000 live birth in very low birth weight <1500 gm) in developed countries [1, 2]. It is estimated that about 5 million neonates die every year in low-income countries. Infection contributes to approximately 30 to 40% of neonatal deaths in these countries [3]. Maternofetal bacterial infection is one of the most common causes of neonatal sepsis. The most common risk factor is PROM occurs in 3% of pregnancies and is responsible for approximately one-third of preterm births [4]. Preterm premature rupture of membranes as well other risk factor like chorioamnionitis, intrapartum fever or untreated or partially treated UTI or foul-smelling liquor is a predisposing factor for serious maternal infections such as intra-amniotic infection, endometritis or septicemia. The fetus is at a greater risk of PROM-related morbidity and mortality than the mother. Fetal infections may appear as early neonatal infections such as pneumonia, meningitis and sepsis and are associated with a serious increase in mortality and morbidity in preterm neonates [5]. The clinical features and laboratory values of neonatal sepsis are often non-specific. As blood culture is the gold standard but takes 24-36 hours to be confirmed which prompts evaluation of CBC and other laboratory marker such as CRP, PCT, the marker of Inflammation eg.IL-6, IL-8, TNF-α as possible tool for diagnosis of EONS [6]. Previously many studies have been performed by measuring inflammatory markers of sepsis in venous blood usually 12-24 hours after birth predicting severe infections in neonates [6-8]. A few has been performed by using umbilical cord blood at birth [9, 10] and to my best knowledge, there has been very few studies aimed at testing these parameters of CBC, I/T ratio, CRP & Blood culture in newborns with PROM and other risk factors by using umbilical cord blood at birth in predicting early onset neonatal sepsis.

This study was designed to assess the usefulness of routine laboratory values measured in umbilical cord blood at delivery and in venous blood at 24 hours for the screening of EONS among those at risk of infection specifically to compare the routine laboratory values of CBC, I/T ratio, CRP and blood culture yields in umbilical cord blood at delivery and neonatal venous blood at 24hrs among those at risk of infection and to see the association between the risk factors of early onset neonatal sepsis with the values of routine laboratory parameters measured in umbilical cord blood.

METHODS

The prospective study was conducted labor ward, postnatal ward and NICU of BSMMU from October 2010 to September 2011. Among the 147 newborns 76 were cases and 71 were control group. Neonates included if their mothers had at least one of the following risk factors for neonatal infection: Prolonged rupture of membranes (PROM > 12 hours), Chorioamnionitis, more than three vaginal examinations after ROM, Intrapartum fever (oral temperature >38º C), Sustained fetal tachycardia (HR >160/min), Maternal Leucocytosis (Total WBC >15000/cmm), Foul-smelling liquor, Untreated or partially treated urinary tract infection in the antenatal period. Newborn babies born with Gestational age less than 28 weeks, Weighing less than 1000 grams, Lethal congenital anomalies and Parental refusal to go through the study despite of having risk factors of infection Newborns having two or more clinical features and one or more abnormal laboratory value(s) were regarded as sepsis Or, Newborns having two or more abnormal laboratory values and one or more clinical feature(s) were regarded as sepsis among the study patients and Newborn babies having clinical feature(s) and laboratory value(s) suggestive of sepsis were categorized as follows: Group A –Proven Sepsis was diagnosed if the newborn baby having clinical features suggestive of sepsis and a positive blood culture with other laboratory values. Group B -Probable sepsis diagnosed in a newborn baby with negative blood culture, but if two or more clinical features had suggestive of sepsis and one or more abnormal laboratory markers, or two or more abnormal laboratory markers with one or more clinical features suggestive of sepsis. The remaining newborns that had no clinical features and/or positive laboratory values but had risk of infection were termed as no sepsis.

Newborn babies with proven sepsis received antibiotics for about 14 days, probable sepsis received antibiotics for about 7-10 day and the remaining no sepsis which received antibiotics for an average of 3 days. Group C – Control group-newborns with similar demographic features with the case group and appropriate for gestational age (AGA) birth weights, delivered spontaneously/elective LUCS by healthy women having no history of risk factor(s) of bacterial infection and laboratory evidence of infection and who did not receive antibiotics during or prior to delivery.

Sample size estimation: The calculated sample size should have been 236. Total 76 neonates who had above criteria were taken as cases for the study in case group and 71 neonates were taken as control group who had no risk of infection. Study procedure and Laboratory Techniques: Approximately 5 ml of blood was collected for culture and other routine laboratory tests from the umbilical cord after clamping and cutting of the cord aseptically in the delivery room. Blood was collected in the automated BACTEC 9240 blood culture media, CBC vial and test tube other routine laboratory tests. Samples were transported to the laboratory without delay for blood culture, total leukocyte count, absolute neutrophil count, immature to total leukocyte ratio and CRP estimation. Neonatal blood at 24 hours of age approximately 3 mL of blood was collected by

venepuncture aseptically from the new born for total leukocyte count, Absolute neutrophil count, Immature to total leukocyte ratio (IT ratio) and CRP estimation. CBC was analyzed by automated Hematology analyzer SYSMEX XT-4000i and CRP levels were determined using a latex agglutination test at Clinical pathology department and. This was a semi-quantitative method with a detection limit of 6 mg/L. Blood cultures were done by fully automated BACTEC 9240 at 37^0C temperature for 48 hours were done from the Microbiology department of BSMMU.

