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Sensitivity and Specificity of Toxic Granules in WBC and Immature to Total Neutrophil Ratio in Diagnosis of Neonatal Sepsis

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Abstract

Background: *Septicemia is the commonest cause of neonatal mortality and severe morbidity. Positive bacterial blood culture result confirms clinically suspected sepsis but it is too late to start antibiotic. We need a easy, reliable and cost effective test to diagnose sepsis in time.*

Objective: *To see the relation of toxic granule and immature neutrophil with neonatal sepsis.*

Material & Methods: *This hospital based, cross-sectional study was conducted on 152 neonate with suspected sepsis admitted in Dhaka Shishu Hospital during January 2013 to April 2014. After enrollment all studied neonate were investigated with complete blood cell count, CRP and Blood culture before starting treatment with antibiotic. Raised immature to total neutrophil ratio >0.2 and toxic granules in neutrophil were correlated with positive blood culture test using x^2 test.*

Result: *Out of 152 studied neonate 61.89% were male, 41.94% premature, 48.85% low weight. Blood culture result was found positive in 7.24% neonate. Leukopenia (WBC<5000/mm^3), Neutropenia (Neutrophil <1750/mm^3), Immature to total neutrophil ratio (I:T) >0.2 and toxic granules were present significantly in sepsis. The sensitivity and specificity of toxic granules to diagnose neonatal sepsis was correspondingly 91.67% and 75% & that of I: T>0.2 were 90.91% and 66.67%.*

Conclusion: *Toxic granules and Immature to total neutrophil ratio (I: T) >0.2 can be considered as useful tools in the diagnosis of neonatal sepsis to start antibiotic earlier, especially where there is limited modern investigation facilities.*

Key words: *Neonate, sepsis, Toxic granule, Blood culture, Immature to total neutrophil ratio (IT ratio).*

Introduction

In Bangladesh it is estimated that up to 20% of the neonates develop septicemia.[1] In the United States and Australia it range from 1.5 to 3.5 per 1000 for early onset sepsis (EOS) and up to 6 per 1000 live births for late onset sepsis (LOS), a total of 6-9 per 1000 for neonatal sepsis.[2] Approximately 98% of neonatal deaths occur in developing countries, and are attributable to infections, asphyxia, and consequences of prematurity and low birth weight.[3]

In developing countries, neonatal mortality from all causes is about 34; most of these deaths occur in the first week of life.[4] Recognition of sepsis in neonate is one of the most difficult problem clinicians facing today, it is essential to prevent morbidity and mortality from sepsis in neonate. That can also help us to achieve SDG4 by 2030. Infants often present with non-specific symptoms and signs so that failure or delay in treatment may result in significant mortality and morbidity. Although various hematological indices have been utilized to screen sepsis, are commonly affected by perinatal factors like maternal hypertension, asphyxia and hemolytic disease.[5] Screening and decision about treatment depend on our understanding,

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how qualitative and quantitative change in the neutrophil links infection. As blood culture for bacteria (confirmation of the diagnosis) is time consuming and not available everywhere, we need a valid, reliable, rapid, easy, cost effective and available diagnostic test along with blood culture to begin empiric antimicrobial therapy that remains "Gold standard" for neonatal sepsis.

Early diagnosis of neonatal sepsis remains challenging and early management of sepsis has become a major problem.[6,7] To combat newborn sepsis, early recognition and treatment of infections with antibiotic, community based interventions like safe delivery, cord hygiene, colostrums and exclusive breast feeding, and follow up care can significantly reduce the newborn death from sepsis.[8] There is an urgent need to know whether the baby has sepsis to start treatment as quickly as possible.[9] This study was done to highlight the easier and cost effective approach to diagnosis of neonatal sepsis.

