SIGNIFICANCE OF RED CELL DISTRIBUTION WIDTH AS A PREDICTOR IN NEONATAL SEPSIS

By

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ABSTRACT

Introduction: *Neonatal sepsis is characterized by presence of bacteremia and clinical manifestations caused by microorganisms and their toxic products, when neonatal sepsis identified early and accurately, the degree of severity can be easily determined which help proper management. Therefore, recognizing a single marker or set of markers for diagnosis of such problem may help decrease the global impact of sepsis.*

Aim of the Work: *Evaluation the role of RDW as a marker for diagnosis of neonatal sepsis and a marker for predicting the clinical outcome of the neonatal sepsis.*

Patients and methods: *The study was a prospective study of 100 neonates (75 cases & 25 controls) who were admitted into the neonatal intensive care unit of Al-Hussein University Hospital during the period from June 2018 to April 2019. A Written consentwas obtained from the parents of patients.*

Results: *mean RDW was significantly higher in cases compared to controls (16.55 ± 2.56 & 14.96 ± 1.63 respectively) (P < 0.006). Significant difference regarding RDW value that increased in parallel with the severity of mild sepsis to severe sepsis and septic shock (14.80%, 16.50% and 19.25% respectively; p < 0.0001). HB was nearly equal in cases and the controls, the difference was not statistically significant (p=0.799), CRP level was normal in 80% of controls, and was elevated in all cases with statistically significant difference (P < 0.001). CRP showed a statistically significant relationship (P < 0.001) with the severity of the disease, higher in septic shock than sever cases than in mild ones (83.03 ± 34.41 & 33.68 ± 9.47 & 20.39 ± 10.40femtoliter respectively). Platelets were lower among septic shock than severe neonatal sepsis and mild forms of sepsis (120.7 ± 41.28 & 172.1 ± 48.88 & 241.4 ±94.95 respectively) it shows statistically significant relation to the severity of the disease (P =<0.001).*

Keywords: *Neonatal; Sepsis; RDW. ; Predictor.*

INTRODUCTION

Neonatal sepsis is characterized by presence of bacteremia and clinical manifestations caused by microorganisms and their toxic products **(Sankar et al., 2008)**.

The incidence of sepsis is significantly higher in premature infants as well as those with extremely low birth weights (ELBW) of less than 1000 grams, compared to term infants and those with weight greater than 1000 grams at birth. African American infants have an increased risk of GBS and LOS, likely secondary to the higher rate of GBS carrier rates in African American females. Among all races, males have a higher risk of sepsis and meningitis, especially with gram-negative enteric bacilli **(Simonsen KA et al., 2014)**.

When neonatal sepsis identified early and accurately, the degree of severity can be easily determined which help proper management. Therefore, recognizing a single marker or set of markers for diagnosis of such problem may help decrease the global impact of sepsis **(Stawicki et al., 2014)**.

Many markers had been evaluated for neonatal sepsis, but no one of these markers was sensitive or specific enough to be adopted as standard of care. Numbers of publications have highlighted the potential use of RDW% in diagnosis and or prognosis of sepsis in neonates. Prognostic markers in sepsis are of great interest **(Pierrakos, 2010)**.The ability to provide diagnosis based on marker, which can be easily available in the complete blood count (CBC) would be greatly helpful in assessing the severity of illness. Recently several studies showed that high RDW value can predict diseases severity especially morbidity and mortality in patients admitted to neonatal intensive care unit (NICU) **(Braun, 2011)**.

RDW considered a measure of the variation of red blood cells (RBC) volume that is reported as part of a standard CBC. Usually RBCs are of standard size (about 6-8μm in diameter). However, certain disorders can cause a significant variation in cell size, the normal reference range of RDW in human RBCs is11.5-15.5%. Mathematically RDW is calculated with the following formula:

RDW = (Standard deviation of MCV÷ mean MCV) × 100

AIM OF THE WORK

Evaluation the role of RDW as a marker for diagnosis neonatal sepsis and outcome predictor of neonatal sepsis.

PATIENT AND METHODS

This study was conducted at neonatal intensive care unit (NICU) at Al-Hussein university hospital, Al-Azhar University.

