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Relationship between Neonatal Sepsis and Red Blood Cell Distribution Width

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Abstract

Recently, studies suggested that Red Blood Cell Distribution Width (RDW) is a useful biomarker of disease in critically ill patients and an increased RDW is an independent predictor of mortality in sepsis. Evaluation of the role of RDW as a marker for diagnosis of neonatal sepsis and to correlate between RDW and other parameters of neonatal sepsis. This case control study was carried on at neonatal intensive care unit (NICU) at National Medical Institute –Damanhor. One hundred neonates were enrolled in this study, fifty neonates with neonatal sepsis either suspected or confirmed according to clinical and laboratory data and for control fifty healthy neonates with age and sex matched with the case group, all participants were investigated for CBC including (RDW, TLC, Hb, Plt, and neutrophil count), CRP and blood culture and sepsis screening. This study was conducted over a period of six month between June 2020 and November 2020. It was found that Sensitivity, specificity and accuracy of RDW CV% as a predictor of infection, it was found that at cut off value 16.2 the sensitivity was 96.0, specificity 93.0 and accuracy 95.0. Red Blood Cell Distribution Width is a cheap, readily available parameter which can be useful for predicting sepsis in neonates. Also, it can be used to predict sepsis severity as a significant difference was found between Red Blood Cell Distribution Width (RDW) value and IT ratio.

Keywords: Red Blood Cell Distribution Width, Neonatal Sepsis, Biomarker.

1. Introduction

Sepsis is a life-threatening disease caused by impaired host response to infections and is responsible for approximately 45% of neonatal emergencies Also, it is a leading cause of neonatal mortality and morbidity, accounting for 14% of deaths in that age group [1]. The early symptoms and signs of neonatal sepsis are usually mild and non-specific but can rapidly progress to septic shock, disseminated intravascular coagulation and death, so early aggressive medical therapy is indicated in neonates with suspected sepsis based on organ source of sepsis [2]. The incidence of sepsis in the newborn infants is greater than any other period of life and varies from one place to another. Although some studies in the developed countries announced that the incidence of neonatal sepsis ranged from 1 to 5 cases per 1000 live births, some other population-based studies in the developing countries reported septicemia rates ranged

49 - 170 per 1000 live births [3]. In Egypt, neonatal sepsis is considered a big problem as with lack of infection control measures and inadequate nursing staff, the incidence range increases more than the documented incidence. Therefore, the present study was undertaken to look for the association of Red Blood Cell Distribution Width with neonatal sepsis [4]. Red blood cells distribution width is a parameter reflecting the heterogeneity of the peripheral red blood volume. In clinic, it can be understood whether the size of RBC volume is uniform through detection of Red Blood Cell Distribution Width. More Red Blood Cell Distribution Width is, the more uneven the RBC size is, and the higher the volume heterogeneity is [5]. It may be elevated in conditions of ineffective production or increased destruction of red blood cells which commonly occur in inflammatory or infectious situations [6].

2. Patients and Methods

This case control study was carried on at neonatal intensive care unit (NICU) at National Medical Institute –Damanhor. One hundred neonates were enrolled in this study, fifty neonates with neonatal sepsis either suspected or confirmed according to clinical and laboratory data and for control fifty **healthy neonates (group 1)** with age and sex matched with the **case group (septic group; group 2**). This study was conducted over a period of six month between June 2020 and November 2020.

2.1 Inclusion criteria:

Full term baby from 0 day to 28 days old presented with suspected (clinically) or confirmed (clinically and laboratory) neonatal sepsis. We selected our patients according to clinical scoring system (Griffin et al clinical score for symptoms and signs of sepsis), Total score = SUM (points for all 7 parameters). Interpretation: minimum score: Maximum score: 13• A score $\geq$ 2 was associated with sepsis Griffin, [9].

2.2 Exclusion criteria:

Newborns suffering from asphyxia at birth, obvious congenital anomalies, cyanotic congenital heart disease, preterm babies less than 37 weeks gestational age, neonates who had recent blood transfusion and neonates born to mothers with severe anemia.

2.3 Technique:

In both groups surgical procedures were performed in a lithotomy position. The procedures were performed as a day case procedure under general or regional anesthesia and the anal canal was visualized with the help of proctoscope.

