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Research Article

Role Of Neutrophil Cd64 in Identification of Neonatal Sepsis

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Abstract

Detection of neutrophil CD64 may help in the early diagnosis of neonatal sepsis and may prevent unnecessary delay in diagnosis, enable prompt start of treatment and will help in reducing mortality and sepsis related complication. Another advantage is that neutrophil CD64 expression is not influenced by antibiotic therapy. Absence of any research in this field in our country has tempted me to undertook this study. This cross-sectional study was carried out in the Department of Clinical Pathology, Neonatology and Microbiology & Immunology, BSMMU, Dhaka. Total 60 neonates who fulfilled inclusion criteria were included in the study. After taking inform written consent from patient's attendant, blood sample were obtained from peripheral venipuncture in all neonates within 24 hours of admission with all aseptic precaution. A total 3.5 ml venous blood was taken of which 1.5 ml was collected in EDTA tube for complete blood count, PBF and for neutrophil CD64 estimation and another 2.0 ml for blood culture. Neutrophil CD64 expression were measured by Flow cytometry. In all observation, early onset of sepsis was observed more (62.5%) than that of late onset of sepsis. Among the infected newborns, male was predominant (57.5%). Preterm (82.5%) and low birth weight babies (77.5%) are more susceptible to infection. Premature rupture of membrane (PROM) >24 hours was found to be an important risk factor in neonatal septicemia. Blood culture was found positive only in 9 (22.5%) cases. Platelet count and IT ratio were found significantly associated with sepsis ($p<0.05$). In the present work neutrophil CD64 showed high sensitivity, specificity, PPV and high NPV (100%, 54.9%, 28.13% and 100% respectively). The results of our study also showed significantly elevated levels of CD64 in septic neonates (36.03±25.70) when compared with controls (4.85±2.95) and also their percentage of expression was higher in culture positive sepsis (77.07±15.07%) than culture negative sepsis (26.56±13.46). Combination of the studied markers such as neutrophil CD64 + IT ratio was associated with higher sensitivity (100%), specificity (62.5%), positive predictive values (32.14%), and high negative predictive value (100%). So neutrophil CD64 is more reliable marker for early diagnosis of neonatal sepsis. It is better than other established marker of neonatal sepsis. It prevents unnecessary delay of treatment and shortened the hospital stay, thereby reduce mortality and sepsis related complications

Key words: neutrophil cd64; identification; neonatal sepsis

Introduction

Diagnosis of neonatal sepsis is one of the most difficult tasks in clinical practice. As the disease progress more rapidly than adult and the mortality rate is higher in neonates, timely diagnosis of neonatal sepsis is essential (Zaki and Sayed, 2009). Several different laboratory determinations are helpful in diagnosis of neonatal sepsis. Among them blood cultures are used as the gold standard for diagnosis of sepsis. It helps to make therapeutic decision, especially in choosing the appropriate antibiotics (Layseca, 2002). The blood cultures have some difficulties. Culture results may be delayed for 24 hours (preliminary report) to 7 days (final report) after collection. Positive cultures ranged from 8% to 73% in the diagnosis of neonatal sepsis (Chiesa et al., 2004). The possibility of sepsis in the presence of negative blood culture is noted in neonates who are exposed to antibiotics in utero (Bhandari et al., 2008). As a results of unnecessary exposure to antibiotics in neonates with clinical suspicion of sepsis, creates an environment for emergence of

bacterial resistance (Magudumana et al., 2000). The negative microbiological cultures do not always exclude the presence of bacterial sepsis (Ng PC et al., 2004). Blood cultures are often negative in some cases of pneumonia and meningitis (Layseca., 2002). As the sensitivity of blood culture is low and longer time required and false negative result may be found, so the other tests in diagnosis of neonatal sepsis are warranted. So early diagnosis of neonatal sepsis is still a great challenge for both the developed and developing countries. Recently numerous cell surface antigens have been studied as promising biomarkers of infection, including CD11b, CD69 and CD64 (Ng PC et al., 2006). In flow cytometric technology neutrophil CD64 is found to be a promising marker for diagnosis of early and late infections in newborns.

