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Mean Platelet Volume and Platelet Function in Neonatal Sepsis

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ABSTRACT

Background: Neonatal sepsis is a clinical disorder developed by bacterial blood stream infections in neonates**.** CBC indices, such as platelet count, mean platelet volume (MPV), PDW have been used as markers of systemic inflammation in children and adults.

Objective: To assess MPV and platelets function in neonatal sepsis.

Patients and Methods: This study was a case control, which included 50 cases and 50 controls. All participants were subjected to history taking, clinical examination, CBC, C-reactive protein, blood cultures and assessment of mean platelets volume.

Results: There was a significant decrease in haemoglobin level, RBCs count, and platelet count. While, there was significant increase in WBCs count and MPV in the patient group than in the control group. Mean value of platelet count was statistically significantly lower among cases than in control. MPV was significantly higher in patients than controls. PDW was statistically significantly lower among cases than in controls. MPV showed significant negative correlation with gestational age, birth weight and platelet count. While it showed statistically significant positive correlation with CRP.

Conclusion: Mean value of platelet count and PDW were statistically significant lower among cases than in control while MPV was significantly higher in patients than in controls. So, platelet function could be a useful early diagnostic marker in neonatal sepsis.

Keywords: Neonatal sepsis, Mean platelet volume, Platelet function.

INTRODUCTION

It is estimated that four million neonatal deaths occur worldwide every year, and approximately one-third of these are caused by infections. Sepsis continue to be one of the main causes of neonatal mortality [(1)]. Neonatal sepsis is a clinical disorder developed by bacterial blood stream infections (BSI) in neonates**.** It is a dangerous and common disease among infants, which is associated with high morbidity and mortality [(2)]**.**

Although blood culture has been considered as the gold standard, this analysis is still too slow and limited by false negative results. There has been constant search of an ideal sepsis biomarker that have high sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) [(3)]**.** The most commonly used biomarkers are C-reactive protein (CRP) and procalcitonin (PCT), but both have shown varied sensitivity, specificity, PPV and NPV in different studies [(4)]**.**

CBC is a simple and routine workup to determine infection in neonates. Low white blood cell count, absolute neutrophil count, and high immature-to-total neutrophil ratio were the most commonly used indices for detection of infection in neonates. Recently, other CBC indices, such as platelet count, MPV and PDW have been used as markers of systemic inflammation in children and adults. In neonates, very limited data are available about the usage of platelet indices for diagnosis of neonatal infection or other inflammatory conditions [(5)]. MPV is a measure of platelet volume. It reveals the presence of inflammatory burden and disease activity in many diseases [(6)]**.** This study aimed to assess platelets' function in neonatal sepsis.

PATIENTS AND METHODS

This study was a case control study, which included 50 patients with neonatal sepsis. In addition, there was 50 age and sex matched healthy neonates who served as controls. The cases and controls were taken from NICU of Benha Children Hospital during the period from October 2018 till July 2019.

Inclusion criteria:

- **Neonates with neonatal sepsis:** A diagnosis of clinical sepsis requires the presence of at least three of the following: bradycardia (<100 beats/min), hypotension, hypotonia, seizures, apnea, tachypnea, cyanosis, respiratory distress, poor skin color and perfusion, feeding difficulty, irritability, lethargy and laboratory results showing elevated levels of CRP or IL-6. Patients with culture positivity were accepted as proven sepsis.

Exclusion criteria:

- Undergoing a course of antibiotics prior to appropriate blood sampling
- Undergoing surgery in the previous week
- Chromosomal abnormality
- Lack of informed consent from the parents

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All participants were subjected to the following:

A. Careful history taking: to detect risk factors for sepsis included obstetric history (death of a previous sibling, previous admission to neonatal intensive care unit, etc.). Prenatal history (diabetes mellitus, maternal fever > 38°C, maternal antibiotics, maternal urinary tract infection (UTI), etc.). Natal history (premature rupture of membrane (PROM), maternal fever, prolonged second stage of labor, etc.). Postnatal history (low Apgar score at 1 and 5 min, aggressive resuscitation, respiratory distress, cyanosis, fever, jaundice, etc.).

