Original Article

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Evaluation of Cord Blood - Haematological Scoring System as an Early Predictive Screening Method for the Detection of Early Onset Neonatal Sepsis

Paediatrics Section

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ABSTRACT

Background: Sepsis is one of the major causes of neonatal morbidity and mortality. Early recognition and diagnosis of neonatal sepsis are difficult because of the variable and non-specific clinical presentation of this condition. Hence, there is a need for early predictive screening method for neonatal sepsis.

Objectives: To evaluate the role of Cord blood Haematological Scoring System as an early predictive screening method for detection of early onset neonatal sepsis and also to identify the neonates who are at risk of developing neonatal sepsis using cord blood.

Settings and Design: The present prospective cross-sectional study was conducted by taking 153 cord blood samples of full term newborns immediately after delivery in the hospital. Pre-term, still birth and emergency deliveries were excluded from the study.

Materials and Methods: The cord blood was collected and analysed for various Haematological parameters like Total leucocyte count, Absolute Neutrophil count, Immature to mature Neutrophil ratio, immature to mature ratio, Neutrophil morphology, nucleated erythrocytes, platelet count, micro erythrocyte sedimentation rate. Blood cultures were performed as gold standard for diagnosing neonatal sepsis.

Statistical Analysis: Chi-square test, Risk ratio, risks in exposed and risk in unexposed were performed.

Results: Of 153 newborns for analysis, 59 (38.56%) developed sepsis. The haematological scoring system found that an abnormal immature to total neutrophil ratio, Neutropenia, micro erythrocyte sedimentation rate followed by an abnormal immature to mature neutrophil ratio were the most sensitive indicators in identifying infants with sepsis. The study also found that higher the score, the greater the certainty of sepsis being present.

Conclusion: The haematological scoring system using cord blood can be considered as an early predictive screening method for detection of early onset neonatal sepsis. Identifying the risk of developing sepsis early can prevent morbidity and mortality of the neonates.

Keywords: Blood culture, Neutrophil count, Nucleated erythrocytes, Platelet count

INTRODUCTION

Neonatal sepsis is a common and major risk factor for neonatal morbidity and mortality in the developing countries [1]. The neonates have prematurity of immune system, hence it is agreed that neonatal sepsis is a syndrome, expressing both metabolic and haemodynamic impairments, brought about by infection. The clinical manifestation of sepsis in newborn infants is usually non-specific; thereby diagnosis of sepsis only by clinical findings is difficult [2]. Thus, diagnosis of sepsis relies on combination of various laboratory tests. The usual practice is, after development of clinical sepsis i.e. significant multisystem disease; blood sample is collected by venipuncture from the neonates and sent for culture and haematology. Collection of blood sample can induce pain or infection or iatrogenic anaemia to the neonates. There are no previous documented reports of early predictive screening methods for neonatal sepsis by using cord blood sample. Neonatal Sepsis can be divided into two groups named as early onset (first 72 hours of life) and late onset sepsis (>72 hours) [3]. The case fatality rate is higher in early onset sepsis as compared to late onset sepsis. Hence, the present study was conducted to evaluate the role of Cord Blood- Haematological Scoring System (CB-HSS) as an early predictive screening method for diagnosis of early onset neonatal sepsis.

MATERIALS AND METHODS

The present prospective cross-sectional study was carried out at Mamata Medical College, Khammam from January 2013 to June 2014. For the purpose of study, 153 cord blood samples were collected immediately after delivery. Cord blood of the newborns were collected and analysed for this study. All live, full term deliveries were included whereas preterm, still birth and emergency deliveries were excluded from the study.

Immediately after the delivery, 5ml of the cord blood was collected using a sterile 22-guage needle with syringe and ejected into vacutainer containing EDTA anticoagulant for assessing haematological parameters. Two ml of cord blood was transferred to anticoagulant EDTA bulb for analysing haematological parameters like total WBC count, WBC Differential count and Platelet count. Zero calibration of the equipment had been performed after each sampling process to avoid error in analysing. Peripheral smear preparation and analysis were also performed for confirmation of parameters including cell counting and to study the morphological changes of Neutrophils and nRBC levels. The remaining blood sample was used for micro ESR estimation and Blood culture which was considered the gold standard for diagnosis of Sepsis. The haematological scoring system was prepared based on re-categorizing the previous study which had been categorized into clinical sepsis, suspected sepsis and proven sepsis [4]. All the haematological parameters were combined [Table/Fig-1] [5,6] and scores were prepared based on the variation from the normal reference values for early and simple approach towards management of the newborn and thereby further prevent morbidity and mortality. The categorized groups (No risk group, Low risk group, High risk group and Sepsis group) were formed based on the results obtained from the screening tests and not related to maternal factors. The feedbacks of the results obtained were given to the respective gynaecologist/paediatrician.