Methods

Study design: Cross sectional

Place of study: This study was conducted in the Department of Clinical Pathology, Department of Neonatology and in the Department of Microbiology and Immunology, BSMMU, Dhaka.

Study population:

- Neonates with signs and symptoms of sepsis, admitted in the Neonatology department in BSMMU
- Neonates with no symptoms or signs of sepsis as control.

Inclusion criteria

1. Neonates (Birth to 28 days)

2. Sex: Both sex

3. Neonates who are clinically diagnosed as sepsis

4. Control: Neonates with no symptoms or signs of infection. Sample was taken from the neonates because of suspicion of other diseases and no illness was detected subsequently. We also took blood for follow-up in neonate with no suspicion of infection and those having physiological jaundice.

Exclusion criteria

1. Neonates with gross congenital anomalies

2. Neonates with chromosomal abnormalities

3. Neonates with severe jaundice due to blood group (ABO, Rh) incompatibilities.

Total sample size was 60

Sampling technique

Purposive sampling. As per inclusion criteria the patient was enrolled in this study. The whole procedure was explained to the patient attendant and informed written consent was taken.

Laboratory assay

1. Neutrophil CD64 assay in flowcytometry technology

2. Complete blood count (Hb%, RBC count, haematocrit, TC, DC, and platelet count) and peripheral blood film (PBF) with IT ratio

3. Blood culture and sensitivity

Specimen collection

After taking informed written consent from attendant blood sample were obtained from peripheral venipuncture in all neonates within 24 hrs of admission. A total 3.5 ml venous blood was taken of which 1.5 ml was collected in EDTA tube for complete blood count, PBF and for neutrophil CD64 estimation. Another 2.0 ml for blood culture for the purposes of this study. Samples was remained acceptable for up to 24 hours after collection when held at room temperature (18-22oC) and for 48 hours when refrigerated (2-8ºC).

To maintain quality assurance and to make the study more authenticated the following steps were done-

1) At first 3 normal healthy neonate′s blood sample ware collected and the neutrophil CD64 expression was measured by BD FACS verse flow cytometer.

2) Then 5 cases were taken as per inclusion criteria, the data sheet was filled up and the laboratory tests were done. The result was compared with the expected outcome. After the pilot study, the original study was commenced.

Data collection

Data were collected by a pre designed proforma. Blood sample was obtained from patients suspected cases of neonatal sepsis or clinically sepsis. Patient information was obtained through using patient's information sheet which involved questionnaire and clinical findings. Data editing, clearing and analysis was done by statistical package for social science (SPSS) 17.0. Sensitivity, specificity, PPV, NPV of neutrophil CD64 was calculate using specific formulas that is specified. Universal precaution was obtained. Gloves, lab coat, and safety glasses were worn when handling all blood products. Disposable plastic, glass, paper and gloves that contact blood were placed in a biohazard bag. Non-disposable materials at the end of working day were disinfected by autoclave. Pipette by mouth was avoided. Washing hands thoroughly was done after removal of personal protective devices used in handling specimens and kit reagents. Eating, drinking or smoking was avoided in designated working areas.

Results

	Neutrophil CD64				
	Positive n=32		**Negative** n=28		**p value**
Age group	**n**	**%**	**n**	**%**	
0-3 days	19	59.38	11	39.29	0.19^{ns}
> 3 days	13	40.62	17	60.71	
Sex					
Male	18	56.25	17	60.71	0.72^{ns}
Female	14	43.75	11	39.29	
Gestational age					
Preterm <37wks	27	84.38	14	50.00	0.004^{s}

Term ≥ 37wks	05	15.63	14	50.00	
Birth weight					
Very low birth weight ≤ 1500 gm	11	34.38	00	0.00	
Low birth weight >1500-2499 gm	15	46.88	09	32.14	<0.001[s]
Normal weight ≥2500 gm	06	18.75	19	67.86	
PROM					
Yes	24	75.00	06	21.43	<0.001[s]
No	08	25.00	22	78.57	

Table I: Neutrophil CD64 and demographic characteristics of study population (n=60).

