COMPARISON OF PROCALCITONIN AND HEMATOLOGICAL RATIOS IN CORD BLOOD AS EARLY PREDICTIVE MARKER OF NEONATAL SEPSIS

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ABSTRACT

Objective: To evaluate the predictive value of neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) with procalcitonin in cord blood.

Study Design: Case control study.

Place and Duration of Study: Study was conducted in Army Medical College and Pak Emirates Military Hospital Rawalpindi, Jul 2018 to Mar 2019.

Methodology: Those mothers, having deliveries with early rupture of membranes (EROM), premature rupture of membranes (PROM), preterm, dai handled, meconium and failure of induction have been included. Total 60 neonates were included in this study. Nineteen neonates were taken as a continuous clinical unsteadiness with a clearly documented suspicion of sepsis and two neonatologists decided sepsis within 1-3 days of life. While remaining neonates were control who have no infection.

Results: Laboratory values show there was a gross difference in mean values of case and control for white blood cell (WBC), platelets, neutrophil, platelet to lymphocyte ratio and procalcitonin with statistical significance except lymphocyte count and neutrophil to lymphocyte ratio. In combined ROC curve. The cutoff of procalcitonin was calculated to be 0.4ng/ml with an area under curve of 84.5%. Similarly, cutoff of neutrophil to lymphocyte ratio was determined to be 1.39 with an area under curve of 65.1% at sensitivity of 63% and specificity of 58.5%. Cutoff of platelet to lymphocyte ratio was determined to be 47.48 with an area under curve of 66.8% at sensitivity of 78.9% and specificity of 49%.

Conclusion: Combined hematological markers neutrophil to lymphocyte ratio and platelet to lymphocyte ratio with procalcitonin in cord blood could be used as a simple, sensitive and predictive parameter for identifying neonates susceptible to sepsis.

Keywords: Early rupture of membrane (EROM), Neutrophil to lymphocyte ratio (NLR), Platelet to lymphocyte ratio (PLR), Prolonged rupture of membrane (PROM).

INTRODUCTION

Establishing a globally accepted case definition in the newborn is tricky due to lack of consensus, lack of optimal diagnostic tests, varied pathologies and nonspecific clinical signs. International consensus definition, new guidelines, and thoughtful practical processes would ensure meaningful reporting and improved patient outcomes[1]. The clinical presentations are variable and nonspecific. According to international sepsis definition conference 2014, sepsis is a clinical syndrome that shows the presence of both infection and systemic inflammatory response based on temperature, heart rate, respiratory rate, and white blood cell count, and hypotension. In 2016, this definition was redefined and updated criteria of sepsis was stated by European society of intensive care medicine (ESCIM) and Society of critical care medicine (SCCM) consensus task force that life threating organ dysregulation caused by host response failure to infection[2]. In early onset neonatal infections, 85% of early onset neonatal sepsis occurs within 24 hours, 5% present between 24-48 hours, and a smaller percentage present between 48-72 hours[3]. The early-onset sepsis occurs within first three days of life (American Academy of Pediatrics definition).

World Health Organization (WHO) reports that approximately 5 million neonatal deaths

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occur per year. The incidence of EONS is estimated as 1-2 cases per 1,000 live births in United States of America while in developing countries it ranges from 2.2 to 9.8 per 1000 live births[4]. A study in United Kingdom has shown that 50% of neonatal sepsis mortalities occur in first 24 hours and half of these neonates die before transfer to NICU. In developing countries, neonatal sepsis is reported to be the leading cause of neonatal mortality with a burden of death tolling almost 27% of the total neonatal deaths[5]. In Pakistan, in past years most of the deliveries were conducted at home but after 1990 this trend changed and approximately 2.5 folds increase in deliveries at healthcare facilities[6]. Cord blood is the first available sample which help the clinician to start therapeutic strategy as soon as possible.

To evaluate for neonatal sepsis, complete blood count (CBC), platelet count, and cerebrospinal fluid examination are practiced in some clinical settings. This hematologic scoring system, although a quick aid to screen for infection in neonates, has poor positive predictive value unless the score of these parameters are too high. These hematological parameters with routinely used biomarkers i.e. lactate, procalcitonin and C-reactive protein might be of value in susceptible neonates in deciding need of antimicrobial agents or whether therapy can be safely discontinued. In CBC, most reliable marker is neutropenia for neonatal sepsis, but different factors affect neutrophil count such as gestational age, planned cesarean delivery has lower counts than vaginal delivery and at high altitude total neutrophil counts are higher. Platelet count are also unreliable in diagnosis of neonatal sepsis[7]. A new emerging ratio neutrophil to lymphocytes (NLR) and platelet to lymphocyte (PLR) is better and easy approach for early prognosis and diagnosis of sepsis. Advantage of these ratios is that it can be measured by using conventional methods without any new sophisticated instrument or tools. The simple and less invasive marker of inflammation whenthere is an increase in neutrophil countand decrease in lymphocytes in the circulation, resulting in high ratio, is a significant marker.