This study was prospectively conducted over a period of 10 months between June 2018 and April 2019.

Written consents were obtained from the parents or care givers.

All neonates were subjected to the following:

Full history was taken including prenatal, natal, postnatal history of symptoms and signs of sepsis and invasive procedures that were done to the baby after delivery.

Full Clinical examination for early and late symptoms and signs of sepsis as: temperature instability, apnea, respiratory distress, bradycardia or tachycardia, hypotension with hypo-perfusion, feeding intolerance and abdominal distension…. etc.

For both patients and control groups, the following investigations were done:

Base line RDW and other routine investigations, including complete blood count (CBC), C-reactive protein (CRP), Blood culture and sensitivity and then analyzed in light of the clinical data and urine culture and cerebrospinal fluid if indicated.

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Ethical consideration:

1. A written informed consent was obtained from all participants (parents) before participation in the study.
2. An approval by the local ethical committee was obtained before the study.
3. The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.
4. All the data and results of the study are confidential and the participants had right to keep it.

At the start of the study, an explanation of the study was provided, to ensure the potential participant had adequate information to provide informed consent.

Statistical analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of

distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

The used tests were

1. Chi-square test

For categorical variables, to compare between different groups

2. Student t-test

For normally distributed quantitative variables, to compare between two studied groups

3. Mann Whitney test

For abnormally distributed quantitative variables, to compare between two studied groups

4. Wilcoxon signed ranks test

For abnormally distributed quantitative variables, to compare between two periods

RESULTS

Table (1): Demographic characteristics of the studied sample

	Cases (n = 75)		Control (n = 25)		χ^2	p
	No.	%	No.	%		
Gender						
Male	40	53.3	12	48.0	0.214	0.644
Female	35	46.7	13	52.0		
Gestational age						
term	36	48.0	13	43.3	0.120	0.729
Preterm	39	52.0	12	56.7		
Postnatal age (days)						
Min. – Max.	1.0 – 23.0		2.0 – 11.0		U= 434.50*	<0.001*
Mean ± SD.	11.64 ± 6.53		5.56 ± 3.08			
Median	12.0		5.0			
Maternal age (years)						
<20	30	40.0	11	44.0	0.150	0.928
20 – 30	30	40.0	9	36.0		
>30	15	20.0	5	20.0		
Maternal smoking	7	9.3	1	4.0	0.725	FEp=0.675
Site of delivery						
Hospital	64	85.3	22	88.0	0.111	FEp=1.000
Home	11	14.7	3	12.0	0.111	FEp=1.000
Maternal diseases						
HTN	9	12.0	3	12.0	0.0	FEp=1.000
DM	12	16.0	4	16.0	0.0	FEp=1.000
Chorioamnionitis	3	4.0	0	0.0	1.031	FEp=0.571
Onset of neonatal sepsis:						
Early onset sepsis	50	74				
Late onset **sepsis**	25	26				
Degree of severity:						
Sepsis	37	48				
Sever sepsis	22	30				
Septic shock	16	22				

This table shows nearly half of the cases had mild disease (48%) and (52%) had severe disease. Early onset neonatal sepsis was seen in nearly three quarters of the cases (74%) while 26% of them had late onset of sepsis.

Table (2): Comparison between the two studied groups regarding to complete blood count parameters

CBC	Cases (n = 75)	Control (n = 25)	Test of Sig.	P
Hemoglobin gm./dl				
Min. – Max.	11.80 – 20.0	11.80 – 20.0	t=0.255	0.799
Mean ± SD.	14.81 ± 1.28	14.72 ± 1.66		
Median	14.70	14.50		
WBCs /cmm				
Min. – Max.	4600 – 27500	4500 – 31000	U= 328.50*	<0.001*
Mean ± SD.	17044 ± 5499	11204 ± 4813		
Median	17000	9800		
Platelet count (1000/cmm)				
Min. – Max.	56.0 – 450.0	145.0 – 402.0	t= 2.937*	0.004*
Mean ± SD.	195.3 ± 88.53	251.5 ± 61.65		
Median	180.0	247.0		
RDW (femtoliter)				
Min. – Max.	12.90 – 23.0	12.80 – 18.70	U= 590.5*	0.006*
Mean ± SD.	16.55 ± 2.56	14.96 ± 1.63		
Median	16.30	14.40		

This table shows that WBCs and RDW were significantly higher among cases compared to controls (17000 vs. 9800 respectively).

