



## Platelet Indices in Confirmed Bacterial Neonatal Sepsis; A Tertiary Referral Teaching Hospital-Based Study

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### ABSTRACT:

#### BACKGROUND:

Neonatal septicemia is a clinical syndrome characterized by signs and symptoms of infection in the first month of life associated with high morbidity and mortality rates if not treated properly. Platelet indices (biomarkers of platelet activation which include mean platelet volume, platelet distribution width, mean platelet mass, platelet large cell ratio, and immature platelet fraction) are of diagnostic value in a variety of settings including the sepsis some are studied very rarely. In automatic complete blood picture platelet count, mean platelet volume and platelet distribution width are a group of platelet indices determined together related to morphology and proliferation kinetics of the platelets.

#### OBJECTIVE:

To determine the role of platelet indices (platelet counts, MPV and PDW) in the diagnosis of neonatal sepsis, to detect the value of these indices in determining the type of bacteria causing the sepsis and to study the association of the platelet indices (platelet count, MPV & PDW) with gestational age, birth weight and onset of sepsis.

#### PATIENTS AND METHODS:

A prospective, case-control study, conducted at Neonatal care unit in Children Welfare Teaching hospital, from the first of June 2019 to the end of June 2020, included 90 neonates with signs and symptoms of sepsis and positive blood culture considered as patients group with other 90 healthy neonates as control group from outpatient clinic. Blood samples were drawn from each neonate of sepsis and control groups for white blood cell, hemoglobin, absolute neutrophil count, platelet count, MPV and PDW and blood culture were sent for patients groups.

#### RESULTS:

55 (61.1%) patients were males and 35(38.9%) patients were females, 24 (26.7%) patients below age of 7 days, 39(43.3%) patients were preterm (GA<37wk), 45(50%) neonates of the sepsis group were low birth weight (less than 2.5kg). In control group, 70(77.8%) neonates below the age of 7 days, 41(45.6%) neonates were low birth weight, there were matching between control and sepsis groups regarding gestational age and gender. Platelet indices including MPV and PDW (except platelet count) were increased with statistical significance in sepsis group in comparison to control group (P<0.05) regardless of gestational age and birth weight. All these indices were statistically significant in early onset sepsis but not in late onset sepsis. There were no statistical significance for platelet indices (platelet count, MPV and PDW) between subgroups of sepsis neonates regarding gram stain, onset of sepsis, gestational age and birth weight (P>0.05).

#### CONCLUSION:

Neonatal sepsis has significantly increased platelet indices (MPV and PDW) compared to healthy neonates regardless of Gestational age and birth weight. Thrombocytopenia and an increase in MPV with PDW are seen significantly in neonates with EOS (Early onset sepsis) but not significant in late onset sepsis compared to control. Platelet indices are not significantly different (decrease in platelet count and increase in MPV with PDW) between Gram-positive and Gram-negative bacteria, so the platelet indices are not specific findings in determining different types of sepsis bacteria.

**KEYWORDS:** Platelet, indices, sepsis, neonate.

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### INTRODUCTION:

Neonatal septicemia is a syndrome manifested clinically by signs and symptoms of infection in the presence or without bacteremia in the first 28 days of life<sup>(2)</sup>. The incidence of sepsis is

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estimated to be 5 to 6 per 1000 live births) among hospitalized patients<sup>(2)</sup> one to four cases per 1000 live neonates in the developed countries, while in developing country estimated ten times greater<sup>(3)</sup>.

Early-onset sepsis is the onset of symptoms within first 7 days of life, (some experts extend the definition to be within the first 72 days of life) and the infections are transmitted before or during delivery (vertical transmission from mother-to-child). There is also variability in the definition of late onset sepsis in which the onset of symptoms after 7 days of age (other definition to be >3 days of age), the infections acquired after delivery from hospital organism or the community acquired). The age at onset depends on the timing of exposure and virulence of the infecting organism. Very-late-onset infections (onset after age 1 month) especially in very-low-birth weight (VLBW) preterm infants or term infants who need prolonged hospitalization in neonatal intensive care<sup>(4)</sup>.