Material & Methods:

This hospital based, cross section study was conducted during May 2013- May 2014, in special care baby unit (SCABU) of the Dhaka Shishu Hospital (DSH), Dhaka. During the study period, all admitted neonates (0-28 days) irrespective of weight & sex and both the term and preterm with suspected sepsis after taking informed written consent from parents/ caregivers were included in the study. Any congenital structural anomalies, suspected congenital TORCH Infection or metabolic diseases were excluded from the study. Clinical signs of sepsis, according to Guidelines for Neonatal Sepsis interventions, National Neonatal Health Strategy & Guidelines for Bangladesh, 2009 were considered.[10] Out of 1196 admitted neonate consecutive 152 suspected neonatal sepsis cases were enrolled according to the inclusion and exclusion criteria.

- ***Suspected sepsis***: Neonate with one or more clinical features of not feeding well, feeding intolerance, lethargy, convulsion, abnormal temperature, poor tissue perfusion, bleeding, abnormal rate and pattern of respiration with or without risk factors were marked as suspected sepsis.
- ***Proven sepsis:*** Infection confirmed by positive bacterial blood culture result in a sick neonate were labeled as proven sepsis.
- ***IT Ratio >0.2*****:** Ratio of total and immature neutrophil observed among 200 neutrophil cells in peripheral blood film under light microscope. Immature neutrophil is more than 20% of total.
- ***Toxic granules:*** Deep blue or purple colored, coarse, membrane bound and numerous granules in cytoplasm of the mature neutrophil.
- ***Outcome variables:*** Presence of toxic granules in neutrophil and raised IT ratio >0.2 of neutrophil in neonates with suspected sepsis.

On admission of neonates met the criteria with suspected sepsis, detail clinical histories were obtained from parents or caregiver and thorough physical examination was performed. After counseling appropriate treatment including the antibiotic was initiated immediate after collection of venous blood under aseptic precaution for hematological, biochemical and microbiological test. Three ml blood was drawn and collected in three sterile test tubes, one ml in each, labeled, recorded and sent earlier to the laboratories. Ethical approval was taken from local ethical review committee.

Neonates were divided in two groups: culture positive (Proven sepsis) and culture negative (Probable sepsis). In this study, positive blood culture result was considered as "Gold standard test" in identifying sepsis. The "IT ratio>0.2 "and presence of "toxic granules" in neutrophil, these two variables were compared with the gold standard. For the purpose of the study, all culture positive and negative neonates were again divided into two according to the presence or absence of raised plasma CRP $\geq$6 mg/L levels. Those babies had negative blood culture result and CRP < 6 mg/L were taken as "true negative" sample and those babies had positive blood culture result were considered as "true positive" sample. Then the "IT ratio>0.2" and presence of "toxic granules" in neutrophil of peripheral blood film were recorded and correlated with both "true positive" and "true negative" sample. All the relevant information was recorded in a pre designed checklist and data was compiled and analyzed. The sensitivity and specificity of both "IT ratio>0.2" and presence of "toxic granules" in neutrophil of peripheral blood were evaluated.

Identification of bacteria in blood was made by growth of bacterial colonies using basic, nutrient, selective, indicator and differentiate media. Gram staining, physical, and physiological and biochemical

characters of bacteria obtained from cultured colonies. The blood was cultured by both conventional or Lysis-direct plating and lysis-centrifugation method in aerobic environment. After overnight incubation of blood in nutrient broth (Basic liquid media) at 37^0 C, next morning growth was collected (If present) and stained with Gram staining followed by first subculture on nutrient media like 5% sheep blood agar and Mac-Conkey agar media for another 24 hours. The colony on nutrient media was stinted on different indicator media, sometime more than one in same other dish. Second subculture was incubated on selective media for 48 hours after stinging of colonies from first subculture. Identification of bacteria colonies was made by physical, physiological and chemical methods after subculture. These included Gram stain, catalase reaction, coagulase reaction, hemolytic activity on sheep blood agar plates for Gram positive bacteria. In case of Gram negative bacteria, Gram stain, morphology on blood agar, Mac-Conkeys agar reaction, Eosin Methiline Blue agar and oxidase, indole, motility tests was used.