2.4 Ethical Considerations

The study protocol was approved by the medical Ethics Committee of Faculty of Medicine for Girls, Cairo, Al-Azhar University and the aim of the study was explained to the parents, Verbal consent was taken from parents, and Privacy of all data was assured.

2.5 Methodology

All newborns in the study were subjected to the following: Full maternal history taking including: Maternal age, gravity, parity, medical and obstetric history, details of labor with stress on any prenatal hazards e.g., pre-eclampsia, premature rupture of membrane PROM, antepartum hemorrhage, fever, urinary tract infection (UTI), chorioamnionitis. Detailed perinatal history of the neonate including Gestational age, mode of delivery, early postnatal cyanosis or using any invasive procedures.

2.6 Examinations

Clinical assessment as regard full general and systemic assessment including Vital

signs: heart rate, respiratory rate, temperature. Anthropometric measures: weight, length and head circumference. Neonatal reflexes e.g., Moro and suckling reflexes. Apgar score at 1 and 5 minutes, and gestational age assessment using new Ballard score. Local examination: To detect clinical signs of sepsis: Respiratory dysfunction: Apnea, intercostal retraction, increase oxygen requirement and signs of respiratory distress. Circulatory dysfunction: Poor peripheral circulation, hypotension, tachycardia, shock and prolonged capillary refill. GIT dysfunction: Abdominal distension, bloody stool, feeding intolerance, hepatomegaly and jaundice. Neurological dysfunction: irritability, poor reflexes, hypotonia and lethargy.

2.7 Investigations

Fully CBC including (RDW, TLC, Hb, Plt, neutrophil count), CRP and blood culture.

2.8 Serum parameters

WBCs count, absolute neutrophil count, Hemoglobin level.

2.9 Sepsis screening

Total leucocytic count, Platelet count, C-reactive protein (CRP) and Erythrocyte sedimentation rate (ESR).

2.10 Cultures

Blood samples were taken at time of presentation of sepsis. Aerobic and anaerobic cultures were done on blood agar plates at 10% Co2 and on MacConkey agar plates.

2.11 RDW measurement from CBC

According to RDW values measured on the same day the blood cultures were obtained. Neonates were divided into normal RDW group (including low RDW) and increased RDW group. (The normal value of RDW in our laboratory was 11.5-14.5%).

2.12 Statistical analysis of the data

Data were fed to the computer using IBM SPSS software package version 24.0. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test. Quantitative data were described using mean and standard deviation for normally distributed data. For normally distributed data, comparison between two independent populations was done using independent t-test. Significance test results are quoted as two-tailed probabilities. ROC curve was used to detect Sensitivity, specificity and accuracy of RDW CV% as a predictor of infection with detection of optimum cut off. A p value $\leq$ 0.05 was considered significant.

3. Results

There was statistically significant increase in both Hb and PLT in control group (group 1) more than cases with sepsis (group 2), while there was a significant increase in WBC and RDW CV% in group 2 more than group 1. Table (1). In cases group (patients with sepsis); Positive culture was higher with 33(66%), IT ration > 0.2 was higher with 29(58%) followed by < 0.2 with 16(32%). Griffin clinical score ranged from 2-6 with mean value 3.960±1.399 and CRP ranged from 12-98 with mean value 64.160±32.604. Table (2) there was highly statistically significant increase in RBS in group 2 more than group 1 ($P < 0.05$) Table (3). Sensitivity, specificity and accuracy of RDW CV% as a predictor of infection, it was found that at cut off value 16.2 the sensitivity was 96.0, specificity 93.0 and accuracy 95.0. Table (4).

Table (1): Comparison between the two studied groups regarding Complete blood count.

Complete blood count	Group 1 "control group." "N=50"	Group 2 "Cases group." "N=50"	t-test P- value
WBC (10^3/ul)			
Range	4.5-20.43	4.96-16.52	2.65
Mean	12.10	13.40	0.022*
S.D.	3.92	2.52	
HGB (g/dl)			
Range	7.9-22.1	7.9-16.7	2.96
Mean	12.77	11.55	0.021*
S.D.	3.54	2.49	
PLT (10^3/ul)			
Range	179-589	7-962	1.96
Mean	362.11	305.88	0.04*
S.D.	103.77	223.12	
RDW CV%			
Range	11.6-17.0	14.8-24.0	7.25
Mean	14.21	18.12	0.001*
S.D.	1.53	2.06	

Table (2): Distribution of patients in group 2 regarding culture, Griffin clinical score, CRP, IT ratio.