The laboratory investigator performing the septic screening was blinded to the clinical status of the newborn babies. Data collection and analyses: Newborn babies were observed for Clinical features of sepsis for at least 72 hrs. Clinical data were collected using a questionnaire. Data were being analyzed using SPSS software version 23.o and double checked before analysis. Means and proportions of the socio-demographic and clinical characteristics were calculated and compared using student t, RR Chi square tests respectively. Probability values < 0.05 were considered as significant for all results. Positive blood culture was considered the "gold standard" against which the performance of CRP was compared. The predictive values of CRP for diagnosing neonatal sepsis have also been calculate.

RESULTS

A total of 147 newborns underwent for the septic screening from the umbilical cord blood at zero hour and neonatal blood at 24 hours of age during the study period among which 76 were case group and 71 were control group. The mean (± SD) maternal age was 24.5±40 years, ranging from 18-35 years among the case group. The mean (± SD) maternal age was 26.76±4.05 year ranging from 20-34 years in the control group. Regarding parity52.6%were primiparous in the case and 33.8% were primiperous in the control group which was statistically significant (P <0.05). Among the cases, most of the babies (67.1%) delivered by LUCS but in the control group mostly (70%) delivered spontaneously. Among the case group of the newborns, the Male- Female ratio was1.1:1 and the mean (±SD) of GA was 33.96±1.82 weeks, ranging from 29-39 wks. The mean (±SD) birth weight was 1900±483.6gms, ranging from 1200–3000 gms among the case group. Of the control group, the mean (±SD) of GA was 34.12±1.5 weeks ranging from 29-40 wks and mean (±SD) of birth weight 1960±212.9 gms ranging from 1300–3100 gms of which male: female ratio was 1.2:1. The base line characteristics were summarized in Table-1. The most common risk factors in the study group were PROM (69.4%), frequent P/V examinations after ROM (28.9%), sustained fetal tachycardia (17.1%) and maternal UTI (3.9%). The results were depicted in the Table-2. The risk ratios (RR) are depicted in the same Table-II. Risk of disease is higher in exposed group than unexposed group according to measurement of risk (RR value >1). The newborns of the case group 22 (28.90%) cases were found to develop sepsis in the first 72 hours based on clinical features and laboratory values and only 2 (2.81%) were found to develop sepsis in the control group. The differences of which were statistically not insignificant (P value <0.05) in comparable to the control group. The case group were categorized in to three group- proven sepsis 6 (7.89%) cases and provable sepsis 16 (21.0%) cases and the remaining 54 (71.1%) cases were no sepsis. In the control group there were 2 (2.8%) found to develop probable sepsis based on clinical features and laboratory values though 2 (2.8%) blood cultures were found positive obtained from umbilical cord blood but had no clinical feature of sepsis or abnormal laboratory values, shown in the control group of Fig-1. Regarding the other Laboratory values of CBC, I/T ratio and CRP, the Total WBC counts were not statistically significant (P value > 0.05) in both umbilical cord blood and neonatal blood sampling at 24 hours of age in comparable to the control group . The mean WBC count of the umbilical cord blood and venous blood at 24 hours, differences were not significant (p>0.05) statistically among the case and control group. Among the case group, Leukopenia/ Leukocytosis were found to develop in the proven sepsis and/or probable sepsis measured in both umbilical cord blood and venous blood at 24 hours which were statistically insignificant (P value > 0.05) between two groups but most likely indicating the severity/progression of the sepsis depicted in the Table-3. The mean (±SD) IT ratio measured in cord blood was 0.29±0.16 ranging from 0.04 to 0.8 in case group and 0.19±0.16 ranging from 0.04 to 0.6 in control group. The mean (±SD) IT ratio measured in venous blood at 24 hours was 0.29±0.14 ranging from 0.12 to 0.59 in case group and 0.16±0.04 ranging from 0.07 to 0.3 in control group. The mean IT ratios of both umbilical cord blood and venous blood at 24 hours, the differences were significant (p<0.05) among the case and control group. The mean (±SD) IT ratio measured in cord blood was 0.3±0.1 ranging from 0.2 to 0.49 in proven sepsis and 0.26±0.09 ranging from 0.14 to 0.4 in probable sepsis among the case group. The mean (±SD) IT ratio measured in venous blood at 24 hours was 0.37±0.1 ranging from 0.24 to 0.48 in proven sepsis and 0.3±0.08 ranging from 0.2 to 0.43 in probable sepsis. Though the mean values of IT ratios in both umbilical cord blood and venous blood at 24 hours were raised to identify sepsis, the differences were insignificant (p>0.05) in both proven and probable sepsis, shown in the Table-3. In the umbilical cord blood, there were 13 (27.63%) cases were found to have positive CRP value (≥ 6mg/L) in the in the case group and only 2 (2.8%) cases were fond CRP positive (≥ 6mg/L) in the control group. In neonatal venous blood at 24 hours of age, 22 (28.94%) cases were found to have CRP positive (≥ 6mg/L) in the case groupwhereas2 (2.8%) cases were found to have positive CRP (≥ 6mg/L) in in the control group depicted in the same Table-3. In both umbilical