The most important risk factors of neonatal infections are: infection in maternal genital tract, total parenteral nutrition, and peripherally inserted of central line.<sup>(1)</sup>

Platelets play an important role in inflammatory process, including the recruitment and activation of white blood cells into inflamed tissue,<sup>(5)</sup>

In automatic CBC profiles, Mean platelet volume (MPV), platelet distribution width (PDW) and Plateletcrit (PCT) are platelet indices as a group determined together. Platelet indices related to proliferation kinetics and morphology of platelets, so they have a definite clinical uses in patients with sepsis. The other platelet indices include mean platelet mass, platelet large cell ratio (P•LCR) and immature platelet fraction (IPF). These indices were very rarely studied. P•LCR often correlates to MPV but is more sensitive to changes in platelet size. The IPF rises in patients with peripheral consumption or destruction of platelets. IPF is normal or low in bone marrow failure patients<sup>(6)</sup>

In the settings of sepsis with increase platelet turnover, increased MPV signifies the release of younger platelets into the blood circulation, new platelets that are larger and active; therefore, increased MPV is a marker of production rate and platelet activation<sup>(7)</sup>. The PDW levels high in platelet utilization when turnover is increased, and behavior similar to MPV during acute severe infections<sup>(8)</sup>

The current study aims to define the platelet indices roles (platelet counts, MPV and PDW) in the diagnosis of neonatal sepsis. Also to determine the value of these indices in

determining the type of bacteria causing the sepsis in neonate in addition to study the association of the platelet indices (platelet count, MPV, PDW) with birth weight, gestational age and sepsis onset.

### **PATIENTS AND METHODS:**

A prospective, case-control study was conducted in Neonatal care unit at Children Welfare Teaching Hospital/ Medical City, from the 1<sup>st</sup> June 2019 to 1<sup>st</sup> June 2020.

All neonates up to 28 days of age were included with signs and symptoms of sepsis including: (temperature instability, poor feeding, jaundice, pallor, shortness of breath, apnea, abdominal distention, vomiting, diarrhea, irritability, lethargy, seizures, cold, clammy skin, petechiae, purpura, bleeding, high-pitched cry, hypotonia, abnormal Moro reflex, full fontanel)<sup>(5)</sup> with positive blood culture. According to these criteria, 90 neonates were enrolled in the study as sepsis group.

The exclusion criteria included neonates with dysmorphic features suggestive of chromosomal abnormalities, neonatal hyperbilirubinemia with kernicterus, as well as neonates with negative blood culture or contaminated blood culture. 20 neonates were excluded from the study according these exclusion criteria (12 patients with contaminated blood culture, 5 patients with neonatal hyperbilirubinemia with kernicterus and 4 patients with dysmorphic feature).

The control group included 90 healthy neonates who attended the outpatient clinic for checkup or vaccination. First, agreement was taken from the parents then history taking and physical examination were done. A special questionnaire was designed for purpose of the study, include (name, age, gender, address, phone number, date of data collection, mode of delivery, date of birth, maternal history of fever, leaking liquor and its duration, vaginal or urinary tract infection, neonatal history and examination for symptoms and signs of sepsis. Measurement of vital signs, weight, height and head circumference. The neonates were divided into groups according to age: Early onset sepsis (less than 7 days age) and late onset sepsis (more than 7 days age). Gestational age: preterm (less than 37 week GA) and term (more than 37-42 week GA). Birth weight: low birth (less than 2500 gm) and normal birth weight (more than 2500 gm). Gram stain: gram positive or negative. Comparisons were done for platelet indices, first between sepsis patients and control neonates then between just sepsis groups.

Laboratory investigations include Blood samples drawn from each neonate (patient and control)

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for: Complete Blood Count (CBCs): 1 ml of blood samples was taken, collected in EDTA containing tubes. (CBCs) performed using a Celltac-G Analyzer (Germany) Manufacturer's, original kits are used in lab test, from which values of Hemoglobin, total leukocyte count, Absolute neutrophil count (ANC), platelet count, PDW (Platelet Distribution Width), Mean platelet Volume (MPV) were derived.

Bacterial Identification (blood culture) by 1-2ml blood samples were drawn aseptically in bottle containing formula that enhance microbial growth, implanted into a heart infusion broth, transferred to lab and incubated at 37 centigrade, then was daily checked for signs of bacterial growth up to seven days. For a positive broth culture, sub-cultures were done on MacConkey agar, blood agar and chocolate agar then incubated at 37 centigrade for 24 hours. Blood culture broths with no bacterial growth after seven days were sub-cultured. If no growth happened, it was reported as a negative. Bacterial isolates were identified by gram staining, colony morphology and standard biochemical tests.