	Group 2"Cases group" "n=50"	
	No.	%
Culture		
Negative	17	34.0
Positive	33	66.0
IT ratio		
<0.2	16	32.0
0.2	5	10.0
>0.2	29	58.0
Griffin clinical score		
Range	2-6	
Mean	3.960	
S.D.	1.399	
CRP		
Range	12-98	
Mean	64.160	
S.D.	32.604	

Table (3): Comparison between the two studied groups regarding RBS.

	Group 1"control group." "N=50"	Group 2"septic group." "N=50"	T-test P- value
RBS	80-133	110-250	
Range	101.574	198.400	0.0001*
Mean S.D.	13.632	29.579	

Table (4): Sensitivity, specificity and accuracy of RDW CV% as a predictor of infection.

Area	Std. Error	Cut off value	P value	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
.951	.018	16.2	.0001*	.915	.987
Sensitivity	96.0				
Specificity	93.0				
Accuracy	95.0				

There was statistically significant relation between IT ratio with RDW, Griffin clinical score and CRP ($P < 0.05$) while there was no statistically significant relation regarding WBC, Hb and Plt ($P > 0.05$). Table (5). There was statistically significant relation between IT ratio and tachycardia ($P < 0.05$) while there was no statistically significant relation regarding tachypnea ($P > 0.05$). Table (6). It was found that there was no significant correlation between hemoglobin level and

RDW in group I and II, i.e., the low hemoglobin level show insignificant relation with RDW in both studied and control group (p >0.05). Table (7).

Table (5): Relation between IT ratio and laboratory findings.

	IT ratio			FP
	<0.2	0.2	>0.2	
WBC (10^3/ul)				
Range	4.50-15.60	6.49-16.95	4.68-20.43	1.60
Mean	11.19	10.32	12.91	0.212
S.D.	3.15	4.97	4.04	N.S.
Hb (g/dl)				
Range	7.90-19.30	8.60-10.90	8.00-53.00	1.98
Mean	12.15	9.68	15.04	0.149
S.D.	3.34	1.04	8.12	N.S.
RDWCV%				
Range	14.80-17.80	16.60-18.90	16.00-24.00	11.30
Mean	16.64	17.14	19.11	0.0001*
S.D.	0.86	1.00	2.12	
Plt (10^3/ul)				
Range	25.00-827.00	7.00-814.00	13.00-962.00	0.260
Mean	272.19	321.80	321.72	0.772
S.D.	224.86	301.51	214.70	N.S.
Griffin clinical				
Score				
Range	2.00-5.00	3.00-6.00	2.00-6.00	40.3
Mean	2.38	4.20	4.79	0.0001*
S.D.	0.81	1.30	0.82	
CRP				
Range	12.00-96.00	24.00-96.00	48.00-98.00	24.3
Mean	32.38	57.60	82.83	0.0001*
S.D.	21.44	36.40	21.88	

F=ANOVA-test, P was significant if ≤ 0.05 NS =Not significant, * Significant at level 0.05

Table (6): Relation between IT ratio, Tachycardia and Tachypnea.

	IT ratio						X2P
	<0.2		0.2		>0.2		
	No.	%	No.	%	No.	%	
Tachycardia							5.624
No	13	81.2	3	60.0	13	44.8	0.05*
Yes	3	18.8	2	40.0	16	55.2	
Tachypnea							1.985
No	10	62.5	2	40.0	12	41.4	0.371
Yes	6	37.5	3	60.0	17	58.6	N.S.

X^2=Chi square-test, P was significant if ≤ 0.05 NS =Not significant, * Significant at level 0.05

Table (7): Correlation between hemoglobin level and RDW in both studied groups.

Hemoglobin # RDW	Group I	Group II
Correlation coefficient(r)	-0.232	-0.069
P value	0.068 N.S.	0.636 N.S.

