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Original Article

Pathology Section

Early Diagnosis of Neonatal Sepsis Using the Leukocyte Alkaline Phosphatase Score: A Cohort Study

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ABSTRACT

Introduction: The clinical features of sepsis in neonates are subtle and non specific, requiring a high index of suspicion for early diagnosis. Blood culture is the gold standard for diagnosis, but it is time consuming. Therefore, there is a need for a cost-effective and reliable screening tool. The Leukocyte Alkaline Phosphatase (LAP) activity of neutrophils is known to increase during bacterial infections in adults.

Aim: To determine the activity of LAP in Neonatal Sepsis (NS) and compare the results with blood culture.

Materials and Methods: This is a prospective cohort study conducted from January 2018 to June 2019 at Mandya Institute of Medical Sciences, Karnataka, India. Total of 200 neonates by their haematological profile were clinically suspected of having sepsis. A peripheral smear was prepared from a drop of blood and stained to assess the LAP activity. In each smear, a total of 100 consecutive segmented neutrophils were examined and were rated from 0 to 4 according to the red granular precipitate intensity within their cytoplasm. The possible range of 0-400. The blood culture report was obtained from the case sheet. The neonates were divided into two groups: Group 1 consisted of culture-proven sepsis cases, and Group 2 consisted of neonates with clinical suspicion of sepsis but negative blood culture results. Descriptive analysis was performed using mean and standard deviation for quantitative variables, and frequency and proportion for categorical variables. Chi-square test was conducted, and a p-value <0.05 was considered statistically significant.

Results: Out of the 200 cases studied, 64 neonates showed a positive blood culture. The most common causative organism observed was *Klebsiella pneumoniae,* which was seen in 56 neonates. The age of the neonates ranged from newborn to 28 days old, with 115 being male. Furthermore, 116 neonates were term neonates, and 146 presented with early onset sepsis. The study revealed a wide range of LAP activity in both groups. In the culture-positive sepsis group, the LAP activity ranged from 36 to 350, while in the clinical sepsis group, it ranged from 10 to 354. Due to the broad range of LAP scores observed, it is concluded that LAP activity is not useful as a screening test.

Conclusion: Findings of the present study indicate that LAP activity exhibited a wide range in both the culture-proven and clinical sepsis groups. However, due to this variability, LAP activity does not appear to be a reliable screening test for NS. While LAP activity assessment may still hold diagnostic value, further research is needed to refine its utility and explore its clinical relevance.

Keywords: Blood culture, Clinical sepsis, Culture proven sepsis, Newborn bacterial infections

INTRODUCTION

Systemic infection in newborns is the most common cause of neonatal mortality [1]. Neonatal Sepsis (NS) is a clinical syndrome characterised by signs and symptoms of infection occurring within the first month of life, with or without accompanying bacteraemia. Clinical features of sepsis in neonates are subtle and non-specific, requiring a high index of suspicion for early diagnosis. Timely diagnosis of NS is crucial because the illness can progress more rapidly in neonates compared to adults.

Blood culture is considered the gold standard for diagnosing septicaemia, but it is time consuming, taking a minimum of 48-72 hours for results and yielding positive results in only 30%-70% of cases [2]. Additionally, under-resourced laboratories may lack the necessary facilities for conducting blood cultures. Other investigations, such as C-reactive protein, when used alone, lack specificity and cannot be relied upon for diagnosing NS [3]. Procalcitonin and newer inflammatory markers like interleukin-6 and interleukin-8, as well as plasma elastase, are highly sensitive and specific for diagnosing NS. However, they require sophisticated and expensive kits [4].

LAP is an enzyme found in the cytoplasmic granules of leukocytes. In healthy adults, the normal LAP level ranges from 15 to 130. In newborns, LAP levels are two to three times higher than those in adults at birth. By 7-14 days, the LAP levels of neonates reach adult LAP levels. In adults, LAP activity is increased during bacterial infections [5]. However, conflicting data exists regarding LAP values in NS. Some studies conducted by Hugo D et al., Sharma SC, and Sharma U et al., showed a decrease in LAP scores during severe bacterial infections in neonates and infancy [6-8]. Conversely, Paul RS and Kumar A in their study, reported an increase in LAP scores during severe bacterial infections [5]. Given the conflicting data on LAP values in NS [9], the purpose of this study is to determine the activity of LAP in NS and compare the results with blood culture.

MATERIALS AND METHODS

The present cohort study examined the haematological profile of 200 neonates admitted to NICU at Mandya Institute of Medical Sciences (MIMS), Karnataka, with clinical suspicion of sepsis, from January 2018 to June 2019. It was initiated after obtaining approval from the Institutional Ethics Committee (IEC no: MIMS/IEC/RP/2017/195).