Procalcitonin (PCT) is protein in nature and is an acute phase reactant. Procalcitonin is prime biomarker which starts to rise in early sepsis. There is rapid rise in the level of PCT within 2–4h, reached its peak level at 6-8h after bacterial endotoxin exposure[8] and return to normal after next 24h. Early rise PCT levels in neonatal sepsis makes it a good marker for early diagnosis of sepsis in neonates as compared to CRP[9]. Procalcitonin has shown highest diagnostic value as compared to CRP in sepsis[10]. Procalcitonin testing can be used to help clinicians diagnose bacterial infection (that can cause sepsis) and make decisions on starting antibiotic treatment.

Numerous sepsis biomarkers have been used in routine for early detection of neonatal EONS, but to date, there is no single or even a group of biomarkers that fulfills all essential criteria. The neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR)combines neutrophils and platelets with lymphocytes in the calculation, is considered comparatively more stable than their values alone. These ratios are inflammatory markers that can be easily, rapidly, and inexpensively measured with routinely used instruments[10].

The objective of this study was to evaluate the predictive value of the neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in cord blood and comparison of these ratios with procalcitonin levels in cord blood which is the biomarker traditionally used for sepsis.

METHODOLOGY

It was a case control study, conducted in Department of Gynecology and obstetrics, Pak Emirates Military Hospital and Department of Pathology Army Medical College, Rawalpindi. This study was started after approval of ethical review committee of the institute. Study population was neonates delivered at Department of Gynecology and obstetrics and neonates being admitted in NICU Department of Pediatrics, Pak Emirates Military Hospital, Rawalpindi for treatment of sepsis from July 2018 to March 2019. Sample size according to WHO calculator was

taken as 60. Those mothers who had given voluntarily consent were included in the study. Consecutive sampling was done. Those having deliveries with early rupture of membrane, preterm, diabetics and having other septic risk factors were included an all healthy mothers, having deliveries at 39 weeks of gestational age were excluded from this study.

Immediately after birth while the placenta was still in situ, four Howard Kelly forceps were placed on the cord to isolate a 20cm segment in the middle. The isolated segment was cut between the two sets of clamps, baby and the placenta still having a clamp in place. 7cc blood (from the placental end of the cord) was collected in a syringe, from which 5ml cord blood was taken in plain tube and 2ml cord blood transferred in EDTA tube.

All the samples were centrifuged after clotting, sample for CBC was processed, and separated sera were stored at -20°C in Eppendorf tubes prior to analysis. Follow up was done within 72 hours. Neonates having a continuous clinical unsteadiness, with a clearly documented suspicion of sepsis confirmed by the neonatologist at 1-3 day of life were taken as cases. Only those neonates which were labelled as having sepsis by two neonatologists independently were taken in case group. Clinical instability was defined as having any of the following clinical signs in neonate: delayed cry, irritability, reluctant to feed, fever or hypothermia, tachypnea or hypotonia. Neonates who did not develop sepsis within 72 hours of birth were considered as controls.

All samples were analyzed in the Department of Chemical Pathology, Army Medical College, Rawalpindi. Complete blood count was performed on 3-part differential automated cell counter Sysmex KX21N (Sysmex, Kobe, Japan). For quality control, two levels of internal quality control materials were used with each batch of analysis. Serum procalcitonin was measured by Roche cobas c411 based on electrochemilumine scence technique. Lowest limit of detection for PCT concentrations was 0.02 ng/mL.

Statistical analysis was done by using SPSS software version 20. Mean and Standard Deviation (SD) was calculated to assess the level of hematological indices, lactate, CRP and PCT. The difference in the level of all biochemical markers among the case groups and control groups were assessed using independent t-test. The difference between the two groups was considered statistically significant, when the *p*-value was ≤0.05 in independent t-test. Receiver Operating Characteristics (ROC) curve was drawn to determine the sensitivity, specificity for the parameters and Area Under the Curve (AUC) was calculated and compared and the most accurate cut-off values were taken to predict sepsis.