Table I shows comparison between demographic characteristics with Neutrophil CD64. Age group 0-3 days 19(59.38%) were in neutrophil CD64 positive cases and 11(39.29%) in neutrophil CD64 negative group (p>0.05). Male were predominant, 18(56.25%) & 17(60.71%) in neutrophil CD64 positive and negative group (p>0.05). Gestational age preterm 27(84.38%) were in neutrophil CD64 positive cases and 14(50.0%) in neutrophil CD64 negative group. Birth weight, VLBW & LBW 26(81.26%) were in neutrophil CD64 positive cases and 09(32.14%) in neutrophil CD64 negative group (p<0.05). Premature rupture of membrane 24(75.0%) were in neutrophil CD64 positive cases group 06(21.43%) in neutrophil CD64 negative group (p<0.05). Chi-square test showed there were significant difference in ggestational age, birth weight and premature rupture of membrane among the two groups with Neutrophil CD64 (p<0.05).

Parameters	Case(n=40)		Control(n=20)		p value
	Mean Min-max	±SD	Mean Min-max	±SD	
Hb (gm/dl)	13.96 7.40-22.20	±3.15	14.98 7.60-20.90	±3.32	0.24[ns]
TLC (/cumm of blood)	14287.50 5000-36000	±8348.10	13475.0 7000-20000	±3625.47	0.68[ns]
ANC (/cumm of blood)	8533.25 1750-29580	±7563.92	7000.50 3420-14000	±2651.31	0.38[ns]
IT ratio	0.22 0.08-0.42	±0.08	0.12 0.08-0.22	±0.02	<0.001[s]
PLT ($x10^9$/L)	159.37 20.0-550	±110.44	264.25 125-800	±152.18	0.001[s]
Neutrophil CD64 (%)	36.03 5.01-96.67	±25.70	4.85 0.61-7.90	±2.95	<0.001[s]

Table II: Laboratory test results of cases and control (n=60).

Table II shows mean difference between cases and control neonates with laboratory findings. Mean Hb were 13.96(±3.15) gm/dl in cases and 14.98(±3.32) gm/dl in controls (p>0.05). Mean total leukocytes count was 14287.50(±8348.10) /cumm of blood in cases and 13475.0(±3625.47) /cumm of blood in controls (p>0.05). Mean absolute neutrophil count was 8533.25(±7563.92) /cumm of blood in cases and 7000.50(±2651.31) /cumm of blood in control (p>0.05). Mean IT ration was 0.22(±0.08) in cases and 0.12(±0.02) in control (p<0.05). Mean platelet count was 159.37(±110.44) x109/L in cases and 264.25(±152.18)x109/L in control group (p<0.05). Neutrophil CD64 were 36.03 (±25.70) % in cases and 4.85 (±2.95) % in control group (p<0.05). Values (mean□SD) were expressed in between groups analysis done by students't' test (un paired).

Parameters	Blood culture		p value
	Positive Mean (±SD)	Negative Mean (±SD)	
Neutrophil CD64	77.07(±15.07)	26.56(±13.46)	<0.001[s]

Table III: Results of neutrophil CD64 detected by flow cytometer in culture positive and culture negative cases of neonatal sepsis (n=40).

Table III shows percentage of expression of neutrophil CD64 was higher in culture positive sepsis (77.07±15.07%) than culture negative sepsis (26.56±13.46%). The difference was statistically highly significant (p<0.001) between culture proven and unproven sepsis with neutrophil CD64.