Inclusion criteria: Neonates admitted to our NICU with clinical suspicion of sepsis [Table/Fig-1] [10] were included in the study.

Exclusion criteria: Congenital disorders that could potentially affect leukocyte count or function, such as severe congenital neutropenias or Chediak-Higashi anomaly, those who had received antibiotics or blood transfusions prior to sample collection and those who had undergone previous surgery were excluded from the study.

General: Fever, temperature instability, not doing well, poor feeding, oedema	**Cardiovascular system:** Cold, clammy skin; pallor, mottling, tachycardia, hypotension, bradycardia
Gastrointestinal system: Abdominal distention, vomiting, diarrhoea, hepatomegaly	**Central nervous system:** Irritability, lethargy, tremors, seizures, hyporeflexia, hypotonia, abnormal moro reflex, irregular respirations, full fontanel
Respiratory system: Apnoea, dyspnoea, tachypnoea, retractions, flaring, grunting, cyanosis	**Haematologic system:** Jaundice, splenomegaly, pallor, petechiae, purpura, bleeding
Renal system: Oliguria	

[Table/Fig-1]: Initial Signs and Symptoms of Infection in Newborn [10].

Procedure

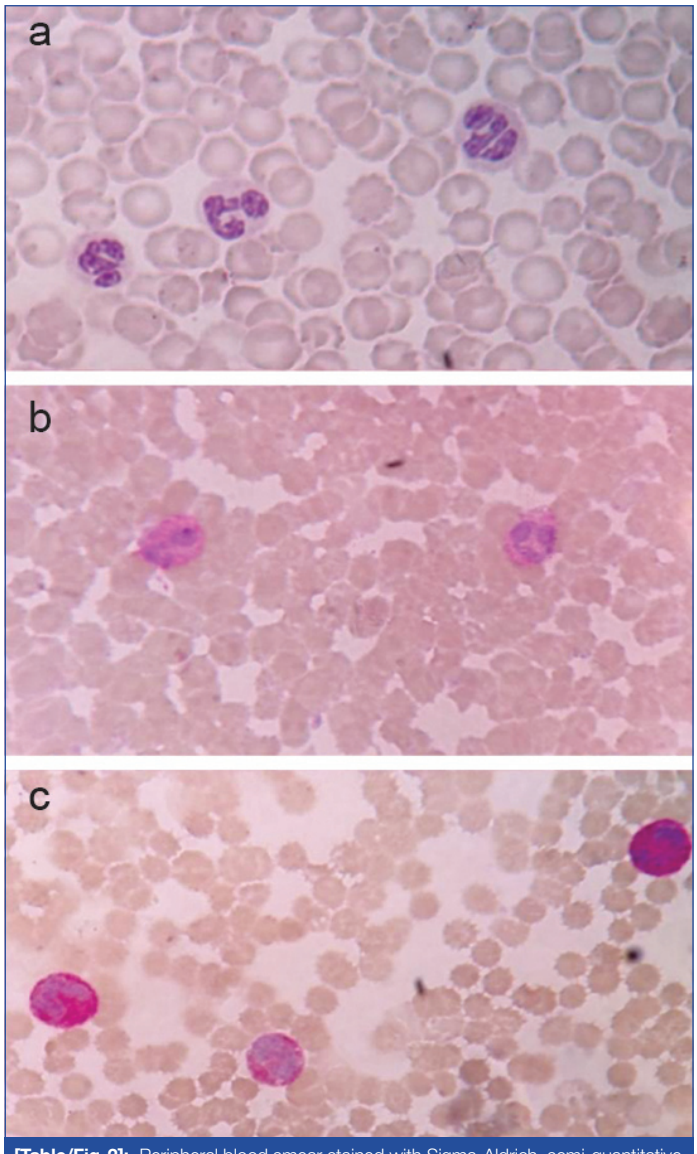
Data collection method involved obtaining clinical information from the newborn and mother's case sheets, including gestational age, age of onset of sepsis, sex, birth weight, mode of delivery, maternal risk factors, and presenting signs and symptoms. The blood culture report was also obtained from the case sheet.

The neonates were divided into two groups:

Group-1: Culture-proven sepsis (neonates with positive blood culture).

Group-2: Clinical sepsis (neonates with clinical suspicion of sepsis and negative blood culture) [Table/Fig-2].

A peripheral blood smear, without the addition of any anticoagulant, was prepared from a drop of venous blood to determine the LAP score. The smear was prepared either during cannula insertion or when blood was withdrawn for routine investigations. Capillary puncture solely for LAP score was avoided to minimise trauma to the neonates.