Statistical Analysis of data was done by using the statistical package of SPSS-26 (Statistical Packages for Social Sciences- version 26). Data were presented in simple measures of frequency, mean, mean, standard deviation, percentage, and range (minimum-maximum values).

The significance of difference of different means (quantitative data) were tested using Students-t-test for difference between two independent means. Statistical significance was considered whenever the P value was equal or less than 0.05.

### RESULTS:

Ninety neonates fulfilling the criteria for diagnosis of sepsis were included in this study, 24 patients (26.7%) below age 7 days and 66 patients (73.3%)  $\geq 7$  days age. Ninety healthy neonates as control group, 70 neonates of them (77.8%)  $< 7$  days age and 20 (22.2%) neonates  $\geq 7$  days age, the range for age at admission from 6 hours to 28 days.

Regarding the gestational age, in sepsis group, 39(43.3%) patients were preterm and 51(56.7%) patients were full term while in control groups 39 neonates (43.3%) were preterm and 51 neonates (56.7%) were full term, the range of gestational age in this study 27-40 weeks. Fifty-five neonates (61.1%) were males with sepsis group and 35(38.9%) neonates were female. In control group, 55 neonates (61.1%) neonates were male and 35 neonates (38.9%). Half of the sepsis group (50%) was low birth weight and 41 neonates (45.6%) of the control, while the others were normal birth weight, with range of birth weight of neonates in this study is (900-4000 grams) (Table 1)

**Table 1: The demographic data for neonates with sepsis and control groups.**

		Sepsis (n=90)	Control (n=90)
		No(%)	No(%)
Age (days)	< 7 days	24(26.7%)	70(77.8%)
	$\geq 7$ days	66(73.3%)	20(22.2%)
	Mean $\pm$ SD (Range)	14.3 $\pm$ 8.6 (6h-28d)	5.2 $\pm$ 5.1 (1d-28d)
Gestational age	Preterm (<37weeks)	39(43.3%)	39(43.3%)
	Full term ( $\geq 37$ weeks)	51(56.7%)	51(56.7%)
	Mean $\pm$ SD (Range)	34.8 $\pm$ 3.9 (27-40)	35.0 $\pm$ 2.6 (28-39)
Gender	Male	55(61.1%)	55(61.1%)
	Female	35(38.9%)	35(38.9%)
Birth weight (gm)	BW<2500 g	45(50%)	41(45.6%)
	BW $\geq$ 2500g	45(50%)	49(54.4%)
	Mean $\pm$ SD (Range)	2399.1 $\pm$ 786.1 (900-4000)	2416.8 $\pm$ 709.1 (1050-3500)

\*Significant difference among two independent means using Students-t-test at 0.05 level.

Regarding the types of bacteria, 50 (55.6%) neonates with positive blood culture results were Gram-negative bacteria and 40(44.4%) were Gram-positive bacteria.

The most common bacteria was *E.coli* 21(23.3%) followed by *coagulase negative staph* 17(18.9%) then *Klebseilla pneumonia* 14 (15.6%) (Figure 1).

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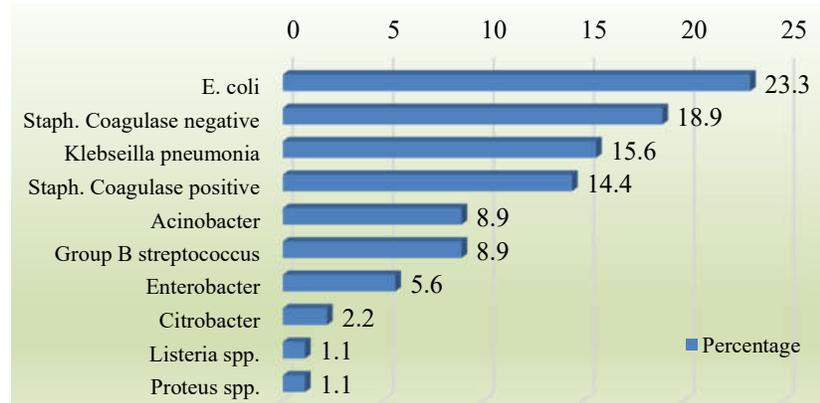


Figure 1: The type of bacteria in sepsis group.