cord blood and neonatal blood, the differences were statistically significant (P value <0.05) among the cases and control group. Organisms isolated included 3(50.0%) E.Coli and1 (16.7%) Coliform 2 (33.3%) Enterobacterbacteria in the cord blood cultures which were statistically insignificant (P value >0.05) in comparable to control group as 2 (100%) cases were found CONS positive in the control group which were false positive Shown in the Table-4.

The Table-5 showed, among the proven sepsis CRP values were positive in 5(83.33%) cases in umbilical cord blood and in venous blood at 24 hours CRP values were positive in6 (100%) cases, on the other hand 8(50%) cases were positive in umbilical cord blood and 16 (100%) cases were positive in the neonatal blood in probable sepsis. In the cord blood CRP had sensitivity, specificity, PPV and NPV of 100%, 89.3%, 43.9% and 100% and in the neonatal blood at 24 hours, CRP had sensitivity, specificity, PPV and NPV of 100%, 77.1%, 27.3% and 100% respectively shown in the Table-6. Receiver operator characteristics (ROC) curve for total WBC and IT Ratios measured in umbilical blood at delivery and venous blood at 24hrs were displayed in Fig 2A & B. The area under the curve (AUC) was 0.321 for WBC count and 0.769 for the IT ratio in the umbilical cord blood and 0.325 for WBC count and 0.859 for the IT ratio in the venous blood at 24 hours depicted in the Table-7. The ROC curve for the IT ratios unlike the WBC count differed significantly from the line of discrimination. The association between the risk factors and the laboratory values of BC, IT ratio, CRP and blood culture were shown in the Table-8. The odd ratio (OR) revealed the IT ratio and CRP values unlike the CBC values and blood culture yields in the umbilical cord blood as well in the venous blood were more likely to be associated with the risk factors of infection.

Table-1: Baseline Characteristics of the study patients (n=147)

			Case Group N=76			**Control Group (N=71)**		
		n	**%**	**Mean ±SD (min – max)**	**n**	**%**	**Mean ±SD (min – max)**	**P - Value**
Maternal age (yrs)	18-25	49	64.5	24.53±4 (18-35)	25	35.2	26.76±4.05 (20-34)	0.002^{s}
	26-30	19	25.0		35	49.3		
	31-35	8	10.5		11	15.5		
Parity	Primi	40	52.6		24	33.8		0.021^{s}
	Multi	36	47.4		47	66.2		
Gravidity	Primi	41	53.9		22	31.0		0.004^{s}
	Multi	35	46.1		49	69.0		
Gestational Age (wks)	<37	62	81.6	33.96 ± 2.8	60	84.5	34.12±1.5	0.5643^{ns}
	>37	14	18.4	(29 – 39)	11	15.5	(29-40)	
Mode of delivery	NVD	25	32.9		36	50.7		0.021^{s}
	LUCS	51	67.9		35	49.3		
Sex	Male	40	52.6		39	54.9		0.780^{ns}
	Female	36	47.4		32	45.1		
Weight (gm)	<1500	21	27.6	1900±483.6	20	28.2	1960±212.9	0.338^{ns}
	1500-2499	46	60.5	(1200–3000)	43	60.6	(1250–3100)	
	≥2500	9	11.9		8	11.3		

S=Significant, NS=Not Significant, P value reached from Chi square test

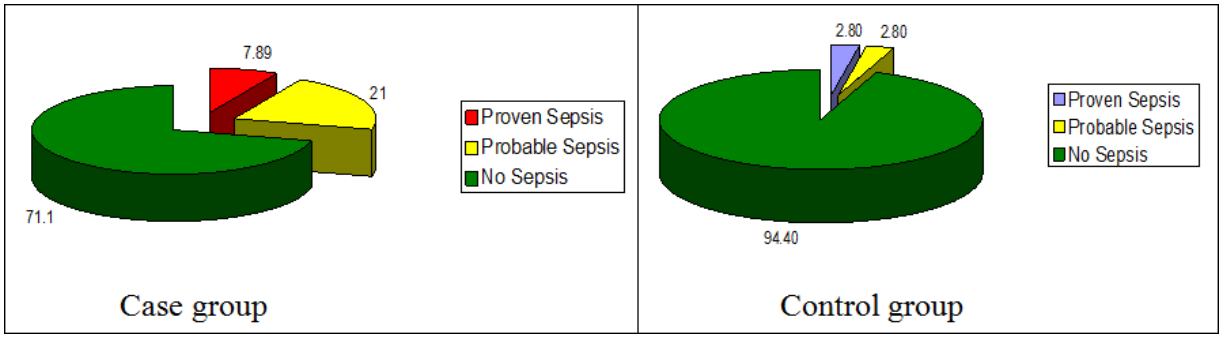

Fig-1: Distribution of Septic newborns among the case and control group

Table-2: Distribution of the study patients according to risk factors (n=147)

