Early diagnosis of neonatal sepsis with special focus on leukergy test – A forgotten tool

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Received - 11 November 2019 Initial Review - 24 November 2019 Accepted - 13 December 2019

ABSTRACT

Background: Sepsis in newborn is a leading cause of mortality and morbidity. Neonates with one or more predisposing factors (namely, low birth weight, prematurity, foul-smelling liquor, prolonged rupture of membranes, and prolonged labor) are at increased risk of sepsis. **Objective:** The objective of the study was to establish laboratory tests that would help in the early diagnosis of neonatal sepsis with a special focus on leukergy test. **Materials and Methods:** The present study was a hospital-based prospective study involving 400 neonates admitted in the Neonatal Intensive Care Unit for clinically suspected sepsis, with different symptoms during the period of October 2018 to September 2019 at a government hospital of Bihar. In this study, we evaluated peripheral smear to establish an appropriate hematological parameter for diagnosis of neonatal sepsis even before blood culture. **Results:** The total neutrophil count was highly sensitive (77.8%) and increased band cell: Mature neutrophil ratio was highly specific (99.2%) for an early diagnosis of neonatal sepsis. The positive predictive value (PPV) was the highest for band cell: Mature neutrophil ratio (97.2%) and negative predictive value (NPV) were the highest for decreased platelet count (78.3%). Leukocyte adhesion/aggregation test had a sensitivity of 59.1%, specificity of 60.2%, PPV being 68.9%, and NPV of 49.5%. **Conclusion:** Positive leukergy test is a good predictor for early diagnosis of neonatal sepsis and can be used in resource poor settings with less time. Hematological parameters are good indicators for early diagnosis of neonatal sepsis. A group of tests is more reliable than any single parameter.

Key words: *Early diagnosis, Leukergy test, Leukocyte adhesion/aggregation test, Neonatal sepsis*

Neonatal sepsis is a disease which depicts systemic response of infection in the newborn. Sepsis in newborn is deadly and can lead to major morbidity and mortality. Neonates with one or more predisposing factors (namely low birth weight, prematurity, foul-smelling liquor, prolonged rupture of membranes, prolonged labor, perinatal asphyxia, and maternal peripartum infection) are at increased risk for sepsis [1]. The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002–2003) is 30/1000 live births in India. Diagnosis of neonatal sepsis can be difficult as the early signs of sepsis may be subtle and variable at different gestational ages. The definitive diagnosis of septicemia is made by a positive blood culture, which requires a minimum of 48 h and yields positive results only in 50–60% cases.

Inability to adequately diagnose neonatal sepsis can result in unnecessary and prolonged exposure of the newborns to antibiotics. The laboratory tests that assist the clinician in diagnosis of infection in neonates have considerable relevance. The objective of this study was to establish laboratory tests that would help in the early diagnosis of neonatal sepsis with special focus on leukergy test.

MATERIALS AND METHODS

The present study was a hospital-based prospective study involving 400 neonates admitted in the Neonatal Intensive Care Unit (NICU) with different symptoms during the period of October 2018 to September 2019 at a government hospital of Bihar. Peripheral blood smears (PBS) were made from freshly collected specimen in anticoagulant containing vial and stained with standard hematoxylin and eosin staining method. All neonates of any gestational age admitted in the NICU of the Department of Pediatrics during the study period for clinically suspected sepsis were included in the study. All neonates with lethal congenital malformations and malignancies at the time of presentation were excluded from this study.

Leukergy test or leukocyte adhesion/aggregation test was one of the hematological parameters evaluated. On each PBS slide, 300 white blood cells of all types were counted and the percentage of leukergy (agglomerated cells) was calculated. Agglomeration was considered to be positive when at least 3 leukocytes were in close proximity, the distance between their nuclei being less than the diameter of 1 cell [2]. The count was

made twice and final leukergy test was the average of the two readings.

Grading of leukergy test was as follows: Grade 0 – leukergy in <10% of leukocytes, Grade 1 – leukergy in 11–19% of leukocytes, Grade 2 – leukergy in 20–34% of leukocytes, and Grade 3 – leukergy in >34% of leukocytes. Leukocyte adhesion/aggregation test presenting with Grade 0 was considered negative while Grades 1, 2, and 3 were considered leukergy positive.

Other analyzed hematological parameters were hemoglobin (by cyanide-free method on automated cell counter – Sysmex XP 300) [3,4], the presence of normoblast, total leukocyte count (TLC), which can be decreased (<4000/mm^3) or increased (>25,000; 30,000; and 21,000/mm^3, respectively, at birth, 12–24 h, and day 2 onward), total neutrophil count which was decreased or increased (normal – 7800–14,500/mm^3 [<72 h] and 1750–4500/mm^3 [>72 h]), band cells increased (normal – 500–1450/mm^3 [<72 h] and <500/mm^3 [up to 28 days]), and band cell: Total neutrophil ratio – increased (>0.2), band cell: mature neutrophil ratio – increased (>0.3), presence of degenerative changes in neutrophils (toxic granules, dohle body, vacuolated cytoplasm), and decreased platelet count (<100,000/mm^3) [5].