There was a significant drop in haemoglobin (Hb) level (mean Hb 12.87 gm /dl) in sepsis group compared to control group (mean Hb 15.42 gm/dl) as (P=0.0001). There was an increase in WBC and ANC in sepsis group when compared to control but it is statistically

not significant (P>0.05) and the decrease in platelet count in sepsis group was statistically not significant (P=0.202). Both MPV and PDW were increased in sepsis group compared to control and was statistically significant as (P<0.05) (Table 2)

Table 2: The laboratory findings of sepsis and control groups.

Laboratory investigations	Total sepsis	Control	P value
Haemoglobin (g/dl)	12.87±3.23 (6.6-20.9)	15.42±2.24 (9.9-19.0)	0.0001*
WBC (x103)	14.48±8.24 (4.3-61.4)	12.88±5.44 (3.7-29.0)	0.128
ANC	6908.2±5420.2 (36-23000)	6834±4743 (1100-22340)	0.922
Platelets count	251.04±145.36 (27-680)	273.90±87.09 (116-500)	0.202
MPV	9.07±1.45 (6.9-15.4)	8.18±0.82 (6.5-11.7)	0.0001*
PDW	21.53±11.63 (7.3-72.0)	14.95±4.67 (7.2-19.5)	0.0001*

\*Significant difference among two independent means using Students-t-test at 0.05 level.

According to Gram-stain of bacteria, there was no statistically difference in sepsis neonates with

gram positive and gram negative culture bacteria regarding platelet indices, as P>0.05 (Table 3).

Table 3: The comparison of platelet indices with Gram stain.

	Gram stain		P value
	Gram +ve (n=50)	Gram -ve (n=40)	
Platelets count	259.22±123.91 (54-570)	244.49±161.26 (27-680)	0.635
MPV	8.88±0.91 (7.0-10.9)	9.21±1.76 (6.9-15.4)	0.290
PDW	20.24±7.83 (8.9-60.9)	22.56±13.94 (7.3-72.0)	0.349

\*Significant difference among two independent means using Students-t-test at 0.05 level.

There was significant drop in platelet count (P=0.01) with significant increase in MPV (P=0.0001) and PDW (P=0.0001) in early onset

sepsis but there were no statistical difference in late onset sepsis (P>0.05).(Table 4)

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**Table 4: The comparison between sepsis and control according to onset of sepsis.**

platelet indices	Sepsis	Control	P value
<b>Early onset</b>			
Platelets count	213.67±115.01	274.84±91.31	0.010*
MPV	8.75±1.22	8.04±0.60	0.0001*
PDW	25.17±13.72	14.66±4.73	0.0001*
<b>Late onset</b>			
Platelets count	264.63±153.32	270.60±72.38	0.867
MPV	9.18±1.52	8.63±1.26	0.147
PDW	20.21±10.57	15.97±4.40	0.085

\*Significant difference among two independent means using Students-t-test at 0.05 level.

There was statistically significant rise in PDW (P<0.05) but no statistical difference in platelet count and MPV in both preterm and full term neonates (P>0.05).(Table 5)

**Table 5: The comparison between sepsis and control groups regarding the gestational age.**

Platelet indices	Sepsis	Control	P value
<b>Preterm</b>			
Platelets count	265.31±141.56	272.59±95.75	0.791
MPV	9.16±1.38	8.24±1.10	0.002*
PDW	21.85±11.75	14.72±4.7	0.0001*
<b>Full term</b>			
Platelets count	240.13±148.48	274.90±80.82	0.145
MPV	9.00±1.51	8.13±0.53	0.001*
PDW	21.28±11.64	15.13±4.67	0.001*

\*Significant difference among two independent means using Students-t-test at 0.05 level.

### DISCUSSION:

Neonatal sepsis is a major cause of mortality and long-term morbidity in neonatal intensive care units<sup>(9)</sup>. As neonates are rapidly deteriorating, so the treatment should be initiated when suspected to have sepsis without any delay<sup>(10)</sup>. Platelet indices are related to proliferation kinetics and morphology of platelets, therefore they have a definitely used clinically in sepsis patients<sup>(11)</sup>.