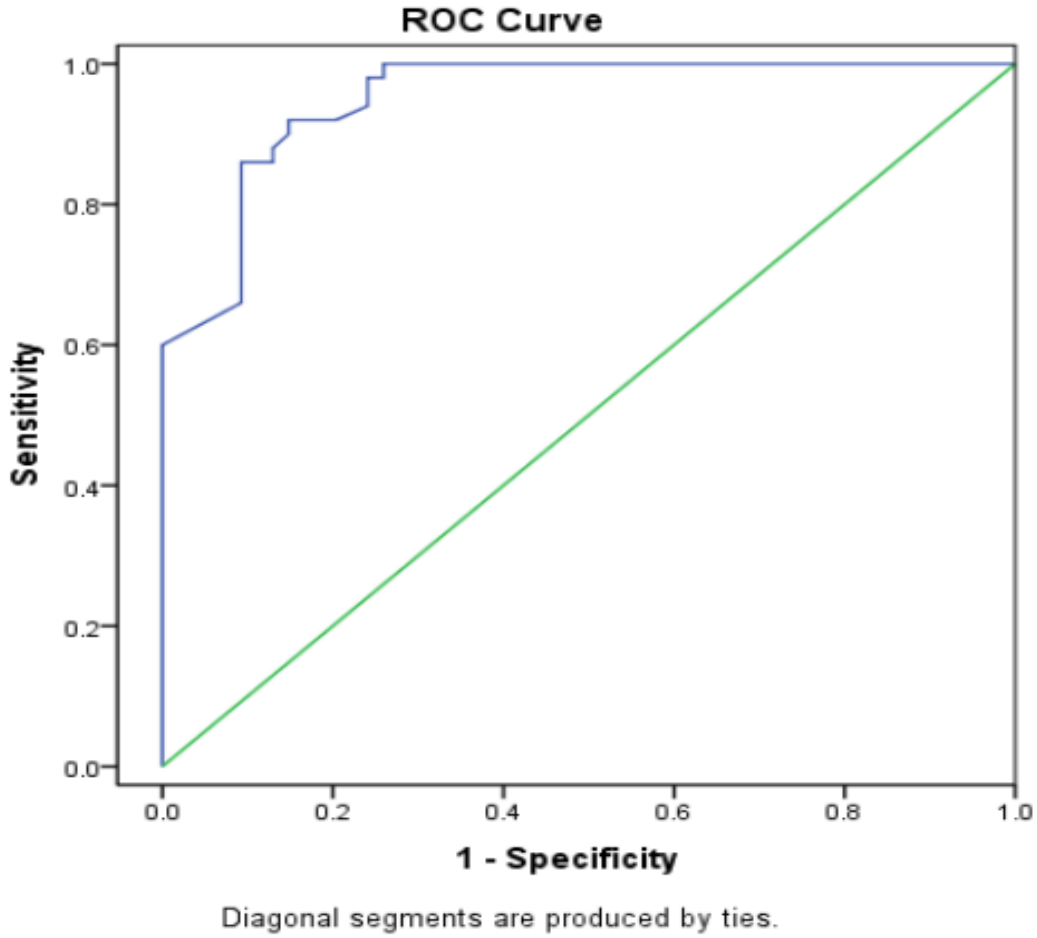

Figure (1): ROC curve to predict the sensitivity, specificity and accuracy of RDW CV% in predict infection.

4. Discussion

Previous studies suggest that RDW is a useful biomarker of disease in critically ill patients and an increased RDW is an independent predictor of mortality in sepsis. Although earlier studies have shown the association of RDW with sepsis, nevertheless the role of increased RDW in neonatal sepsis is not very clear [10]. The aim of the work was to evaluate the role of RDW as a marker for diagnosis of neonatal sepsis and to correlate between RDW and other parameters of neonatal sepsis. This case control study was carried on at neonatal intensive care unit (NICU) at National Medical Institute -Damanhor, one hundred neonates were enrolled in this study, fifty neonates with neonatal sepsis either suspected or confirmed according to clinical and laboratory data and for control fifty apparently healthy neonates with age and sex matched with the case group. This study was conducted over a period of six months between June 2020 and November 2020. Turhan et al., [11] reported in his study that low birth weight newborns have inappropriate immunological response, also they concluded that Birth weight <1000 grams increase the neonatal infection rate by 26 folds when compared to term infants. In our study, when comparing between the two studied groups regarding complete blood count, we found that there was a significant increase in WBCs in septic group more than the control, the hemoglobin level was significantly lower in case group more than the control group, also the platelet count was significantly lower in cases less than the controls. The RDW was significantly