B. Thorough clinical examination: complete clinical examination: (For signs of sepsis, including lethargy, feeding intolerance, fever or hypothermia, tachypnea, cardio-respiratory instability, or hypotonia).

C. Laboratory investigations: CBC, CRP and blood culture.

- CBC was done for all samples using sysmex KX-21N (Sysmex Corporation, New York, USA) for red blood cell (RBC) count, hemoglobin level, hematocrit value, WBC count (total and differential), and platelet count.

- Quantitative measurement of the level of C-reactive protein (CRP):

Estimation was carried out using the test kit (Cromatest) at 0 h of clinical presentation. The AVITEX- CRP latex particles are coated with antibodies to human CRP. When the latex suspension is mixed with serum containing elevated CRP levels on a slide, clear agglutination was seen within 2 minutes.

- Specimen collection and storage: Fresh sample of venous blood was allowed to clot form and retract centrifuge clotted blood sample and collect serum, store at 2-8 ^{o}C AVITEX-CRP had a detection limit of 6 mg/L of CRP in the patient's serum.

- Blood cultures:

Blood for culture was collected and dispensed with great care as indicated to avoid contaminating the specimen and culture medium. Wearing gloves thoroughly disinfect the venepuncture site as follows:

- Using 70% ethanol; cleanse an area about 50 mm in diameter and allow to air dry.
- Using 2% tincture of iodine and a circular action, swab the area beginning at the point where the needle will enter the vein. Allow the iodine to dry on the skin for at least 1 minute. Using a sterile syringe, withdraw about 1 to 2 ml of blood and inoculate it immediately in the blood culture bottle.

1- The foil cap of the blood culture bottle was held cut and then the rubber cap was wiped using an ethanol swab, then perforated with the syringe containing the collected blood.

2- The top of the culture bottle was wiped again with ethanol swab and the foil cap was replaced.

3- The blood was mixed with the broth without delay.

4- The bottles were labeled with the name, the number of the case and the date of collection.

5- The blood culture bottles were incubated at 37 °C.

6- Subculture was done after the first night incubation on:

- Blood agar that was aerobically incubated at 37 °C for 24 hours
-MacConkey agar that was aerobically incubated at 37 °C for 24 hours

The plates were examined after the incubation period for growth.

7- Negative blood culture bottles were checked every other day for evidence of microbial growth (hemolysis, turbidity, gas production, or presence of visible growth). If there is no evidence of microbial growth after 10 days of incubation, Gram stains and terminal subcultures were done before considering the culture as negative**.**

8- +ve blood cultures were identified using Vitec 2 with subsequent antibiotic sensitivity.

D. Assessment of mean platelets volume.

2 milliliters of blood were drawn in sodium citrate (3.8%) tubes (Terumo, Tokyo, Japan) from venous puncture. Platelet-rich plasma (PRP) was immediately prepared from the whole blood by centrifugation at 90 × g for 10 min at 20 °C

Blood for complete blood counts was obtained either by venipuncture, by arterial puncture, or through a central catheter. PC and MPV determinations were performed using a twice daily calibrated Cell- Dyn 3700 automated hemocytometer (Abbott, IL).

MPV ranges between 7.5 and 12.0 fl.

Platelet Aggregation Systems (Aggregometer):

Platelets are small cell fragments most commonly known for playing a role in wound repair and blood clotting via a clumping process known as platelet aggregation. Platelet aggregation systems (also referred to as an aggregometer) can be used to determine how well platelets stick together.

Such a test measures platelet aggregation with the use of a platelet antagonist, such as ADP, thrombin, and ristocetin. An aggregometer measures the turbidity in the sample and records how quickly the platelets clump together by measuring the increased light and clarity in the sample. Additionally, a platelet aggregation system can encompass several test modes where the number of channels available in an aggregometer is equivalent to the number of tests that can be run simultaneously and independently. Certain aggregometers can test and measure platelet function from a patient and can be used to diagnose patients with von Willebrands disease. Platelet aggregation systems and aggregometers are reliable diagnostic tools in a clinical research laboratory.