Table (3): Comparison between the two studied groups regarding to indicators of infection

	Cases (n = 75)		Control (n = 25)		Test of sig.	p
	No.	%	No.	%		
WBCs						
Leukopenia 5000)	2	2.7	1	4.0	χ^2= 26.033*	<0.001*
Normal (5000-15000)	25	33.3	22	88.0		
Leukocytosis (>15000)	48	64.0	2	8.0		
I/T ratio						
Positive (≥0.2)	38	50.7	5	20	χ^2= 7.194*	0.007*
Negative (<0.2)	37	49.3	20	80		
Platelets (1000/cmm)						
Normal (≥150)	22	29.3	24	96	χ^2= 6.795*	0.009*
Thrombocytopenia (<150)	53	70.7	1	4		
Blood culture						
Positive	75	100	0	0.0	χ^2= 94.872*	<0.001*
Negative	0	0	25	100.0		
CRP mg/dl						
Negative**<6**	0	0.0	20	80.0	χ^2= 75.0*	<0.001*
Positive>6	75	100.0	5	20.0		

This table shows that WBCs is statistically significant correlated with cases and controls , I/T ratio showed statistically significant differences, platelets were lower among cases compared to controls , CRP was higher in all cases ,the difference was statistically significant andBlood culture was positive in all cases and negative in all controls the difference was statistically significant (0.001).

Table (4): Comparison between the two studied groups regarding to red cell distribution width on admission and on discharge

RDW (femtoliter)	**Cases (n = 75)**		**Control (n = 25)**
	On admission	**On discharge**	
Min. – Max.	12.90 – 23.0	12.90 – 20.0	12.80 – 18.70
Mean ± SD.	16.55 ± 2.56	15.81 ± 1.53	14.96 ± 1.63
Median	16.30	15.40	14.40
Sig. bet. Grps	$p_1=0.005^*, p_2=0.006^*, p_3=0.016^*$		

This table shows that the mean of RDW on admission is higher than the mean of RDW on discharge.

Table (5): Relation between severity of sepsis and different parameters in the cases group (n= 75)

	Sepsis			Test of Sig.	p
	Mild Sepsis (n= 37)	Severe sepsis (n= 22)	Septic Shock (n= 16)		
Hemoglobin (gm/dl)					
Min. – Max.	11.80 – 20.0	12.20 – 16.0	12.50 – 16.40	F= 1.445	0.243
Mean ± SD.	15.04 ± 1.44	14.46 ± 0.96	14.74 ± 1.21		
Median	14.70	14.50	14.70		
WBCs /cmm					
Min. – Max.	7300 – 25000	4600 – 27500	14000 – 27500	H= 21.247*	<0.001*
Mean ± SD.	14830 ± 4688	17120 ± 5629	21060 ± 36.19		
Median	15000	17250	22600		
Sig.bet.Grps	p_1=0.061,p_2<0.001*,p_3=0.008*				
I/T ratio					
Min. – Max.	0.01 – 0.23	0.09 – 0.28	0.14 – 0.28	H= 10.682*	0.005*
Mean ± SD.	0.16 ± 0.05	0.18 ± 0.04	0.22 ± 0.04		
Median	0.17	0.20	0.21		
Sig.bet.Grps	p_1=0.202*,p_2=0.001*,p_3=0.054				
Platelets (1000/cmm)					
Min. – Max.	60.0 – 450.0	75.0 – 260.0	56.0 – 195.0	F= 16.160*	<0.001*
Mean ± SD.	241.4 ± 94.95	172.1 ± 48.88	120.7 ± 41.28		
Median	215.0	180.0	120.0		
Sig.bet.Grps	p_1=0.003*,p_2<0.001*,p_3=0.097				
RDW (femtoliter)					
Min. – Max.	12.90 – 20.30	14.20 – 22.0	13.0 – 23.0	H= 28.986*	<0.001*
Mean ± SD.	15.15 ± 1.65	16.88 ± 2.02	19.31 ± 2.63		
Median	14.80	16.50	19.25		
Sig.bet.Grps	p_1=0.003*,p_2<0.001*,,p_3=0.018*				
CRP mg/dl					
Min. – Max.	12.0 – 48.0	24.0 – 48.0	36.0 – 166.0	H= 48.363*	<0.001*
Mean ± SD.	20.39 ± 10.40	33.68 ± 9.47	83.03 ± 34.41		
Median	18.70	32.0	73.50		
Sig.bet.Grps	p_1<0.001*,p_2<0.001*,,p_3=0.001*				