Parameters	Findings	Score
Total leukocyte Count	≤ 5,000 /mm^3	02
	≥ 25,000 /mm^3	01
Absolute Neutrophil Count	No mature Neutrophils seen	02
Absolute Neutrophil Count [Mature Neutrophils]	< 1,500 /mm^3	02
	> 8,000 /mm^3	01
I/T Ratio	> 0.2	01
I/M Ratio	> 0.3	01
Neutrophil Morphology	> 30%	01
nRBCs	> 10 /100WBCs	01
Platelet Count	< 1,50,000 /mL	01
Micro ESR	> 15mm/hr	01
Total		12

[Table/Fig-1]: CORD BLOOD - Haematological Scoring System for Early onset Neonatal Sepsis [5,6]

Ethics

With the help of gynaecologist, informed consent from the mother was taken on the day of admission in Mamata General Hospital for delivery. Details and advantage of the study were explained, and only those who gave consent were included.

STATISTICAL ANALYSIS

Chi-square test, Risk ratio, risk in exposed and risk in unexposed were performed.

RESULTS

Of the total 153 full term normal delivery newborns, 59 (38.56%) developed early onset sepsis which were confirmed by positive cord blood culture. All the haematological parameters in the present study used in CB-HSS showed statistically significant values. The abnormal variation in parameters like nRBCs (nucleated red blood cells), ANC (Absolute Neutrophil count), I/T ratio (Immature/Total count ratio), I/M ratio (Immature/Mature ratio) and Neutrophil morphology showed > 65% risk in developing sepsis among newborns, whereas remaining parameters like Total count, Platelet count and micro ESR showed >50% risk in developing sepsis. Parameters like Neutrophil morphology and Platelet count showed abnormal variation in >33% of newborns without sepsis [Table/Fig-2].

In 153 neonates, 47 (30.71%) had abnormal morphological changes. The commonest morphological abnormality observed in sepsis was Toxic granules (18/47, 38.29%) followed by combination of toxic granules with cytoplasmic vacuolations (15/47, 31.91%) and cytoplasmic vacuolations alone (08/47, 17.02%). The cytoplasmic vacuolations alone (04/47, 8.51%) was frequently seen in neonates without sepsis [Table/Fig-3].

All the neonates in Sepsis group had CB-HSS value between10-12 whereas 90.62% neonates developed sepsis among high risk group and 15% neonates developed sepsis among Low risk group [Table/Fig-4].

Parameters	N-N	N-D	A-N	A-D	Chi square test	RE	RUE	Relative Risk	p-value
nRBC's	83	15	11	44	62.23	80%	15.3%	5.23	< 0.001*
Total Count	44	01	50	58	35.53	53.7%	2.2%	24.4	< 0.001*
ANC	77	01	17	58	93.34	77.3%	1.3%	59.46	< 0.001*
I/T ratio	69	02	25	57	71.45	69.51	2.8%	24.82	< 0.001*
I/M ratio	66	02	28	57	65.55	67.1%	2.9%	23.13	< 0.001*
Neut Morp	88	18	06	41	56.8	95.3%	34.8%	2.74	< 0.001*
Platelet Count	76	39	18	20	4.22	52.6%	33.9%	1.55	<0.05
microESR	62	03	32	56	54.97	54.9%	4.6%	11.9	< 0.001*

[Table/Fig-2]: Evaluating various parameters of CORD BLOOD- Haematological Scoring System
* Highly significant

Neutrophil Morphology	With Sepsis	Without Sepsis	Total
Toxic granules	18	01	19
Cytoplasmic vacuolations	08	04	12
Toxic Granules + cytoplasmic vacuolations	15	01	16
Total	41	06	47

[Table/Fig-3]: Morphological changes in Neutrophils in relation with Neonatal sepsis

The relative risk of Absolute Neutrophil Count (59.46), I/T ratio (24.82), Total Count (24.4) and I/M ratio (23.13) suggests that they are more reliable markers in identifying the risk of developing neonatal sepsis.

EONS was frequently seen on day 1 (37/59, 62.71%) of neonates followed by day 2 (14/59, 23.73%) and day 3 (08/59, 13.56%) [Table/Fig-5].

The most common microorganism isolated among sepsis group was Group B *Streptococcus* while in High risk group *Staphylococcus aureus* was frequently observed. In low risk group, *Klebsiella* and *Acinetobacter* were seen [Table/Fig-6].

