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Original Article

Scope of mean neutrophil volume as an indicator of neonatal sepsis

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ABSTRACT

Background: Neonatal sepsis is one of the leading causes of mortality in developing countries because of its non-specific presentation. Blood culture is the gold standard for diagnosing sepsis, but culture results take 48 – 72 hours. Therefore, there is a need for an indicator that could be used as a simple parameter to indicate the possibility of evolving sepsis to the clinician. The efficacy of mean neutrophil volume as an indicator must be evaluated.

Materials and methods: The study aims to evaluate the utility of the mean neutrophil volume in the early diagnosis of neonatal sepsis. Following ethical clearance, the study involved analyzing mean neutrophil volume from peripheral smears of 50 newborns clinically diagnosed with neonatal sepsis. The control group included mean neutrophil volume findings from peripheral smears of normal newborns. Maternal or fetal factors like pregnancy-induced hypertension and birth asphyxia that could cause changes in neutrophils were excluded. The mean neutrophil value was calculated after establishing the mean diameter of 100 neutrophils per case using the National Institutes of Health Image J software.

Results: The average mean neutrophil volume was found to be significantly increased in the sepsis group (181x104fl) than in the control group (739x103fl). A statistically significant difference (p= < 0.0001) in the mean neutrophil volume was observed.

Conclusions: Mean neutrophil volume is a potential indicator to distinguish neonates with and without sepsis and help clinicians in the early diagnosis and management of sepsis.

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INTRODUCTION

"Neonatal sepsis" or "Sepsis Neonatorum" is defined as a generalized bacterial infection during the first month of life. India has a very high incidence of neonatal sepsis (17,000/ 1,00,000 live births) with a case fatality rate as high as 25% to 65%, highlighting the magnitude of the problem.[1] This mortality rate can be reduced by early intervention and effective management. Bacterial culture is the gold standard for diagnosis of sepsis but it takes 48-72 hours. Various hematological parameters like white blood cell (WBC) count, absolute neutrophil count, immature neutrophil count,

and morphological changes like toxic granules/ vacuoles have shown promising results in the diagnosis of sepsis. However, these morphological changes are not seen in all cases and are noted in the advanced stages of sepsis. Therefore, we need to find a parameter that can serve as an early and consistent indicator of sepsis. Mean neutrophil volume (MNV) can be one such parameter. Thus, in our study, we have tried to establish the utility of MNV as an ideal marker for the early diagnosis of sepsis.

MATERIALS AND METHODS

This is a prospective study conducted from August 2018 to July 2020. A total of 100 samples were evaluated and categorized into sepsis group (n =50) [sepsis proven (n =30), sepsis probable (n =20)], and no sepsis/control (n =50).

Neonates with a clinical suspicion of sepsis and/or blood culture-positive were included in the study. Cases with positive blood culture were grouped as sepsis group and cases with negative blood culture report but high clinical suspicion of sepsis with raised biochemical parameters like C reactive protein (CRP)was considered as sepsis probable cases. Any maternal or fetal factors like PIH / birth asphyxia that could cause changes in the morphology of neutrophils in neonates were excluded from the study.

A complete blood count was done using a Sysmex KX21 analyzer. Peripheral smears were prepared using Leishman stain and evaluated for routine parameters like hemoglobin level, total leukocyte count, absolute neutrophil count, immature neutrophil count, immature neutrophils to total neutrophil ratio i.e. I/T, and platelet count. The relevant clinical details and laboratory parameters like CRP and blood culture findings were collected from the case sheets.

The morphometric analysis of 100 neutrophils per case was performed using "NIH Image J software"[2]. Images generated by the Olympus camera linked to the Olympus CX31 microscope were transferred to Image J software. The mean neutrophil diameter was measured using this software and MNV was calculated (fig. 1). "Modified Neubauer's chamber" was used for calibration. After measurement, the data was transferred to a MicrosoftExcel sheet for further statistical analysis.