	Sensitivity	Specificity	PPV	NPV	Accuracy
Neutrophil CD64	100%	54.9%	28.13%	100%	61.67%
IT ratio	66.6%	58.82%	22.22%	90.90%	60.0%
PLT	50.0%	47.06%	20.59%	77.42%	47.69%
IT ratio + CD64	100%	62.75%	32.14%	100%	68.33%
PLT +CD64	100%	60.50%	27.50%	100%	65.0%

Table IV: Validity of different laboratory tests with blood culture (n=60).

Table IV shows that sensitivity of Neutrophil CD64 was 100%, specificity 54.9%, accuracy 61.67%, positive and negative predictive values were 28.13% and 100% respectively. Table shows that sensitivity of IT ratio was 66.6%, specificity 58.82%, accuracy 60.0%, positive and negative predictive values were 22.22% and 90.90% respectively. Table shows that sensitivity of PLT count was 50.0%, specificity 47.06%, accuracy 47.69%, positive and negative predictive values were 20.59% and 77.42% respectively. Table shows that sensitivity of IT ratio+CD64 was 100% specificity 62.75% accuracy 68.33%, positive and negative predictive values were 97.44% and 100% respectively. Table shows that sensitivity of PLT+CD64 was 100%, specificity 60.50%, accuracy 65.0%, positive and negative predictive values were 27.50% and 100% respectively.

	Cut of value	Sensitivity	Specificity	Area under the ROC curve	95% Confidence interval (CI)	
					Lower bound	Upper bound
Neutrophil CD64	>10 %	100%	54.9%	0.970	0.470	0.755
Blood culture	--	22.5	100.0	0.613	0.934	1.006

Table V: Receiver-operator characteristic (ROC) curve of neutrophil CD64 and blood culture for diagnosis of neonatal sepsis.

Table V showed Neutrophil CD64, with a cut off value >10% sensitivity 100%, specificity 54.9% and area under the ROC curve was 0.970. Blood culture sensitivity and specificity were 22.5% and 100% with area under the ROC curve was 0.613.

Discussion

Diagnosis of neonatal sepsis is still a challenge, as there is no single reliable test for early diagnosis. Currently blood culture is the most reliable method for detection of bacterial infections. But the sensitivity of blood culture is low, longer time required for report (preliminary 24hours, final 7 days) and false negative result may be found. Culture positive sepsis is a small proportion of a larger group of clinical sepsis (with negative blood cultures). So it is clear that to manage neonates with sepsis properly, a single reliable marker of infection is needed, to avoid unnecessary antibiotic therapy. In this study, we tried to determine the neutrophil CD64 expression as an immunological marker for rapid diagnosis of neonatal sepsis. This study included 60 patients with a mean age of 5.9±6.49 days. There was 40 clinically diagnosed sepsis neonates and 20 control neonates who did not have any symptom or sign of sepsis. In sepsis group, early onset was observed more (62.5%) than that of late onset of sepsis (37.5%). This observation is consistent with the findings of others (Noor et al.,2008; Khaleda et al., 2010) in BSMMU. Preterm (82.5%) and low birth weight babies (77.5%) are more susceptible to infection. Higher susceptibility of infection in preterm and low birth weight babies might be due to low level of IgG and lower defense mechanism. There were significant differences in means of gestational age and birth weight between neonates. These findings showed that prevalence of infection in neonates is inversely related to gestational age and birth weight. Duration of premature rupture of membrane (PROM) for >24 hours have to be an important risk factor in neonatal septicemia because PROM poses of ascending infection to the fetus. In our study, PROM was 75% in septic neonates and none in control group. These findings are consistent with the study of Khaleda et al., (2010) and Kuruvilla et al., (1998). In this study, out of 40 clinically diagnosed neonatal sepsis, blood culture was found positive in 9 (22.5%) cases. Khaleda et al., (2010) in BSMMU, found 12% neonates as culture positive sepsis. In the present

study, there was high percentage of expression of CD64 on neutrophils in patients (36.03 ±25.70) when compared with controls (4.85±2.95) and also their percentage of expression was higher in culture positive sepsis (77.07±15.07%) than culture negative sepsis (26.56±13.46). These results are consistent with another study (Azza et al., 2013;). This may be due to