This table shows that: -

RDW, WBCs and CRP were significantly increased in parallel with the disease severity from mild sepsis, severe sepsis and septic shock while platelet count was significantly decreased in parallel with the same disease severity.

DISCUSSION

Sepsis is a clinical syndrome characterized by a series of immunological, metabolic, hemodynamic and respiratory alterations secondary to an infective process caused by an abnormal SIRS to the organism. Systemic neonatal infections are an important long-term cause of mortality and morbidity for preterm and hospitalized newborn babies **(Dessì A et al., 2014)**.

The early symptoms and signs of neonatal sepsis are usually mild and nonspecific but can rapidly progress to septic shock, disseminated intravascular coagulation (DIC), and death. It is therefore of paramount importance to find a tool for prediction of neonates who are more likely to experience a worse clinical outcome so that closer monitoring and more aggressive treatment would be offered to them.

Despite the many and extensive investigations that have been performed in the last decades, there is still no single test that satisfies the criteria as being the ideal marker for the early diagnosis of neonatal sepsis.

Early detection and timely treatment of neonatal sepsis is of extreme importance in reducing mortality. Although many biomarkers are useful, they are expensive and not easily available.

This study showed that the mean gestational age of the septic neonates and the controls was (35.75 ± 2.34& 37.36 ± 1.44) and their birth weight was (2.70 ± 0.59& 3.15 ±0.59) respectively.

Sepsis was more prevalent among male cases (53.3%) than female (46.7%) and the difference between cases and control was not statistically significant as shown in table (1) Also, this result agreed with **(Shehab El-Din et al., 2015)** who reported that among the studied neonates, 56.7% were males and 43.3% were females resulting in an overall male to female ratio of 1.3: 1. without significant difference ($P > 0.05$).

The prevalence of sepsis was higherin hospital deliveries than home deliveries as in table1.

The prevalence of early onset sepsis (EOS) in our study was 74% while that of late onset sepsis (LOS) was 26%. Similarly, a prior study reported a higher prevalence of EOS of about 73% and attributed this to the mode of delivery and consideration of timeframe of EOS as 72 hours **(Pal et al., 2014)**. In contrast, another study found a higher prevalence of LOS (about 68%) and attributed this to some factors like the definition of EOS as sepsis occurring within 48 hours of delivery **(Medhat et al., 2017)**.

Maternal age in our study was below (20) year old 40%, between (20-30) years old (40%) and above 30 years old 20%. **(VanDykeMK et al., 2009)** said that maternal age is not a significant risk factor for group beta hemolytic streptococci (GBS-specific or non-GBS EOS).

The continued rise in caesarean section (C-section) deliveries raises a major public health concern worldwide. In our study the percentage of C-section was (64%) among the cases and the controls groups and (36%) normal deliveries.

Within the Arab region, rates of CS are far higher in Egypt than any other Arab country through the distribution of CS rates in the Arab region, the second highest rate is recorded in Jordan (28 percent) and the lowest was recorded in Yemen (5 percent) **(Betrán et al.,2016)**.

Blood culture remains the "gold standard" for diagnosing neonatal sepsis, even though, in many cases, it is negative. Regarding blood culture 100% of cases shows positive blood culture while none of controls shows positive blood culture with statistically significant difference (P=0.001) (table 3).

Although the results of **(Neal et al., 2011)**, who reported that as many as 60% of blood cultures would be falsely negative for common neonatal pathogens and this may be explained by the fact that maternal antibiotics given in the majority of preterm deliveries may suppress the growth of bacteria in culture and subsequently give negative blood culture results but we depended on the cases with positive blood culture to be sure definite sepsis. Most of blood cultures were Positive for Klebsiella42% and reflects our main problem in our NICU this agrees with **(Verma et al., 2015)** who found that Klebsiella was the most common pathogen (48.21%) in both early and late onset septicemia.