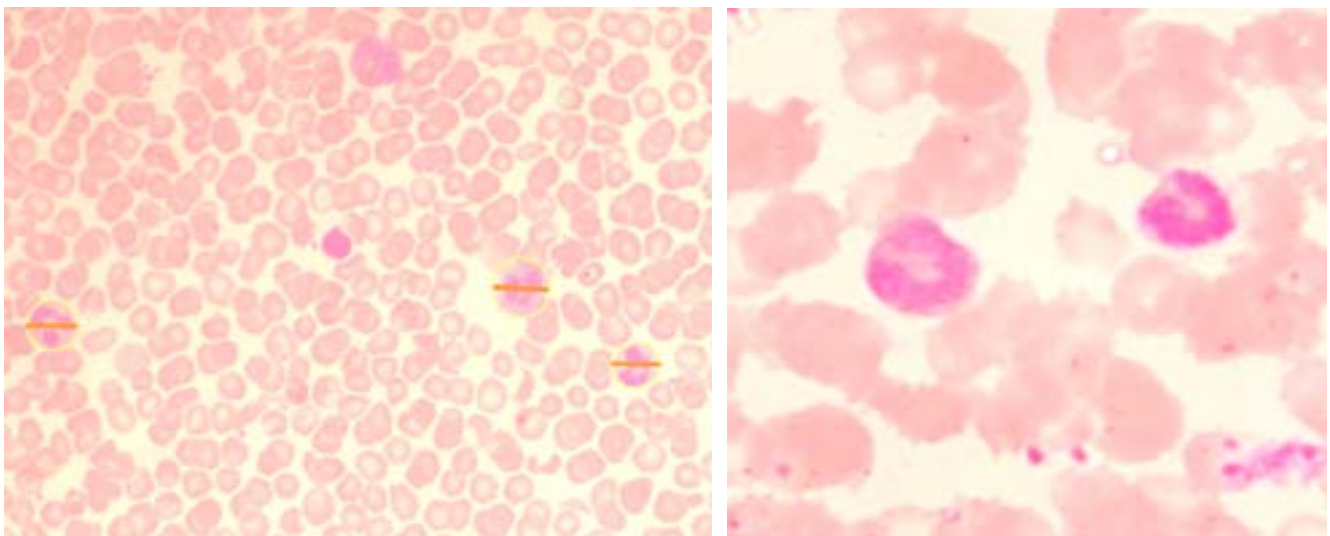

Figure 1: *A. Measuring the MNV using Image J software. B. Giant immature Neutrophils (Band forms) (Leishman stain, 100x)*

RESULTS

A total number of 100 cases evaluated included 50 cases of neonatal sepsis and 50 control cases i.e., normal newborns. A positive culture was seen in 30 of the 50 cases of clinically suspected sepsis (sepsis proven). In the remaining 20 cases, sepsis was strongly suspected based on clinical findings or findings of other tests like CRP (sepsis probable). The most common causative organism found in culture was *Klebsiella pneumoniae* (21 cases, 70%) while the least common was 2 cases of *Acinetobacter* and 1 case of *Pseudomonas aeruginosa*.

There was no significant difference in the baseline parameters like age of the neonate, gestational age, birth weight, and mode of delivery between "sepsis-proven", "sepsis probable" and control groups (Table 1). Haemoglobin level was reduced in the "sepsis group" (Mean – 14.6 gm/dl) compared to the control group (Mean – 17.3 gm/dl). Total leukocyte count, absolute neutrophil count, and immature neutrophil count were found to be increased and platelet count was found to be reduced in the sepsis group compared to the control group. CRP was positive in all the 50 cases in the "sepsis group".

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Table 1: Comparison of clinical and laboratory findings in sepsis and control groups