Risk factors	Case (n=76)		Control (n=71)		RR(95% CI)	P Value
	n	%	n	%		
PROM						
≥12 Hours	10	13.2	0	0.0	2.08(1.74 - 2.47)	
≥18 Hours	22	28.9	0	0.0	2.31(1.89 -2.83)	0.001[s]
≥24 Hours	20	26.3	0	0.0	2.27(1.86 - 2.76)	
Not found	24	31.6	71	100.0	0.25 (0.10 – 0.36)	
Chorioamnionitis						
Found	4	5.3	0	0.0	1.99(1.69 – 2.34)	0.068[ns]
Not found	72	94.7	71	100.0		
Maternal peripartam fever (>38 C/100.4 F)						
Found	6	7.9	0	0.0	2.01(1.71 – 2.38)	0.017[s]
Not found	70	92.1	71	100.0		
Sustained fetal tacyhycardia (HR>160/min)						
Found	13	17.1	0	0.0	2.13 (1.78 – 2.55)	0.001[s]
Not found	63	82.9	71	100.0		
Maternal Leucocytocis (TWBC>15000/cmm)						
Found	6	7.9	0	0.0	2.01(1.71 – 2.38)	0.017[s]
Not found	70	92.1	71	100.0		
Foul smelling liqor						
Found	6	7.9	0	0.0	2.01(1.71 – 2.38)	0.017[s]
Not found	70	92.1	71	100.0		
Maternal UTI						
Found	3	3.9	0	0.0	1.97(1.68 - 2.32)	0.135[ns]
Not found	73	96.1	71	100.0		
PV Examination (>3 times after PROM)						
Done	22	28.9	0	0.0	2.31(1.89 - 2.83)	0.001[s]
Not done	54	71.1	71	100.0		

S=Significant, NS=Not Significant, P value reached from Chi square test

Table-3: Distribution of the study patients according to total WBC, IT ratio and CRP values from umbilical cord blood and venous blood at 24 hours (n=147)

	Laboratory test	Case (n=76)		Control (n=71)		P Value
		Mean	± SD	Mean	± SD	
umbilical cord blood	Total WBC/cmm	15238	± 6727	15133	± 3950	0.941[ns]
	Range (min – max)	(4500	-30000)	(8000	-24000)	
Neonatal blood	Total WBC/cmm	15644	±10428	14060	±3123	0.584[ns]
	Range (min – max)	(4500	-27000)	(9000	-22000)	
Umbilical cord blood	IT ratio	0.29	± 0.16	0.19	± 0.16	0.001[s]
	Range (min – max)	(0.04	- 0.8)	(0.	04-0.6)	
Neonatal blood	IT ratio	0.29	±0.14	0.16	±0.04	0.001[s]
	Range (min – max)	0.12	-0.59	0.07	-0.3	
Umbilical cord blood	<6 mg/L	63	82.9	69	100.0	0.016[s]
	CRP ≥6 mg/L	7	9.2	0	0.0	
	≥12 mg/L	5	6.5	2	2.8	
	≥24 mg/L	1	1.4	0	0.0	
Neonatal blood	<6 mg/L	54	71.1	69	97.2	0.002[s]
	CRP ≥6 mg/L	6	7.9	0	0.0	
	≥12 mg/L	13	17.1	1	1.4	
	≥24 mg/L	3	3.9	1	1.4	

NS=Not Significant, P value reached from unpaired t-test

Table-4: Distribution of the study patients according to blood culture in umbilical cord blood (n=147)

Blood culture	Case(n=76)		Control(n=71)		PValue
	n	%	n	%	
No growth	70	92.1	69	97.1	0.161[ns]
Growth of the Organism (s)	6	7.9	2	2.9	
E-Coli	3	50.0	0	0.0	
Coliform	1	16.7	0	0.0	
Enterobacter	2	33.3	0	0.0	
CONS	0	0.0	2	100.0	

S= SignificantP value reached from Chi square test

Table-5: CRP statusof the septicpatients among the case group measured in Umbilical Cord Blood (n=76) and Venous Blood at 24 hours (n=76)

	CRP	Proven case n=6 (7.9%)		Probable n=16 (21.1%)		P value	No sepsis n=54 (71.1%)	
		n	%	n	%		n	%
	< 6 mg/L	1	16.7	8	50.0		53	98.1
Umbilical cord blood	≥6 mg/L	2	33.3	5	31.3	0.234[ns]	1	1.9
	≥12 mg/L	2	33.3	3	18.8		0	0.0
	≥24 mg/L	1	16.7	0	0.00		0	0.0
	<6 mg/L	0	0.0	0	0.0		54	100.0
Venous blood	≥6 mg/L	2	33.3	5	31.3	0.508[ns]	0	0.0
	≥12 mg/L	4	66.7	8	50.0		0	0.0
	≥24 mg/L	0	0.0	3	18.8		0	0.0