higher in cases group more than the control group, (18.12 ± 2.06, 14.21 ±1.53 respectively). This can be explained by inflammation may cause an increase of neurohormone and endocrine hormone in the body including noradrenaline, angiotensin I and other angiotensin levels and renal ischemia. These neurotransmitters can stimulate red blood cell proliferation through increasing the secretion of erythropoietin (EPO) leading to increase in RDW and inflammatory factors which may affect marrow hemopoietic function and iron metabolism in the body to cause RDW increase [12]. Ellahony et al. [13] studied the red cell distribution width among neonatal sepsis, they found that RDW can predict the prognosis of critical patients. Although the exact mechanism of the correlation between RDW and mortality in septic children is unclear, it may involve the changes of red cell balance caused by the body's inflammatory response and oxidative stress. In agreement with our results, Deka et al., [14] studied the red cell distribution width as a diagnostic marker in neonatal sepsis, this was a prospective observational study, 50 normal and 50 sepsis neonates were considered for the study, they found that mean RDW (%) was significantly higher in sepsis neonates (18.59±1.28) than in normal newborns (16.21±1.35). The ROC curve analysis of RDW in the diagnosis of neonatal sepsis is shown that the Area under Curve was found out to be high (0.938) which proves that RDW is a very useful test in the diagnosis of neonatal sepsis [14]. An RDW cut off of 16.95% has a sensitivity of 96%, specificity of 80%, PPV of 82.75%, NPV of 95.23% and an accuracy of 93.8% in the diagnosis of neonatal sepsis. According to a study by Tonbul et al,[15] mean normal range of RDW in neonate's ≤34 weeks was 17.8±2.1 and that of neonates ≥35 weeks was 16.7±1.6. According to a study by Martin et al, [16] the normal range of RDW in newborns was 17.1±1.7, independent of gestational age. The range of RDW in normal neonates in our study is more or less similar to the values obtained by the authors Martin et al, [16]. Also in agreement with our results of RDW, Salim et al., [17] studied the Correlation between Neonatal Sepsis and Red Blood Cell Distribution Width (RDW), they found that the mean RDW was significantly higher in sepsis cases compared to controls (P = 0.001), also this finding was in agreement with Jianping et al., [18]who reported that RDW value of sepsis group (19.61±1.48 &18.35± 1.79 respectively) was much more higher than that of normal control group. Snehal L. Martin et al., [19] observed that RDW values were higher in the neonatal sepsis cases than those of the control group (p<.001). Similarly, Cosar et al., [20] found in their study that RDW indices were higher than those of the control group (p<0.001) in term and near-term newborns with EONS. But Abbasoglu et al., [21] did not find any change in RDW values in neonatal sepsis. However, these studies were retrospective in nature with a small sample size. In our study, it was found that the relation between Sepsis score and C reactive protein show a significant relation between results of C-reactive protein positive and high probable sepsis score, 94.9% of high probable group was positive CRP (p <0.05). In our study, the serum C-reactive protein (CRP) level was significantly higher in the clinically suspected neonatal sepsis groups than the control group and it showed higher specificity than other markers. This result is consistent with other studies [22]. A study done by Prashant et al., [23] showed that CRP has high specificity which correlates with our study results, and it also suggested that the high C-reactive protein (CRP) level is a better indicator of severe bacterial infection in neonates. CRP is widely studied, and commonly available laboratory test used for the diagnosis of neonatal sepsis. Hofer et al., [24] in their study, stated that serial CRP measurement along with other markers such as interleukins improves the diagnostic

accuracy of neonatal sepsis There were studies which stated that CRP level is a good predictor of severe bacterial infection in neonatal sepsis, but the increase in CRP level is low in case of sepsis due to Coagulase Negative Staphylococci (CONS) infection which correlates with our study, and it was proposed that it causes less inflammation and tissue damage as it is a low-level pathogen [14].

5. Conclusion

RDW is a cheap, readily available parameter which can be useful for predicting sepsis in neonates. Also, it can be used to predict sepsis severity as a significant difference was found between RDW value and IT ratio.

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