One ml whole blood was collected in EDTA containing tubes. Peripheral blood smears on glass slide was stained with Leishman stain, Total cell count reading was obtained by MS 95 automated analyzer. Differential counts were performed on these smears by counting manually at least 200 cells and using Beckman Coulter HMX automated haematology analyzer (U.S.A), differential leucocytes counts (DLC), total neutrophil count (TNC), immature neutrophil count (including band form) and mature neutrophil count (M) were performed. Toxic granules were observed as blue colored rough granules in cytoplasm of segmented and band form neutrophil in Giemsa stained. The plasma CRP of studied neonates were measured by ELISA method along with other biochemical tests in biochemistry laboratory.

After compilation of data separate multivariable analyses were carried out to identify the significant demographical risk factors, common presenting features and different laboratory findings. Data was analyzed using SPSS for windows version 17.0. Statistical test between dependent and independent variables was done using Chi square test (χ^2). Probability values ≤0.05 with 95% confidence interval was considered statistically significant. Interpretation of different analysis result was done to detect the sensitivity and specificity of IT ratio >0.2 and toxic granules.

Results:

Among the enrolled 152 studied neonates, 7.89% (12/152) were found culture positive and 92.11 % (140/152) culture negative.

Table-I

Distribution of infected neonates in relation to specific organism and presenting age.

Organism	Age ≤7days *41.64%(n=5)*	Age >7 days *58.34%(n=7)*	Total *(n=12)*
Klebsiella pn.	16.67%(2)	16.7%(2)	33.34%(4)
E. coli	8.33%(1)	16.67% (2)	25%(3)
Acinetobacter	0	8.33%(1)	8.33%(1)
Pseudomonas	8.33%(1)	0	8.33%(1)
Staph. Aureus	0	8.33%(1)	8.33%(1)
Strep. Pn.	0	8.33%(1)	8.33%(1)
Group B Strept.	8.33%(1)	0	8.33%(1)

Table-II

Distribution of studied neonates according to WBC count

WBC Count	Total n = 152	Culture (+) ve n = (12)	Culture(-) ve n = (140)	*p*-value*
TC <5000/cumm	27.11% (42)	58.34% (07)	25% (35)	<0.05
TC ≥5000/cumm	72.88%(110)	41.66% (05)	75% (105)	

***** Chi square test

Table-III

Distribution of studied neonates according to total neutrophil count considering Â1750/cumm, I:T> 0.2 and Toxic Granules

	Culture +ve n= (12)	Culture –ve n= (140)	*p*-value*
Total neutrophil			0.027
<1750/cu mm	75% (09)	23.60% (33)	
≥1750/cu mm	25% (03)	76.40% (107)	
I:T > 0.2			0.024
Present	91.67% (11)	22.85% (32)	
Absent	8.33% (01)	77.15% (108)	
Toxic Granules			0.0121
Present	91.67% (11)	21.55% (30)	
Absent	8.33% (1)	78.45% (110)	

***** Chi square test

Plasma CRP >6mg/L was present in 91.66% (n=11) babies with proven sepsis and in 58.57% (n=82) newborn with probable sepsis. Rest, 41.43% (58) of probable sepsis had plasma CRP< 6mg/L.

In this study sepsis in neonates were labeled as,

True positive (TP) : Both blood culture positive and CRP > 6mg/L (n= 11)
True negative (TN) : Both blood culture negative and CRP < 6mg/L (n= 58)
False positive (FP) : Blood culture negative but CRP > 6mg/L (n= 82)
False negative (FN) : Blood culture positive but CRP < 6mg/L (n= 01)

Table IV

Distribution of neonate groups according to the presence of Toxic granules and IT ratio >0.2 of neutrophil.(n=152)

	Studied neonate (n = 152)	TG		I:T > 0.2	
		Present	Absent	Present	Absent
TP	11	11	0	10	01
TN	58	03	55	04	54
FP	82	01	81	02	80
FN	01	01	0	01	0

Then the true positive (TP) group babies (n=11) and true negative group babies (n=58) were regrouped according to presence or absence of Toxic granules (TG).

Then the true positive (TP) group babies (n=11) and true negative group babies (n=58) were regrouped according to raised IT ratio>0.2 also.