S= Significant P value reached from Chi square test

Table-6: Sensitivity, specificity, accuracy, positive and negative predictive values of the CRP (≥6mg/L) in diagnosis of sepsis

Test of validity		Percentage
	Umbilical blood	Venous blood at 24 hrs
Sensitivity	100.0	100.0
Specificity	89.3	77.1
Accuracy	89.7	78.9
Positive predictive value	43.9	27.3
Negative predictive value	100.0	100.0

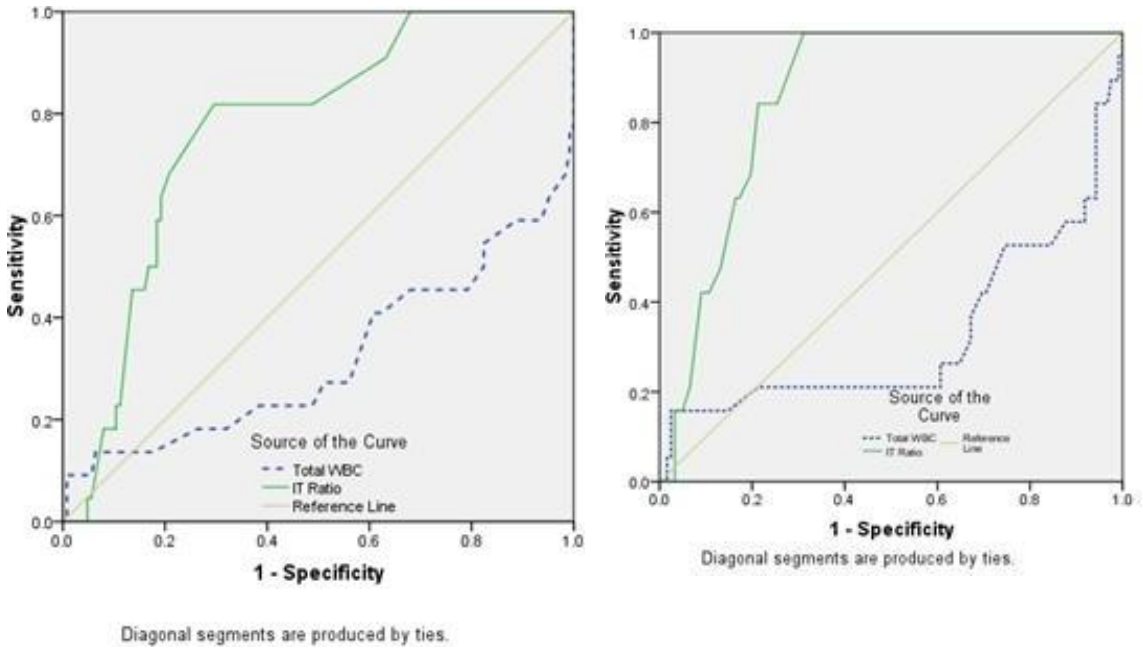

Fig-2A & B: Receiver operator characteristics curve of total WBC and IT Ratio in cord blood and venous blood at 24 hrs

Table-7: Receiver operator characteristics curve of total WBC and IT Ratio in cord blood and venous blood at 24 hrs

	Test Result			**Asymptotic 95%**	**Confidence l**	**Interval**
	Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
Cord	Total WBC	0.321	0.075	0.008	0.174	0.468
Blood	IT Ratio	0.769	0.048	0.001	0.675	0.863
Venous	Total WBC	0.325	0.081	0.014	0.167	0.483
Blood	IT Ratio	0.859	0.031	0.001	0.797	0.921

Table-8: Association of risk factors with positive CBC, IT Ratio, CRP & Blood culture findings in the umbilical cord blood and venous blood at 24 hrs