Blood culture is the gold standard test used to diagnose neonatal sepsis and this has also been used as gold standard in our study. Blood was collected using aseptic technique and inoculated on blood agar and MacConkey agar, reported after 48 h of incubation [6]. Positive results were further confirmed by Gram staining and other biochemical tests [7]. The various blood parameters were then compared with the gold standard to find the usefulness in diagnosing neonatal sepsis with a special focus on leukergy test. The present study was conducted in accordance with the current version of the Declaration of Helsinki. Informed written consents were taken from guardian/parents of children involved.

RESULTS

Of 400 cases, 201 were male (50.2%) and 199 were female (49.8%). The number of cases belonging to the age group of <24 h was 20 (5.0%), between 25 and 48 h was 48 (12.0%), between 49 and 72 h was 128 (32.0%); of 73–96 h was 60 (15.0%) and between 97 and 120 h was 64 (16.0%); and those belonging to the age group of >120 h was 79 (20.0%). The maximum number of cases (32.0%) belonged to the age group of 49–72 h (day 3).

The results of various hematological changes are summarized in Table 1. Blood culture was positive in 60% (240) cases. The positive predictive value (PPV) was the highest for increased band cell: Mature neutrophil ratio (97.2%) while the highest negative predictive value (NPV) was of decreased platelet count (78.3%) followed by the presence of normoblast (72.6%) which indicated that neonates did not have any evidence of sepsis [8]. Leukergy or leukocyte adhesion/aggregation test had a sensitivity of 59.1%, specificity of 60.2%, PPV of 68.9%, and NPV of 49.5%.

DISCUSSION

In the present study, it was observed that the total neutrophil count showed maximum abnormality (77.8% sensitive) if a neonate was having septicemia. Band cell: Mature neutrophil ratio would be normal if a neonate is not having sepsis (99.2% specific). Increased band cell: Mature neutrophil ratio had maximum PPV (97.2%) while low platelet count has the highest NPV (78.3%) which indicated that neonates had no evidence of sepsis. A group of tests is more reliable in the early diagnosis of neonatal sepsis than any single laboratory parameter [9].

Blood culture may come out to be sterile if antibiotics are administered before withdrawing the blood sample for laboratory investigations [10]. In such situations, hematological changes are much more useful for diagnosis and management. Leukergy test had a sensitivity of 59.1%, specificity of 60.2%, PPV of 68.9%, and NPV of 49.5%. Leukergy test and degenerative changes in neutrophils are good predictors for an early diagnosis of neonatal sepsis. Otremski *et al*. found that leukergy test was even more accurate than erythrocyte sedimentation rate (ESR), TLC, and blood culture in diagnosing septic process that too in a rapid and inexpensive way [11].

Maharshak *et al*. conducted a prospective study involving 121 patients with non-viral acute febrile illness with no abnormality in TLC and found that leukocyte aggregation was one of the earliest responses of the host to the inflammatory/septic process and can help in the early diagnosis [12]. Rotstein *et al*. clearly demonstrated that increased TLC and leukocyte aggregation do not result from a simple chance collision, rather they conveyed a

Table 1: Performance of individual laboratory parameter

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Leukergy test	59.1	60.2	68.9	49.5
Total leukocyte count	50.4	80.6	79.6	52.0
Total neutrophil count	77.8	15.3	57.9	31.2
Band cell count	71.1	55.5	68.9	56.3
Band cell: Total neutrophil ratio	33.1	86.4	78.2	46.2
Band cell: Mature neutrophil ratio	29.5	99.2	97.2	48.3
Degenerative changes in neutrophil	14.3	92.1	72.3	41.6
Platelet count	41.4	92.5	89.2	78.3
Hemoglobin level	59.2	37.1	58.4	37.6
Normoblast	46.2	81.4	78.7	72.6

NPV: Negative predictive value, PPV: Positive predictive value

message of biological relevance, reflecting both the appearance of cell adhesive molecules during activation as well as the contribution of plasmatic sticky proteins [13].

Urbach *et al*. found greater sensitivity, specificity, and PPV of leukergy test than TLC, differential count, and ESR in differentiating children with a viral and bacterial infection in a rapid and inexpensive way [14]. Rotstein *et al*. found that capillary and venous leukergy test had the best specificity and PPV in diagnosing an acute bacterial infection [15]. The leukergy test with validity features at par in all aspects can be used as a tool or one of the parameters of septic screen to help diagnose neonatal sepsis in an inexpensive and rapid way. As this was a hospital-based study, the exact validity of the ascribed tests may vary than what prevails in the community, which is the limitation of this study and a field survey is required.

CONCLUSION

Positive leukergy test is a good predictor for the early diagnosis of neonatal sepsis and can be used in resource poor setup and requiring less time to perform. To the best of our knowledge, studies involving leukergy tests are very limited and more elaborate studies need to be warranted for its routine use in the diagnosis of neonatal sepsis.

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Funding: None; Conflicts of Interest: None Stated.

How to cite this article: Prakash A, Richa R, Narain B. Early diagnosis of neonatal sepsis with special focus on leukergy test – A forgotten tool. Indian J Child Health. 2019; 6(12):673-675.

Doi: 10.32677/IJCH.2019.v06.i12.009