[Table/Fig-2]: Peripheral blood smear stained with Sigma-Aldrich, semi-quantitative LAP score kit (86R-1KT) for LAP activity (20X view). These neutrophils are showing (a) 0+; (b) 1+; (c) 2+ (middle), 3+ (left) and 4+ (right) lap activity.

LAP activity was studied using the Sigma-Aldrich (Bengaluru) semi-quantitative LAP score kit (86R-1KT). Peripheral smears were fixed to microscopic slides with a citrate acetone formaldehyde solution. The film was then incubated in a mixture of naphthol AS-BI alkaline solution with fast red violet LB. The alkaline phosphatase activity is indicated by an insoluble diffuse, red dye deposit in the cytoplasm of Polymorphonuclear Neutrophils (PMNs). LAP scoring was performed according to the method described by Kaplow [9]. Each individual neutrophil was rated from 0 to 4 [Table/Fig-1,3] based on the intensity of red granular precipitate within their cytoplasm. A total of 100 consecutive segmented neutrophils were examined and rated. The LAP score is the sum of the ratings of the hundred consecutive segmented neutrophils examined. The possible range of the LAP score is 0-400. For example, if all the hundred consecutive neutrophils studied in a smear show no staining (rating 0+), the LAP score would be 0. Similarly, if all the hundred neutrophils show a rating of 4+, the LAP score would be 400.

Precipitated Azo Dye in Cytoplasm				
Cell rating	**% of volume of cytoplasm occupied by precipitate**	**Size of granules**	**Intensity of staining**	**Background of cytoplasm**
0+	None	-	None	None
1+	50	Small	Faint to moderate	Colourless to very pale pink or blue
2+	50-80	Small	Moderate to strong	Colourless to pale pink or blue
3+	80-90	Medium to large	Strong	Colourless to pink or blue
4+	100	Medium to large	brilliant	Not visible

[Table/Fig-3]: LAP activity of an individual neutrophil is rated on a scale of 0-4. LAP score is obtained by summating the ratings ofhundred consecutive.

Neonatal sepsis is classified as [10]:

- Early onset Neonatal Sepsis (NS): Infections occurring before one week of life.
- Late onset Neonatal Sepsis (NS): Infections occurring after one week of life.

Birth Weight is categorised as [11]

- Very low birth weight: <1500 g.
- Low birth weight: 1500-2499 g.
- Normal birth weight: ≥2500 g.

STATISTICAL ANALYSIS

Descriptive analysis was conducted using mean and standard deviation for quantitative variables, and frequency and proportion for categorical variables. Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) version 22.0. The chi-square test was used, and a p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 200 neonates were included in the final analysis. Out of the 200 neonates, 64 showed positive blood culture. The study cohort predominantly consisted of male neonates (117), term deliveries (116), and early onset NS (146) [Table/Fig-4]. The most common organism isolated from blood cultures was *Klebsiella pneumonia* [Table/Fig-5]. The mean LAP score was not statistically significant between the two groups in terms of age, gestational age, onset of sepsis, and birth weight [Table/Fig-6].

N=200	Culture-proven sepsis (64)	Clinicalsepsis (136)
0 to 3-day-old (146)	47	99
4 to 7-day-old (31)	8	23
>7-day-old (23)	9	14
Male (115)	37	78
Female (85)	27	58
Preterm (84)	32	52
Term (116)	32	84
Early-Onset sepsis (146)	47	99
Late-Onset sepsis (54)	17	37
Very low birth weight (23)	10	13
Low birth weight (71)	29	42
Normal birth weight (106)	25	81

[Table/Fig-4]: Demography of Group-1 and Group-2.

Organisms isolated	No. of cases	Percentage
Klebsiella pneumoniae	56	87.5%
Staphylococcus haemolyticus	3	4.68%
Enterococcus aerogenes	2	3.12%
Achromobacter spp	1	1.56%
Escherichia coli	1	1.56%
Pseudomonas aeruginosa	1	1.56%

[Table/Fig-5]: Organisms isolated on blood culture (N=64).