Risk factors	**CBC**		**IT Ratio**		**CRP**		**Culture**	
	Cord blood n= 7 (OR)	Venous blood n=9 (OR)	Cord blood n=21(OR)	Venous blood n=25 (OR)	Cord blood n=13(OR)	Venous blood n=22 (OR)	Cord Bloodn=6 OR	P value
PROM(n=52) Positive/Negative	5/47 1.17(0.18-9.53)	5/47 0.53(0.11-2.69)	19/33 6.33(1.22-43.75) *	19/33 3.39(0.91-13.71) *	12/40 6.90(0.83-151.12) *	19/33 4.03(0.95-19.58) *	4/48 0.83(0.12-7.20)	0.581[ns]
Chorioamnionitis (n=4) Positive/Negative	0/4 0.00(0.00-18.38)	0/4 0.00(0.00-13.61)	2/2 2.79(0.26-30.51)	2/2 0.67(0.03-7.88)	1/3 1.67(0.0-21.05)	2/2 2.60(0.24-28.34)	0/4 0.00(0.00-22.62)	0.741[ns]
Maternal fever (n=6) Positive/Negative	2/4 6.50(0.63-62.15)	3/3 10.67(1.31-2.36) *	4/2 6.24(0.86-54.46) *	4/2 12.50(1.27-301.47) *	4/2 13.56(1.74-128.54) *	5/1 15.59(1.56-378.74) *	1/5 2.60(0.0-33.51)	0.400[ns]
Sustained fetal Tachycardia (n=13) Positive/Negative	1/12 0.82(0.12-5.42)	2/11 1.45(0.18-9.46)	3/10 0.75(0.14-3.49)	3/10 0.56(0.11-2.56)	4/9 2.67(0.55-12.62)	3/10 0.69(0.13-3.22)	1/12 0.97(0.15-6.26)	0.128[ns]
Maternal Leukocytosis (n=6) Positive/Negative	3/3 16.50(1.84-169.33) *	2/4 4.50(0.47-38.51)	4/2 6.24(0.86-54.64) *	4/2 2.18(0.32-15.09)	4/2 13.56(1.74-128.54) *	5/1 15.59(1.56-378.74) *	1/5 2.60(0.0-33.51)	0.400[ns]
Foul smelling liquor(n=6) Positive/Negative	1/5 2.13(0.0-26.00)	2/4 4.50(0.47-38.51)	4/2 6.24(0.86-54.64) *	5/1 12.50(1.27-301.47) *	4/2 13.56(1.74-128.54) *	5/1 15.59(1.56-378.74) *	2/4 8.25(0.76-86.46)	0.068[ns]
Maternal UTI(n=3) Positive/Negative	½ 5.58(0.0-102.60)	1/2 4.06(0.0-69.72)	3/0 -*	3/0 -*	2/1 11.27(0.70-346.58) *	3/0 -*	1/2 6.80(0.0-132.37)	0.221[ns]
P/V Exam. (n=22) Positive/Negative	3/19 1.97(0.31-11.92)	4/18 2.18(0.43-10.89)	10/12 3.26(0.99-10.91) *	13/9 5.06(1.55-16.98) *	7/15 3.73(0.93-15.27) *	14/8 10.06(2.80-38.01) *	2/20 1.25(0.14-8.97)	0.563[ns]

DISCUSSION

In the present study, male newborns (Ratio 1.1:1) were predominantly distributed in case group as the existing literature suggested that male were equally or predisposed to development of neonatal sepsis [11, 12]. The mean (±SD) maternal age was 24.53±4 in case group and 26.76±4.05 years in control group. Maximum number 49 (64.5%) was observed in the age group 18 to 25 years in case group and 35(49.3%) was observed in 26 to 30 years in control group. A study found a 1.5-fold risk of sepsis in neonates born to mothers under the age of 25 years [13]. Research has showed that very low birth weight infants were more susceptible to sepsis [14] which stresses the potential nature of this condition as an aggravating factor for sepsis. Study has also shown that very low birth weight infants were at a 25-fold risk of developing sepsis when compared with normal birth weight infants [15]. In this study, most of the neonates 62(81.6%) were preterm (gestational age < 37 completed weeks) and 60.5% had low birth weight (1,500 to < 2,500 g) and 26.71% had very low birth weight (< 1,500 g to >1000g). Despite extensive efforts to isolate causative pathogens, blood cultures were positive in only 6(7.9%) cases among those having at risk of infection though positivity ranges widely, from 9 to 64% in one study [16]. The other studies reviewed; positive cultures ranged from 8% to 73% in the diagnosis of potential neonatal sepsis [17]. In this study 6(7.9%) blood cultures were found positive among the case group with E. coli and Coliform and Enterobacter in contrast with control group where 2 blood cultures were found positive with CONS. In the present study 2 organisms were isolated in control group but did not match with the clinical manifestation and/or laboratory parameters which were regarded as contamination or

bacteremia that would be manifested afterwards. Similar observation also found in other study [18]. We found Gram negative organism like E.Coli is the most common cause of neonatal sepsis rather than Gram positive Group B Streptococcus as fond in other study in the developing countries like Bangladesh, india and Pakista [19-21]. Many known factors influence the sensibility of blood cultures like maternal intrapartum antibiotic prophylaxis or time for sample collection and volume of sample [22]. Intrapartum antibiotic prophylaxis for a maternal genital tract or urine infection, maternal fever, prolonged rupture of membranes or fetal tachycardia difficult bacterial grow in culture media [18]. The varying results may be due to different study population and different defining of proven sepsis. It seems the prevalence rates for a specific bacterial pathogen vary from NICU to NICU and may change with time. Thus, the data about most commonly isolated bacteria in a NICU must be periodically reviewed and antibiotic policy revised according to susceptibilities of these organisms.

CRP has been used as an important early indicator of sepsis development, and declining CRP levels in the presence of clinical improvement is used as a parameter for discontinuing antibiotic therapy [11]. The findings of the 147 study neonates demonstrated, in the cord blood 13(17.1%) cases were found to have positive CRP values (> 6mg/L) in the case group and in the control group only 2(2.8%) newborns were found to have positive CRP values (≥6mg/L) which was statistically significant (P-value < 0.05). On the other hand, CRP values in the venous blood at 24 hours, 22(28.9%) cases were found to have positive CRP values (≥ 6mg/L) in the case group and only 2 (2.8%) newborns were found to have positive CRP values (> 6mg/L) in the control group. The differences were also statistically significant (P-value < 0.05). The findings of CRP values corroborate the findings of other study [23].