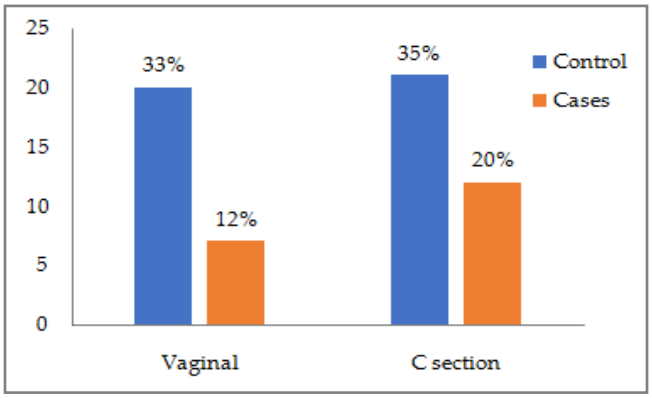

Figure-1: Mode of delivery.

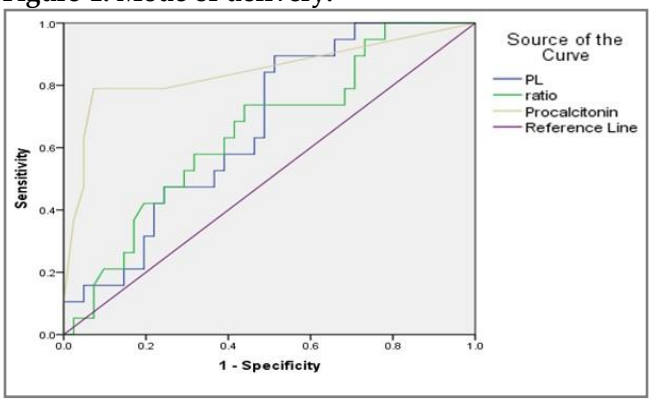

Figure-2: ROC curve for procalcitonin, neutrophil to lymphocyte ratio and platelet to lymphocyte ratio.

PL= Platelet to Lymphocyte Ratio, Ratio=Neutrophil to Lymphocyte Ratio

RESULTS

Study participants was included 60 neonates delivered with high risk mothers, divided in two groups. Nineteen neonates were taken as cases on neonatologist decision with positive clinical presentation and lab results and being admitted in neonatal intensive care unit. Other group included 41 neonates having no infection were taken as controls. Some controls although have shown mild clinical unsteadiness so kept under observation butafter management discharged by neonatologist. Demographic data showed that male neonates delivered with emergency caesarian section in presumed mothers have higher risk of neonatal sepsis.

Neonates included in study showed 45% were born with normal vaginal delivery and 55% via caesarean section. Neonates delivered through cesarean section had higher risk of developing neonatal sepsis as compared to neonates delivered by vaginal deliveries.

For cases, WBC count mean 14.79 ± 2.75 x 10^3 cells/µL *p*=0.04, platelets 301.5 ± 42.12 x 10^3 cells/µL, *p* 0.00, neutrophil count varied between cases 8.35 ± 2.4 to 6.02 ± 2.22 x10^3 cells/µ for controls, *p* 0.00l ymphocyte count were 4.9 ± 1.59 and 4.60 ± 1.82 x 10^3 cells/µL for cases and controls, *p*=0.47, NLR 1.91 ± 0.93 for cases and 1.54 ± 0.99 for controls, *p*=0.17, PLR for cases and controls were 69.90 ± 33.9 and 51.91 ± 26.60, *p*=.03. Procalcitonin values were also as significant as WBC, platelets and neutrophil counts. Procalcitonin has mean value of 0.968 ± 0.13 ng/mL for cases and 0.0288 ± .0176 ng/mL for controls, *p* 0.002. Cord blood Procalcitonin levels were raised almost in all cases, so out of 19 babies (thirteen males and six females) showed presence of sepsis, 18 have raised procalcitonin levels. Lab Values shows there was a gross difference in values of case and control for WBC, platelets, neutrophil, lymphocyte, NLR, PLR and procalcitonin with statistically significance except lymphocyte and NLR which did not show any significance between case and control groups.

The combined ROC curve for NLR, PLR and PCT is shown in figure. The cutoff of PCT was calculated to be 0.04 with a CI of 95% (71.9%-97.1%), with an AUC of 84.5%. Similarly, cutoff of NLR was determined to be 1.39 with a CI of 95% (50.5%-79.7%) with an AUC of 65.1% at sensitivity of 63% and specificity of 58.5%. Cutoff of PLR was determined to be 47.48 with a CI of 95% (53.0%-80.5%) with an AUC of 66.8% at sensitivity of 78.9% and specificity of 49%.

Table-I: Values of different parameters in cord blood.