Ethical approval: This work was done in coordination with the Molecular Biology and Biotechnology Unit. **The study was approved by Benha Children Hospital' Ethical Committee.** Written informed consent obtained from the parents of all babies enrolled in the study.

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 22 software (Spss Inc, Chicago, ILL Company). Categorical data were presented as number and percentages. Chi square test (X2), or Fisher's exact test (FET) were used to analyze categorical variables. Quantitative data were tested for normality using Kolomogrov Smirnove test assuming normality at P > 0.05. Quantitative data were expressed as mean ± standard deviation, median and range. Student "t" test was used to analyze normally distributed variables among 2 independent groups, or Man Whitney U test for nonparametric ones.

Difference among 3 independent means was analyzed using ANOVA for parametric variables or Kruskal Wallis test (KWT) for nonparametric ones. Spearman's correlation coefficient (rho) was used to assess correlation between non-parametric variables.

The accepted level of significance in this work was stated at 0.05 ($P \leq 0.05$ was considered significant), P value > 0.05 is non-significant (N-S). $P \leq 0.05$ is significant (S). $P \leq 0.001$ is highly significant (HS).

RESULTS

There was no statistically significant difference between cases and controls regarding age (days) and Sex. There was statistically significant difference between cases and controls regarding MOD. **Table (1).**

Table (1): Comparison between cases and controls regarding demographic data

			Cases (No.= 50)	Controls (No.= 50)	X2	P. value
Age(days)	Rang		5 - 25	3 - 25	t. test	.717
	Mean ± SD		23.06 ± 1.57	22.08 ± 1.43	0.363	
Sex	Male	No.	35	29	1.56	.211
		%	70.0%	58.0%		
	Female	No.	15	21		
		%	30.0%	42.0%		
MOD	NVD	No.	4	24	19.84	.000
		%	8.0%	48.0%		
	CS	No.	46	26		
		%	92.0%	52.0%		

There was statistically significant decrease in RBCS, HB, HCT, PLATLET COUNT, PLATLET AGGREGATION IN ADP and RISTOCETIN among cases than controls. There was statistically significant increase in TLC among cases than in Controls (Table 2).

Table (2): Comparison between cases and controls regarding laboratory parameters

		Cases (No.= 50)	Controls (No.= 50)	t. test	P. value
RBCS (cells/mcL)	Mean ± SD	3.55 ± 0.735	4.008 ± 0.69	-3.210-	.002
HB(gm/dl)	Mean ± SD	11.56 ± 2.626	12.92 ± 3.0139	-2.399-	.018
HCT (%)	Mean ± SD	34.346 ± 7.68	39.72 ± 8.619	-3.294-	.001
TLC(10^3/cmm)	Mean ± SD	15.17 ± 3.532	9.92 ± 2.09	4.083	.000
PLATLET COUNT(10^3/cmm)	Mean ± SD	220.64 ± 77.735	375.76 ± 44.143	-4.793-	.000
PLATLET AGGREGATION IN ADP %	Mean ± SD	35.84 ± 3.68	75.52 ± 3.96	-11.684-	.000
RISTOCETIN %	Mean ± SD	51.08 ± 3.546	84.04 ± 4.62	-7.544-	.000

There was statistically significant increase in MPV among cases than in Controls. There was statistically significant decrease in PDW among cases than in controls (Table (3).

Table (3): Comparison between cases and controls regarding MPV and PDW

		Cases (No.= 50)	Controls (No.= 50)	t. test	P. value
MPV(fl)	Mean ± SD	7.79 ± 1.008	7.38 ± 0.49	2.558	.012
PDW(fl)	Mean ± SD	13.43 ± 3.4057	15.026 ± 1.0759	-3.156-	.002

Regarding CRP, positive was 90% and negative was 10%. Regarding blood culture, positive was 32% and negative was 68%. Regarding organism, positive was 87.5% and negative was 12.5% (Table 4).