	Infection status		
	Culture-proven sepsis (64) Lap score (Mean±SD)	Clinical sepsis (136) LAP score (Mean±SD)	p-value
0 to 3-day-old (146)	195.11±75.39	167.97±90.14	0.076
4 to 7-day-old (31)	197.5±84.03	150.7±86.19	0.194
>7-day-old (23)	179.11±89.41	137.29±81.75	0.261
Preterm (84)	185.32±78.53	164.58±84.99	0.271
Term (116)	200.52±76.69	160.17±91.42	**0.027**
Early-Onset sepsis (146)	195.11±75.39	167.97±90.14	0.076
Late-Onset sepsis (54)	187.76±84.71	145.62±83.64	0.093
Very low birth weight (23)	176±98.28	162.15±85.4	0.722
Low birth weight (71)	194.66±63.53	158.45±88.58	0.063
Normal birth weight (106)	198.28±84.98	163.63±90.25	0.092

[Table/Fig-6]: Comparison of LAP score between 2 groups based on age, gestational age, type of onset of sepsis and birth weight (N=200).

DISCUSSION

Out of the 200 neonates with clinical suspicion of sepsis, 64 (32%) neonates had a positive blood culture, and 73% of the neonates had early onset sepsis. Among the culture-proven sepsis group, 37 (57.8%) neonates were male. The most common presenting features of NS were respiratory distress, poor feeding, and lethargy. The most common pathogen isolated in the blood culture was *Klebsiella pneumonia* [Table/Fig-5]. Previous studies conducted by Hugo D et al., Sharma SC, Sharma U et al., reported a decrease in LAP score during severe bacterial infections [6-8]. However, Paul RS and Kumar A showed an increase in the LAP score during severe bacterial infections [Table/Fig-7] [5,6-8].

The present study showed a wide range of LAP activity in both groups. The LAP activity ranges from 36 to 350 in the culture-proven sepsis group and 10 to 354 in the clinical sepsis group [Table/Fig-8].

Variable degrees of haemolysis are observed in neonatal infections, suggesting that a haemolytic crisis may induce subtle changes in neutrophil metabolism and lead to depressed LAP activity. Neutrophils in the bone marrow exhibits 50% less LAP levels than circulating neutrophils, indicating that the decreased LAP activity

Study done by	Study conducted in the year	Place of study	LAP Score-Range
Hugo D et al., [6]	1979	Argentina	74-138
Sharma SC [7]	1980	Military hospital, Jabalpur Cantt, India	<132
Sharma U et al., [8]	1983	Jaykaylon Mother and Child Health Institute and state Zenana Hospital, Jaipur, India	79-202
Paul RS et al., [5]	1984	Saint Luke's Hospital, Ohio, USA	202.5- 262.9
The present study			
Culture-proven sepsis group	2019	MIMS, Mandya, Karnataka, India	36-350
The present study			
Clinical sepsis group	2019	MIMS, Mandya, Karnataka, India	10-354

[Table/Fig-7]: Comparison of LAP score range in Neonatal Sepsis (NS) among various studies [5,6-8,Present study].

	Infection status	
	Culture-proven sepsis (64) LAP score range	Clinical sepsis (136) LAP score range
0 to 3-day-old (146)	40-344	10-344
4 to 7-day-old (31)	72-350	14-354
>7-day-old (23)	36-336	11-288
Preterm (84)	36-344	10-332
Term (116)	77-350	10-354
Early-Onset sepsis (146)	40-344	10-344
Late-Onset sepsis (54)	36-350	14-354
Very low birth weight (23)	36-344	10-264
Low birth weight (71)	64-310	12-332
Normal birth weight (106)	72-350	11-354

[Table/Fig-8]: LAP score range of the 2 groups based on age, gestational age, type of onset of sepsis and birth weight (N=200).

in some infected infants might be due to the rapid release of functionally immature neutrophils from the bone marrow. Steroids are known to increase LAP activity. Normally, the steroid levels in infected newborns are either normal or increased due to stress-induced endogenous steroid secretion. This may explain the increased LAP activity in some children [12].

In the authors' opinion, the difference in LAP scores observed in different studies is possibly due to the variation in the time between blood sample collection and the onset of symptoms. The Total Leukocyte Count (TLC) varied from case to case in the present study, ranging from neutropenia to severe neutrophilia. The correlation between TLC and LAP activity was not analysed.

Limitation(s)

The exact time between the onset of the disease and the collection of the blood sample was not fixed. The sample was collected only after the clinical suspicion of sepsis was established. Although the LAP score is an unpredictable marker of NS, it can still provide value as a clinical pointer.

CONCLUSION(S)

This study highlights the challenges in using LAP activity as a practical screening test for NS. The wide variation in LAP activity observed within both the culture-proven and clinical sepsis groups, limits its effectiveness as a standalone diagnostic tool. While LAP activity assessment may not be suitable as a primary screening method, it could still be useful as part of a comprehensive diagnostic approach when combined with other clinical and laboratory indicators. However,

it is important to note that blood culture remains the gold standard for definitive diagnosis in NS cases, and further research is needed to develop improved diagnostic strategies.

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