	Case	Control	*p*-value
	Mean ± SD	Mean ± SD	
WBC (x10^3 cells/µL)	14.79 ± 2.75	13.15 ± 2.95	0.04
Platelet (x10^3 cells/µL)	301.52 ± 42.12	207.46 ± 72.7	0.00
Neutrophil (x10^3 cells/µL)	8.35 ± 2.04	6.02 ± 2.24	0.00
Lymphocyte (x10^3 cells/µL)	4.96 ± 1.59	4.60 ± 1.82	0.47
Neutrophil to lymphocyte ratio	1.91 ± 0.93	1.54 ± 0.99	0.17
Platelet to lymphocyte ratio	69.90 ± 33.97	51.91 ± 26.60	0.03
Procalcitonin (ng/mL)	0.0968 ± 0.13	0.0288 ± 0.02	0.002

Table-II: Area under the curve for different parameters.

Test Result Variable(s)	Area	Asymptotic 95% Confidence Interval	
		Lower Bound	Upper Bound
Neutrophil to lymphocyte ratio	0.651	0.505	0.797
Platelet to lymphocyte ratio	0.668	0.530	0.805
Procalcitonin	0.845	0.719	0.971

DISCUSSION

In developing countries, it is affirmed that the burden of neonatal sepsis is still high and the clinical presentations; symptoms and signs are variable and non-specific, leading to delay in

diagnosis[11-15]. Thus, there is a dire need for early diagnosis and timely administration of antibiotics. Blood culture-based diagnosis is no doubt a first-class way to find the causative organisms, but well-equipped laboratory and trained personnel are required for this gold standard that are lacking in most of the hospitals in our population. Neonatal sepsis has rapid course and multiple system involvement. To address the problems of delay and false negative results associated with blood culture-based diagnosis, an algorithm of simple, rapid and economical markers is required. Procalcitonin is now widely used in clinical laboratories in Europe and USA with FDA recommendation for the evaluation of risk factors as well as for diagnosis and monitoring of sepsis[13]. PCT cut-off limit >0.5 ng/mL indicates a two-fold probability of sepsis. In 2010, in a meta-analysis results, PCT have shown a higher pooled sensitivity (72%) p=<0.05[14] and PCT is a sensitive marker in detection of early onset neonatal sepsis[15,16]. These finding are same as our results which showed the significance of PCT AUC 84.5%, p<0.05.

In addition, by using the multiple markers combining early sensitive marker with hematological indices will further enhance the predictive accuracy of these mediators for susceptible cases. These tests are simple and cost effective so help clinicians to avoid unnecessary antibiotic therapies. In these aspects, hematological ratio such as NLR and PLR are emerging in early detection of neonatal sepsis because no extra blood sample or equipment is required for these ratios[17]. To our knowledge there have been no such study in cord blood which show the prediction value of NLR and PLR in EONS. Platelet-lymphocyte ratio appears to be more closely related to sepsis as indicated in previous studies that platelets are tightly associated with severe inflammatory diseases[18-19]. Previous studies have shown these ratios as predictor of birth outcome if neonate is presumed to be affected because neonatal NLR reflect the maternal chorioamniotic inflammation[11,20]. NLR and PLR could be a predictor of poor birth outcome. The prognostic impact of these ratios in disease prediction is still unknown[18]. A previous study showed PCT and NLR have similar predictive values for sepsis[21] but our study didn't show any significance of NLR in sepsis but showed strong significant of PLR with sepsis. These finding are consistent with studies which shows PLR is more valuable than either platelet or lymphocyte count alone in the prediction of various septic conditions[22,23].

Mean value for NLR, reported higher in Non-Hispanic Blacks was 2.24, in Whites It was 1.76 less than blacks in a study conducted in United States[24]. Another study conducted in Chinese population, mean baseline value for NLR was 1.5 ± 0.05 in healthy adults. Different studies used different reference value but the use of these arbitrary cut off points are characteristically misleading for risk stratification because NLR and PLR values are affected by age, environment, cardiovascular diseases etc[25]. So, there is a need for establishment of reference value and cut off points according to local factors. In our study, cutoff of NLR was determined to be 1.39 with a CI of 95% (50.5%-79.7%) with an AUC of 65.1% at sensitivity of 63% and specificity of 58.5%. Cutoff of PLR was determined to be 47.48 with a CI of 95% (53.0%-80.5%) with an AUC of 66.8% at sensitivity of 78.9% and specificity of 49%.

These stable values, neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) combines neutrophils and platelets with lymphocytes in the calculation, are comparable with procalcitonin. So, these ratios can be used conveniently in combination with procalcitonin without requirement of additional sample or equipment.

CONCLUSION

Combination of hematological markers, NLR and PLR, with procalcitonin in cord blood could be used as a simple, sensitive and predictive parameter for identifying neonates susceptible to sepsis.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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