Table (4): CRP and blood culture among cases

			Cases (No.= 50)
CRP (mg/L)	**Positive**	**No. (%)**	45 (90.0)
	Negative	**No. (%)**	5 (10.0)
Blood Culture (mL)	**Positive**	**No. (%)**	16 (32.0)
	Negative	**No. (%)**	34 (68.0)
Organism	**Gram -ve bacilli (klebsiela)**	**No. (%)**	14 (87.5)
	Gram +ve cocci	**No. (%)**	2 (12.5)

DISCUSSION

This study showed that, there were no statistically significant difference between cases and controls regarding age and sex. This agrees with **El-Mashad *et al.*** [7] who found no significant difference between cases and controls in relation to gender and age.

In the current study, it was found that CS was significantly associated with increased frequency of sepsis. This agrees with **Omran *et al.*** [5] in Egypt who found that CS was significantly associated with increased frequency of sepsis. This is in disagreement with **Gebremedhin *et al.*** [8] who found that the mode of delivery was not significantly associated with increased frequency of sepsis.

This study showed that mean value of hemoglobin was significantly lower among cases than in controls ($P < 0.05$). This result is in agreement with **Nupponen *et al.*** [9]**, Kuboyama *et al.*** [10] who reported that mean value of hemoglobin was significantly lower among cases than controls.

In our study, the findings of white blood cells in the sepsis group were significantly increased compared to control group, with mean level in sepsis group of 15.1, and in no sepsis of 9.9 ($P < 0.05$). This agrees with the results of **Fathy *et al.*** [11] who found that white blood cells were significantly increased in sepsis group compared to control group.

In this study, there was a significant decrease in haemoglobin level, RBCs count, and platelet count & significant increase in WBCs count and MPV in the patient group than in the control group. This agrees with the results of **Annam *et al.*** [12].

This study showed that mean value of platelet count was statistically significantly lower among cases than controls ($P < 0.05$). This agrees with **Powell and Marcy** [13] who stated that mean value of platelet count was statistically significantly lower among cases than control. This thrombocytopenia could be due to direct toxic injury of platelets, megakaryocytic suppression, increased peripheral consumption as in disseminated intravascular coagulopathy (DIC) or presence of immune component due to increased level of platelet associated immunoglobulin's [14]**.**

In the current study, we found that MPV was significantly higher in patients than in controls. This comes in agreement with the study of **Aydin *et al.*** [15], who found that MPV in newborns with septicemia was significantly higher than in control group. Similar results were found by **Oncel *et al.*** [16] who studied MPV in neonatal sepsis and found that there was a statistically significant increase with regard to MPV values in patients with sepsis. This agrees with **Omran *et al.*** [5] who found that MPV showed significant difference between septic neonates and controls (10.2 ± 1.2fL vs.8.0 ± 0.5fL respectively).

This study showed that mean value of PDW was statistically significantly lower among cases than controls ($P < 0.05$). This disagrees with the study of **Catal *et al.*** [17] who found that there were no statistically significant differences between the control and sepsis group in PDW levels.

This study showed that CRP level was positive in 90% of patients group. This comes in agreement with the results of **Ganesan *et al.*** [18]. In addition, this agrees with the results of **El Sebaie *et al.*** [19] who found that C-reactive protein level was higher in group I than in group II ($P < 0.001$).

This study showed that 68% had negative blood cultures and 32% of the case group had positive blood cultures. Klebsiella was the most common organisms in our patients followed by E. coli. This is in agreement with the study of **Dzwonek *et al.*** [20] in which nearly half of the positive blood cultures showed K. pneumoniae. Moreover, this comes in agreement with another study in which the most common organism in positive blood cultures was Klebsiella pneumonia [21]. **Hisamuddin *et al.*** [22] found that culture proven sepsis occurred in 30% of cases with sepsis.

In the current study, MPV showed significant negative correlation with gestational age, birth weight and platelet count.

The gold standard for diagnosing of neonatal sepsis remains the blood culture. However, negative blood cultures are difficult to interpret because bacteremia can be intermittent in neonates with sepsis. Therefore, biomarkers with high accuracies are desirable in order to identify infants who are truly septic or to minimize inappropriate exposure of those infants with low probability of sepsis to antimicrobials [23].

CONCLUSION

Present study concluded that mean value of platelet count and PDW were statistically significantly lower among cases than among controls while MPV

was significantly higher in patients than in controls. So, platelet function could be a useful early diagnostic marker in neonatal sepsis.

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