In the present study, late onset sepsis is found to be more common than early onset sepsis which is similar to other studies such as in study conducted in Egypt (Omran *et al*;2017)<sup>(12)</sup>, this may be due to advances in prenatal care and use of prophylactic antibiotics to prevent vertical infection caused by GBS bacteria which is a major cause of early onset sepsis.

The neonates with sepsis clinically presented as lethargy, shortness of breath, poor sucking in most cases of sepsis in the current study, which agrees with Pakistan study (Mustafa; 2005) that mention them as the major clinical presentations of sepsis.<sup>(13)</sup>

Gram-negative bacteria were more commonly observed in comparison to Gram-positive of sepsis in this study, in which *E.coli* and *coagulase negative staph*, *Klebsella pneumonia*

were the most common bacteria identified in this study, These results agree with other studies as Rajnesh; 2015<sup>(15)</sup>, Krishna *et al*<sup>(16)</sup> and Kumhar *et al*<sup>(17)</sup>. These findings may be related to high late onset sepsis in this study, those with risk of invasive interventions, such as mechanical ventilation and catheterization, delay in starting breast milk, a long period of parenteral nutrition, prolonged time of hospital admission, surgery, underlying lung disease and cardiovascular compromise<sup>(14)</sup>.

In the current study, leukocytosis was observed in neonates with sepsis group as compared to control healthy neonates but it is not statistically significant. In Oncel *et al*; 2012<sup>(18)</sup>, WBC significantly increased in sepsis group in comparison to the control group along with other markers of sepsis.

In this study, there was significant decrease of hemoglobin in sepsis group than control group which may be related to frequent blood sampling with decrease production of RBC, as in study of Hellerqvist<sup>(19)</sup> and study of Pauly<sup>(20)</sup>.

Platelet indices changes frequently in ill neonates, especially in sepsis. In this study, sepsis group has statistically significant increase in MPV and PDW as compared to control group

regardless gestational age and birth weight, but in platelet counts there was not statistically significant difference. This comes in agreement with Oncel *et al* ; 2012<sup>(18)</sup> but disagrees with the study of Aksoy *et al.*<sup>(21)</sup> who found that there was no significant difference in MPV between septic patent and control infants, the cause of this disagreement may be due to the difference in the demographic data of the studied groups as they focused on MPV than other indices in the sepsis of very low birth weight preterm neonates.

In this study, no statistically significant difference in platelet counts, MPV and PDW in Gram-negative infections in comparison to gram-positive infection, which agreed with Manzoni *et al.*<sup>(22)</sup>.

We observed that there was statistically significant decrease in platelet counts, increase in MPV and PDW in early onset sepsis as compared with control group, it was significant in early onset sepsis but not significant in late onset sepsis which was similar to study of Aydemir *et al* but disagree with Choudhary *et al*<sup>(23)</sup> who reported that high MPV, high PDW and low platelet count are common finding in late onset rather than early onset sepsis.

In the current study, there was statistically significant difference in platelet indices according to the onset of sepsis (early onset or late onset), this comes in agreement with study in Iran (Madani *et al*; 2019)<sup>(24)</sup>, and study in India (Bhakri *et al*; 2017)<sup>(25)</sup> but disagrees with other studies as in Kudawla *et al.*<sup>(26)</sup> and study in India (Mittal *et al*; 2018)<sup>(27)</sup> who concluded that decreased platelet count and significantly higher MPV were seen more frequently in LOS group (P<0.05) but the difference in PDW was not significant.

There was no significant difference for platelet indices in relation to birth weight as low birth weight or normal birth weight in sepsis groups in the current study agrees with other studies as a study in India (Mittal *et al*; 2018)<sup>(27)</sup>.

**CONCLUSION:**

Neonatal sepsis have significantly increased platelet indices (MPV and PDW) compared to control neonates regardless Gestational age and birth weight. Thrombocytopenia and an increase in MPV with PDW are seen significantly in neonates with EOS (Early onset sepsis) but not significant late onset compared to control. Platelet indices are not significantly different (decrease in platelet count and increase in MPV with PDW) between Gram-positive and Gram-negative bacteria, so the platelet indices are not specific findings in determining different types of sepsis bacteria.

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