The findings in the study had a high NPV (100%) but low PPV (43.9%) with the sensitivity and specificity of (100%) and 89.7% respectively in cord blood and there were also high NPV (100%) but low PPV (27.3%) with the sensitivity and specificity of 100% and 77.1% respectively in venous blood. Similar findings of high NPV (97%) but low PPV (36.0%) also observed with the sensitivity and specificity of 79% and 85% respectively in neonatal blood at 24 hours of age [24]. In this study CRP was measured semi-quantitatively in the cord blood and in the neonatal blood at 24 hours of age among those at risk of infection.

There is not an established standard practice for the use of CRP in infants and a variety of approaches are described in the literature [25]. A CRP level measured at the beginning of septic work-up having a sensitivity of 67% and NPV of 87% in Garland SM et al study [26]. These figures were 76% and 96% in another study [23]. Which measured CRP qualitatively, with cutoff point of >20 mg /l, reported 75% of sensitivity and 86% of specificity rate. The most studies measured CRP quantitatively with different cut off point and different times from onset the signs of infection. Nuntnarumit P et al., [28] reported a sensitivity of 100%, specificity of 94% PPV and NPV of 91.6% and 100% respectively of CRP for detecting proven sepsis and localized infection at cut off point >or =5mg/l.

Like CRP, the Immature-to-Total neutrophil ratio (I: T ratio) has been suggested as a good indicator of neonatal sepsis [29]. In the study it has been found an association between CRP levels and I: T ratio. The former were statistically higher in neonates with abnormal I:T ratios, whether in the cord blood or in the neonatal blood. Similar finding was found in previous study [30]. The Mean (±SD) I:T ratio in the umbilical cord blood (0.28±0.15) ranging from 0.04 to 0.8 in the case group and 0.15±0.15 ranging from 0.04 to 0.6 in the control group which was statistically significant (P-value < 0.05). I:T ratio was also statistically significant (P-value < 0.05) in the neonatal venous blood at 24 hours in between case and control group. Leukopenia was the most common findings among the proven sepsis with Neutropenia on the differentials as found in other study [31] indicating most likely the progression and severity of sepsis. But the mean (+SD) of total WBC/cmm was not significant statistically (P value >0.05) in both case and control group as well as proven or probable sepsis in both the umbilical cord blood or in the neonatal blood at 24 hours of age. Similar findings of total WBC were observed in the other study [32] where the ROC curve for the WBC count did not differ significantly from the line of nondiscrimination as found in this study.

CONCLUSION

The IT ratio and CRP unlike the CBC values are found to be raised and better indicator to identify early onset sepsis in the umbilical cord blood as well in the venous blood among those with risk of infection in comparison to those without risk of infection. Negative CRP values were to be more useful in excluding infection. Cultures obtained in umbilical cord blood were not a good way for etiological diagnosis of early onset sepsis. Several prenatal and intrapartum risk factors for EONS cause raised CRP values and I/T ratio in the umbilical cord blood as well as in the venous blood in newborns.

RECOMMENDATIONS

More surveillance and studies are required in the hospitals as well as in the community. The larger studies are necessary to establish- which prenatal risk factors must be taken to obtain the umbilical cord blood sample, what are the accurate methods to measure the

laboratory tests and what is the correct way to control these patients.

LIMITATION

This was a hospital-based study. The drawback of this study was small sample size and short duration. Most of the blood culture could not be obtained from the venous blood at 24 hours of age.