PARAMETERS	SEPSIS GROUP [n=50]			CONTROL GROUP [n=50] Mean (Range)
	Sepsis proven [n=30] Mean (Range)	Sepsis probable [n=20] Mean (Range)	TOTAL [n = 50] Mean (Range)	
Age in Days	6 (1-23)	7 (1-28)	7 (1-23)	7 (1-28)
Gender (Male/Female)	16/14	11/9	27/23	22/28
Gestational age (Term/Preterm)	13/17	10/10	23/27	25/25
Birth weight in kg	2 (1.04- 4.5)	2.2 (1- 3.3)	2.3(1.04-4.5)	2 (0.9- 3.5)
Mode of delivery (NVD/LSCS)	15/15	08/12	23/27	19/31
Hemoglobin (gm/dl)	14.6 (8.1- 21.3)	14.4 (8.7- 20.1)	14.6 (8.1-21.3)	17.3 (14- 23.1)
Total leukocyte count (x 103 / mm3)	13.5 (4.9- 30.0)	10.1 (1.8- 25.2)	17.7 (2.4-30.0)	12.3 (8.2- 22.0)
Absolute Neutrophil count (x 103 / mm3)	8.9 (1.2- 24.9)	6.9(0.9 - 19.1)	8.8(1.2- 24.9)	3.9 (1.2 – 5.6)
Immature Neutrophil count (x 103 / mm3)	1.4(0.13 – 6.8)	1.4 (0.068 – 4.9)	1.3(0.136-6.8)	0.199 (0.08 – 0.4)
Immature neutrophil /Total neutrophil count ratio(I/T)	0.272(0.01 – 0.82)	0.161(0.02 – 0.39)	0.15(0.02-0.39)	0.077 (0.05 – 0.1)
Platelet count (lakh/ mm3)	1.4(1000 – 3.3)	1.3(1000 – 3.1)	1.3(1000-3.3)	2.4 (1.5 – 4.2)
CRP (Positive/Negative)	30/0	20/0	50/0	-
Culture (Positive/Negative)	30/0	0/20	30/20	-

Table 2: Comparison of Mean Neutrophil Volume in sepsis and control groups

STUDY GROUP		MNV		p-value
SEPSIS GROUP	Sepsis proven group	$191x10^4$fl	$181x10^4$fl	< 0.0001 (less than 0.0001)
	Probable sepsis group	$174x10^4$fl		
CONTROL GROUP (no sepsis group)			$739x10^3$fl	

The MNV was significantly increased in the sepsis group than in the control group (Table 2). There was a statistically significant difference in the mean MNV values between the sepsis group and control group, p-value< 0.0001. A statistically significant difference in the mean MNV values was noted when proven sepsis and probable sepsis were compared with control individually (p-value < 0.0001 in both); however, there was no statistically significant difference in the mean MNV values between the sepsis-proven group and probable sepsis group (p-value <0.03).

The mean MNV value showed 100% sensitivity, 71% specificity, 60% PPV, and 100% NPV for detecting the sepsis state. According to the criteria put forth by Jin lee et al and Fernanda Chaves et al, a cut-off of 156 x 10^4 fl was considered for comparison between the control and sepsis groups.[3,4] As compared to the control group, MNV values were found to be increased in the sepsis group irrespective of normal or raised WBC counts (Table 3). MNV was found to be increased in the sepsis group even when the absolute neutrophil counts were normal/ decreased (Table 4).

Table 3: Comparison of MNV between sepsis & control groups concerning total WBC count

MNV value	Sepsis group			Control group		
	WBC count	Cases		WBC count	Cases	
MNV > 156	5000- 10000	20	Total 50	5000- 10000	0	Total 0
	10000-15000	18		10000-15000	0	
	15000-20000	6		15000-20000	0	
	20000-30000	6		20000-30000	0	
	>30000	0		>30000	0	
MNV < 156	5000- 10000	0	Total 0	5000- 10000	19	Total 50
	10000-15000	0		10000-15000	23	
	15000-20000	0		15000-20000	7	
	20000-30000	0		20000-30000	1	
	>30000	0		>30000	0	

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Table 4: Comparison of MNV between sepsis & control groups with reference to Absolute neutrophil count

MNV (x104 fl)	Sepsis Group			Control Group		
	Absolute Neutrophil count	Cases		Absolute Neutrophil count	Cases	
MNV > 156	<2000	6	TOTAL 50	<2000	0	TOTAL 50
	2000- 4000	8		2000- 4000	0	
	4000-6000	7		4000-6000	0	
	6000-8000	7		6000-8000	0	
	8000-10000	6		8000-10000	0	
	10000-13000	10		10000-13000	0	
	>13000	6		>13000	0	
MNV < 156	<2000	0	TOTAL 50	<2000	2	TOTAL 50
	2000- 4000	0		2000- 4000	21	
	4000-6000	0		4000-6000	27	
	6000-8000	0		6000-8000	0	
	8000-10000	0		8000-10000	0	
	10000-13000	0		10000-13000	0	
	>13000	0		>13000	0	

DISCUSSION

Early diagnosis and treatment are essential for reducing neonatal mortality due to sepsis. In routine practice, an increase in WBC count, absolute neutrophil count, and CRP levels are used as screening tools for diagnosing sepsis. Morphological changes in leucocytes can be determined by using conventional methods such as peripheral smear examination. Parameters like cytoplasmic granules/ vacuolation and toxic granules are commonly used in diagnosing sepsis. MNV is a new parameter that can also aid in the diagnosis of sepsis.