Risk of Sepsis vs New Haematological Scoring System			
	NHSS	Death	Developed sepsis
No Sepsis [n-74]	<4	0	0
Low risk [n-20]	4-6	01 [5%]	03 [15%]
High Risk [n-32]	7-9	04 [12.5%]	29 [90.62%]
Sepsis [n-27]	10-12	06 [22.22]	27 [100%]

[Table/Fig-4]: Neonates at risk of developing sepsis by CB-HSS

	Day 1	Day 2	Day 3
No Sepsis [n-74]	--	--	--
Low risk [n-20]	00	02	01
High Risk [n-32]	15	08	06
Sepsis [n-27]	22	04	01
Total	37	14	08

[Table/Fig-5]: Correlation of early onset neonatal sepsis with risk groups

Microorganisms	Sepsis	High Risk	Low risk	No risk	Total
Group B *Streptococcus*	18	09	00	00	27
E.coli	02	05	00	00	07
Staphylococcus aureus	07	13	00	00	20
Klebsiella	00	02	02	00	04
Acinetobacter	00	00	01	00	01
No Growth	00	03	17	74	94
Total	27	32	20	74	153

[Table/Fig-6]: Correlation of risk groups with umbilical cord blood culture

DISCUSSION

Sepsis is one of the most common infectious conditions in the neonatal period, and remains a major source of morbidity and mortality despite extraordinary progress in the field of neonatology in recent years [1]. Early-onset neonatal sepsis usually occurs in the first 72 hours of life, with 80 to 90% of cases presenting upto 48 hours after birth [3]. Early onset sepsis typically manifests as a fulminant, multisystem illness usually acquired by vertical transmission from the mother. Late-onset sepsis may occur as early as postnatal day 3, but is more common after the first week of life and is usually a progressive illness characterized by focal infection. The case fatality rate is higher in Early onset neonatal sepsis (EONS) as compared with Late onset sepsis In the present study, the incidence of EONS

among full term newborn was 38.56% (59/153) which can suggest the burden of disease.

It is well-known that withdrawing large amounts of blood from a neonate can be challenging and may also lead to necessary blood transfusions due to iatrogenic anaemia [7]. For infants and children, blood sampling by venipuncture can be difficult. It has been supported that, in routine clinical practice a large proportion of negative blood cultures were almost inevitable because of the submission of an inadequate volume of blood [8]. Repeated needle prick for adequate blood sample can cause more pain to the neonate resulting in abnormal neurobehavioral development [9]. There are two documented reports concluding that umbilical cord blood may prove to be a satisfactory alternative to infant blood for neonatal sepsis evaluation by blood culture and total leucocyte count [10,11]. Hence, we used umbilical cord blood for the study.

Although various tests [12,13] like complete blood count and Neutrophil parameters, are used as a diagnostic tool for neonatal sepsis, the complete blood count with differential is widely used, either singly or in conjunction with other test or clinical findings. The advantage of Haematological Scoring System lies in the fact that it is easy and applicable to all infants, including those who have received antibiotic. In the present study, along with existing HSS parameters like total leucocyte count, I/T ratio, I/M ratio, ANC and microESR, we included new HSS parameters like nRBCs, morphological characteristics of neutrophils and platelet counts. The combination of all these parameters gives better results rather than individual or in conjunction. The abnormal variations in parameters like nRBCs, ANC, I/T ratio, I/M ratio and Neutrophil morphology showed > 65% risk in developing sepsis among newborns, whereas other parameters like Total leucocyte count, platelet count and microESR showed >50% risk in developing sepsis. This suggests that immature RBCs and WBCs play important vital role in neonatal sepsis compared to platelets.

The major problem in neonatal infections is identification of newborn's at risk of developing sepsis because of its nonspecific clinical symptoms. In the present study, 90.62% high risk group and 15% low risk group developed sepsis but lacked clinical evidence. These are the neonates who pose a diagnostic and therapeutic dilemma because fatal infections occur in EONS. We also observed that most of the EONS (62.71%) were seen within 24 h of delivery. The most common microorganism isolated among the sepsis group was Group B *Streptococcus* while in High risk group *Staphylococcus aureus* was observed. In low risk group, *Klebsiella* and *Acinetobacter* were seen.

CONCLUSION

Cord blood can reduce the complex sample collection procedure of obtaining neonatal blood. CB-HSS of cord blood can be performed rapidly and results are obtained early when compared to culture which is the gold standard. These results if informed early to the Paediatrician/gynaecologist, antimicrobial regimen can be started to prevent septic attack and further complications. Haematological parameters used in CB-HSS can be easily performed in laboratories with basic facilities. Thus, CB-HSS of cord blood can be used as an early predictive screening method for all the newborns to identify early onset neonatal sepsis.

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