faulty sterile technique in collection procedure, insufficient sample volumes, intermittent or low-density bacteraemia, or suppression of bacterial growth by earlier antibiotic administration and delayed arrival of patients. Total leukocyte count (TLC) and absolute neutrophil count are of little clinical use in the diagnosis of neonatal sepsis because of wide variation in values. Neutropenia has been more common in association with sepsis, compared with neutrophilia (Rodwell et al.,1998), probably because of increased adherence to altered endothelial cells and utilization at the site of infection. IT ratio is the ratio between immature neutrophil count (band form) and the total neutrophil in a blood smear. As a marker of sepsis in newborn babies, the IT ratio should be >0.2(Khaleda et al., 2010). In the present study, I/T ratio >0.2 had a sensitivity, specificity, PPV and NPV of 66.6%, 58.82%, 22.22% and 90.90% respectively. While an I/T ratio >0.2 suggested by Khaleda et al., (2010) had a sensitivity of 100% specificity 04%, PPV13% and NPV of 100%. Specificity and positive predictive value were low because of large number of false positive results. Therefore, this parameter alone should not be evaluated for diagnostic purpose. Neonates with sepsis develop thrombocytopenia, possibly because of disseminated intravascular coagulation (DIC) and the damaging effects of endotoxin on platelets. In this study, we found thrombocytopenia with cut off value<150x109/L had sensitivity of 50.0%, specificity 47.06%, PPV 20.59% and NPV 77.42%. This parameter could be used as an early but nonspecific marker for sepsis. These results are consistent with other study (Khaleda et al., 2010; Shirin et al.,2005). There are many advantages of using neutrophil CD64 expression as an indicator of neonatal sepsis, as the quantitation of neutrophil CD64 is rapid (<60minutes) and only minimal blood volume (100 µl) is used, which is a real advantage in neonates (Davis BH., 2006). In fact, for the present study, no extra blood was obtained from the neonates to perform this test; the samples sent for the complete blood count is adequate. In the present study, neutrophil CD64 showed high sensitivity 100%, specificity 54.9%, PPV, 28.13% and also high NPV 100%. Specificity and PPV were low because of large number of false positive result. This may be due to small sample size and blood culture was found positive only in 22.5% cases of neonatal sepsis. The results of our study also showed significantly elevated levels of CD64 in septic neonates when compared to healthy controls. This finding coincided with the outcome of numerous studies done for the diagnostic performance of neutrophil CD64 in neonatal sepsis in view of the high sensitivity and negative predictive values (Azza et al., 2013; Young et al., 2012; Dhlamini et al., 2011; Minoo et al., 2006; Bhandari et al., 2007). The results of present study clearly indicated that measurement of neutrophilCD64 can be useful for diagnosis of neonatal sepsis in early phase. Because of high cost and skilled technology, it will be difficult to introduce this test in all level in our country. In our study, combination of the studied markers such as neutrophil CD64 + IT ratio showed both higher sensitivity (100.0%), specificity (62.75%), high negative predictive value (100%). Also, neutrophil CD64 + platelet count had higher sensitivity (100%), specificity (60.5.0%) and negative predictive value (100%). In a previous work by

Bhandari et al., (2007), the use of CD64 in combination with IT ratio to improve the sensitivity and Specificity and negative predictive value. An accurate inflammatory marker with high diagnostic sensitivity and negative predictive value for neonatal sepsis would be a valuable tool for therapeutic decision-making process and may reduce the unnecessary use of antibiotics. In that case, neutrophil CD64 surface antigen can replace and, in many cases, may be an adjunct the conventional and routine markers of neonatal sepsis.

Conclusion

Flow cytometric assessment of neutrophil CD64 may be considered as a rapid and reliable marker for the diagnosis of bacterial neonatal sepsis in comparison to other conventional and routine diagnostics markers. However, important issues of cost and availability are required to be evaluated in routine clinical setting.

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