Table V

Distribution of toxic granules (TG) in neutrophil and IT ratio > 0.2

	TG	I:T>0.2
Sensitivity = TP / (TP + FN)	11/(11+01)	10/(10+01)
Specificity = TN / (TN + FP)	3/(3+1)	4/(4+2)
PPV = TP / (TP + FP)	11/(11+1)	10/(10+2)
NPV = TN / (TN + FN)	3/(3+1)	4/(4+1)

Table VI

Performance evaluation of toxic granule & IT ratio in neonatal septicemia

Name	Sensitivity	Specificity	PPV	NPV
Toxic granules	91.67%	75%	91.67%	75%
IT Ratio>0.2	90.91%	66.67%	83.34%	80%

Discussion

In this study presence of toxic granules in neutrophil and raised Immature to total neutrophil ratio> 0.2 were correlated with positive blood culture result. All-Party Parliamentary Group (APPG) on Pneumococcal Disease Prevention in the Developing World in 2008 reported that most affected children that reach the hospitals receive empirical antibiotic therapy prior to coming to the hospitals, thus rendering blood and CSF cultures sterile and non-diagnostic.[11] A study on Maternal and neonatal colonization in Bangladesh: prevalence, etiologies and risk factors, the investigators found that Maternal colonizers colonize the umbilicus, an important precondition causing neonatal sepsis.[12] Among the studied 152 neonate, total 7.9% (12/162) neonate had positive blood culture and 75% babies were infected with Gram negative bacteria. This finding is close to study that was evaluating the causative agents in sepsis at Sir Salimullah Medical College & Hospital (SSMCH), the investigators found that 79% of sepsis was caused by Gram negative bacteria.[13]

The common isolated Gram-ve organisms were found *Klebsiella pneumoniae* in 33.34% cases, which was similar to study of Mannan et al.[14] *E. coli* was found in in 25% case which was similar to another study of DSH where they found *Klebsiella spp* in 43% & *E. coli* in 23% in 13% of neonates with sepsis.[15,16]

Infection causes arrest of maturation, immature neutrophil count increases in peripheral blood.[17] Among the neonates with positive blood culture result, the low number of total neutrophil count (<1750/cumm) was found in 75% of culture positive sepsis showing significant relationship of total neutrophil count <1750/ cu mm (Neutropenia) with proven sepsis in neonate (p=<0.05) as supported by Lambert RM et al.[18] In this study, Immature to total neutrophil ratio was markedly raised (I:T>0.2) in 91.67% neonates with proven sepsis and in 22.85% babies with negative blood culture result (p=<0.05). The sensitivity, specificity, positive predictive values and negative predictive values of IT ratio>0.2 were evaluated, those were 90.90%, 66.67%, 83.34% and 80% respectively which were 93.75%, 94.44%, 93.75% and 89.47% in the study of Makkar M et al.[19]

Toxic granules in neutrophil were found in 91.67% neonates with positive blood culture in this study. The sensitivity, specificity, PPV and NPV of toxic granules were found 91.66%, 75%, 91.66% and 75% in diagnosis of sepsis. Those parameters were found as 78.12%, 94.44%, 92.59% and 82.92% in study of Narasimha A et al.[20] In performance evaluation of a diagnostic test to identify sepsis, sensitivity and positive predictive value is more important than specificity and negative predictive value.

In Bangladesh there is still lack of diagnostic facilities. In case of suspected sepsis, we can do CBC and peripheral blood film even in primary health care centre, as no sophisticated instrument is required to perform the test. Then earlier administration of antibiotic will be possible to limit the neonatal morbidity and reduce neonatal mortality.

Conclusion:

Toxic granule in neutrophil and raised IT ratio>0.2 were found useful, simple and effective test to early screening of neonatal sepsis. Presence of toxic granules in the cytoplasm of neutrophil proved more efficacy in diagnosis of sepsis. The sensitivity and specificity of toxic granules to diagnose neonatal sepsis were 91.67% and 75% respectively.

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