REFERENCES

1. Jeffrey SG, Richard P. Early diagnosis and treatment of neonatal sepsis. Indian Journal of Pediatrics. 1998; 65: 63-78.
2. Stoll BJ, Gordon T, Korones SB, Shankaran S, Tyson JE, Bauer CR, Fanaroff AA, Lemons JA, Donovan EF, Oh W, Stevenson DK. Early-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. The Journal of pediatrics. 1996 Jul 1;129(1):72-80.
3. The WHO young infants study group, Bacterial etiology of serious infections in young infants in developing countries: results of a multicentric study. Pediatr Infect Dis J. 1999; 18: 17-22.
4. Hutzal CE, Boyle EM, Kenyon SL. Use of antibiotics for the treatment of preterm parturition and prevention of neonatal morbidity: a meta-analysis. Am J Obstet Gynecol. 2008; 199: l-8.
5. Satar M, Turhan E, Yapicioglu H, Narli N, Ozgunen FT, Cetiner S. Cord blood cytokine levels in neonates born to mothers with prolonged premature rupture of membranes and its relationship with morbidity and mortality. Eur Cytokine Netw. 2008; 19: 37-41
6. Chan DK, Ho LY. Usefulness of C-reactive protein in the diagnosis of neonatal sepsis. Singapore medical journal. 1997; 38:252-55
7. Himayun M, Sheikh MA, Akhter R. Role of CRP in early onset neonatal sepsis. The Internet Journal of Pediatrics and Neonatology. 2010; 11(2).
8. Koksal N, Harmanci R, Cetinkaya M, Hacimustafaoglu M. Role of procalcitonin and CRP in diagnosis and follow-up of neonatal sepsis. Turk J Pediatr. 2007; 49: 21-29.
9. Fos NI, Gomis R, Gomis CV, Rubio J, Justich P, Valera JC. Blood culture from the umbilical vein in the diagnosis of neonatal sepsis. Internet J Pediatr Neonatol. 2010;12(1).
10. Kordek A, Torbé A, Podraza W, Łoniewska B, Jursa-Kulesza J, RudnickiJ. Does prenatal antibiotic therapy compromise the diagnosis of early-onset infection and management of the neonate? J. Perinat Med. 2011; 39:337-42.
11. Dulcimar PC, Marcos VS, Juliana RM, Lucio RC, Virmondes R, Barata CH. Early-onset neonatal sepsis: cord blood cytokine levels at diagnosis and during treatment. Jornal de Pediatria. 2010; 86:509-514.
12. Oddie S, Embleton ND. Risk factors for early onset neonatal group B streptococcal sepsis: case-control study. BMJ. 2002; 325:308.
13. Goulart AP, Valle CF, Dal-Pizzol F, Cancelier AC. Fatores de riscopara o desenvolvimento de sepse neonatal precoceem hospital da redepúblicadoBrasil. Rev Bras TerIntensiva. 2006; 18:148-53.
14. Stoll BJ. The global impact of neonatal infection. Clin Perinatol. 1997; 24:1-21
15. Benitz WE. Perinatal treatment to prevent early onset group B streptococcal sepsis. Semin Neonatol. 2002; 7:301-14.
16. Carvalho PR, Trotta Ede A. Avanços no diagnóstico e tratamento da sepse. J Pediatr (Rio J). 2003; 79:195-204.
17. Chiesa C, Pellegrini G, Panero A, Osborn JF, Signore F, Assumma M, Pacifico L. C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. Clinical chemistry. 2003 Jan 1;49(1):60-8.
18. Fos NI, Gomis R, Gomis CV, Rubio J, Justich P, Valera JC. Blood culture from the umbilical vein in the diagnosis of neonatal sepsis. Internet J Pediatr Neonatol. 2010;12(1).
19. Ahmed AS, Chawdhury MAKA, Houque M and Dermstad GL. Clinical and Bacteriological profile of Neonatal septicemia In a Tertiary level Paediatric Hospital in Bangladesh. Indian Pediatrics. 2002; 39:1034-39
20. Roy I, Jain A, Kumar M, Agarwal SK. Bacteriology of neonatal septicemia in a tertiary care hospital of northern India. Indian J Med Microbiol. 2002; 20(3):156 -159.
21. Bhutta ZA, Naqubi SH, Muzaffar T, Farique BJ. Neonatal sepsis in Pakistan. Acta Pediatric Scand. I991; 81:596-601.
22. Schrag SJ, Hadler JL, Arnold KE, Martell-Cleary P, Reingold A, Schuchat A. Risk factors for invasive, early-onset Escherichia coli infections in the era of widespread intrapartum antibiotic use. Pediatrics. 2006; 118: 570-576.
23. Borna H, Borna S. Value of Laboratory Tests and C - reactive protein In the Detection of Neonatal Sepsis. The Internet Journal of Pediatrics and Neonatology TM ISSN: 1528-8374. 2005; 5(2).
24. Koksal N, Harmanci R, Cetinkaya M, Hacimustafaoglu M. Role of procalcitonin and CRP in diagnosis and follow-up of neonatal sepsis. Turk J Pediatr. 2007; 49: 21-29
25. Hengst JM. The role of C - reactive protein in the evaluation and management of infants with suspected sepsis. Adv neonatal Care. 2003; 3-13.
26. Garland SM, Bowman ED." Reappraisal of C-reactive protein as a screening tool for neonatal sepsis. Pathology. 2003 Jan; 35(3):240-3.

27. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr. 1988; 112:761-7.
28. Agnieszka K, Stefania GK, Beata P. Umbilical Cord Blood Serum Procalcitonin Concentration in the Diagnosis of Early Neonatal Infection. J Perinatology. 2003; 23: 148–153.
29. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr. 1988; 112:761-7.
30. Nupponen I, Andersson S, Jarvenpaa AL, Anna LJ, HannuK,Repo H. Neutrophil CD11b expression and circulating interleukin-8 as diagnostic markers for early-onset neonatal sepsis. Pediatrics. 2001; 108:12.
31. Yoon BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, Jun JK. The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6 concentration, amniotic fluid infection, and neonatal sepsis. American journal of obstetrics and gynecology. 2000 Nov 1;183(5):1124-9.
32. Agnieszka K, Stefania G-K, Beata P. Umbilical Cord Blood Serum Procalcitonin Concentration in the Diagnosis of Early Neonatal Infection. Journal of Perinatology. 2003; 23: 148–153.