There are various automated hematology analyzers like Coulter LH analyzer which use conductivity and scatter technology to measure Mean Neutrophil Volume (MNV), Volume Distribution Width (VDW), and Mean Neutrophil Scatter (MNS). Chaves F et al in their study have observed that MNV was elevated in cases of sepsis even when the total leucocyte count and neutrophil count were low. Thus, they have opined that MNV has the potential to be an additional indicator for acute bacterial infection.[4] Similar findings were noted in our study as well. In a study conducted by Nesargi P et al, it was observed that Neutrophil Volume Conductivity Scatter (VCS) parameters like MNV and Volume distribution width (VDW) showed high sensitivity (95%) and specificity (86%) in the diagnosis of neonatal sepsis.[5]

Şafak Bet al have proved in their study that MNV can be a useful parameter in distinguishing between gram-positive and gram-negative sepsis, thus MNV values can aid in initiating appropriate antibiotic therapy at the earliest for better patient management.[6]It was observed in a study carried out by Suresh PK et al that though MNV is significantly raised in cases of acute infections, there was no statistically significant difference in the MNV values of individuals with systemic and localized infections.[7]Celik HI et al. have proven that neutrophil volume conductivity scatter (VCS) parameters are useful in early diagnosis and evaluation of treatment efficacy of neonatal sepsis.[8]Similarly various other studies have shown MNV as a promising indicator and a more sensitive parameter than total leukocyte count and absolute neutrophil count in diagnosing sepsis.[4-9]

In this study, we have tried to evaluate the utility of MNV in the early diagnosis of neonatal sepsis using “Image J software”. We found MNV to be significantly increased in the sepsis group when compared to the control group. MNV was 100% sensitive and 71% specific in the detection of neonatal sepsis.

Table 5: Significance of MNV for detection of sepsis in various studies

MNV	Age	Sensitivity	Specificity
Nesargi et al[5] (n = 304)	Neonates	95%	86%
Birol Safak et al[6] (n = 148)	Adults	79%	61%
Pooja K Suresh et al[7] (n = 94)	Adults	72%	70%
Celik et al[8](n = 304)	Neonates	79%	82%
Raimondi et al[9](n = 120)	Neonates	95%	88%
Mardi et al[10](n = 80)	Adults	76%	63%
Chaves et a[14](n = 69)	Adult	83%	54%
Present study (n = 100)	Neonates	100%	71%

The diagnostic utility of MNV in diagnosing sepsis has been studied by various authors, sensitivity and specificity of MNV for the detection of sepsis ranged from 100% to 72% and 88% to 54% respectively (Table 5). The sensitivity and specificity of MNV in the detection of sepsis were relatively lower in studies carried out on adults as compared to those done on neonates.

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A similar study conducted by Raimondi et al showed a higher sensitivity and specificity of MNV in detecting sepsis.[9] In the above studies authors have obtained MNV values from an automated hematology analyzer that measures VCS (Volume, Scatter, Conductivity) whereas in our study the MNV was measured manually using the Image J application. Another reason for this disparity may be the larger sample size in a few of these studies compared to ours.

CONCLUSIONS

MNV might emerge as a potential and reliable indicator in early diagnosis of neonatal sepsis and thus help clinicians in timely management. Since these parameters were established from a simple peripheral smear examination, it will be advantageous over other expensive biochemical or microbiological tests requiring more blood and time. However, one of the disadvantages of this technique is that it is labor-intensive and requires a specialist to measure MNV. A more practical and simpler alternative to measuring MNV manually would be obtaining these values from automated analyzers using the volume conductivity and scatter (VCS) graphs. These graphs can give a few more measurements like volume distribution width (VDW) and mean neutrophil scatter (MNS)which can be interpreted in association with MNV for diagnosing sepsis.

But not all laboratories or hospitals are equipped with these hematology analyzers which can give VCS graphs, especially in resource-poor settings. In such circumstances picking up these large Neutrophils while reporting neonatal smears might help in the early diagnosis of sepsis. We need to train our minds to look for this parameter and compare it with other known indices to derive better results from simple peripheral smear examinations for diagnosing sepsis.

Conflict of interest: None

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