In our study, mean RDW was significantly higher in cases compared to controls (16.55 ±

2.56 & 14.96 ± 1.63 respectively) (P < 0.006), this finding is in agreement with **(Chenet al., 2015)** who reported that RDW value of sepsis group (19.61±1.48) was much more higher than that of normal control group (16.04±1.25), and there was a significant difference (F=15.6, P=0.0001).

Our patient sub-groups showed a significant difference regarding RDW value that increased in parallel with the severity of illness from sepsis to severe sepsis and septic shock (14.80%, 16.50% and 19.25% respectively; p < 0.0001). **(Chen et al., 2015)** found a similar phenomenon in full term neonates where RDW in sepsis, severe sepsis and septic shock were 16.59%, 18.88% and 19.71% respectively. Conversely, another study, conducted on 122 septic patients with and without shock, detected that RDW is not associated with microcirculatory alterations or prognosis in septic patients **(Fontana et al., 2017)**.

Regarding other markers of diagnosing sepsis, CRP level was normal in 80% of controls, and was elevated in all cases with statistically significant difference (P < 0.001), this finding is in agreement with **(Younis et al., 2014)**, who founded that mean CRP level was significantly higher in patients with sepsis than controls, also in agreement with **(Buch et al., 2011)**, who reported that CRP has high sensitivity and specificity for establishing the diagnosis of neonatal sepsis which is comparable to that of blood culture results.

On the other hand, WBCs were statistically significantly (P = <0.001) higher among cases compared to normal controls, as the vast majority of controls had normal WBCs count (88%), while only two cases had leucocytosis (8%). with high statistically significant difference (p value <0.001) different.

As regards severity of neonatal sepsis RDW is significantly correlated with neonatal sepsis (P < 0.001). It is higher in septic shock than severely cases than in mild cases (19.31 ± 2.63 & 16.88 ± 2.02 & 15.15 ± 1.65 respectively), this suggests that septic neonates with RDW ≥ 17% may have a higher severity of illness and RDW may have value in differentiating between more severe and less severe cases of neonatal sepsis, this finding is in agreement with **(Kader et al., 2015)** who reported that incidence of RDW increase in neonatal sepsis and increased with increasing severity of the disease. He also stated that mean RDW

value in less severe patients were 16.04 ± 0.7 and mean RDW value in more severe patients were 19.75± 1.9. This mean RDW difference in both groups was statistically significant (P<0.001) which points to the fact that raised RDW is associated with increasing severity of neonatal sepsis.

CRP showed a statistically significant relationship (P < 0.001) with the severity of the disease, higher in septic shock than severely cases than in mild ones (83.03 ± 34.41 & 33.68 ± 9.47 & 20.39 ± 10.40 respectively), this finding is in agreement with **(Hofer et al., 2012)** who reported that CRP is higher in severe cases than in mild ones with high sensitivity and specificity in predicting neonatal Gram-negative sepsis, while IgM and IL6 are inferior to it.

CONCLUSION

This study revealed that RDW may become a new indicator for diagnosis and risk stratification of sepsis in newborns due to being simple, less expensive, available and easily repeated as it is routinely done with CBC. The study of the relationship between sepsis and RDW% can make us have a better exploration of the relevant pathological mechanism from a new aspect, to look for the new treatment methods which may block the progressive development of sepsis. On the light of the above results we recommend that future studies with larger samples are needed to confirm these findings, with added emphasis on multivariable diagnostic models that incorporate other biomarkers in addition to the RDW.

AUTHOR STATEMENTS

All authors declare that the study was approved by the Institutional Ethical Committee, of faculty of medicine, Al-Azhar University and written consents were obtained from the parents, and all steps of the research take into consideration guidelines of Helsinki declaration.

Authors also declare that there is no conflict of interest regarding financial or other relationships in this research. We here confirm that this work has not been published in its current form and not accepted for publication elsewhere.

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أهمية قياس توزيع كريات الدم الحمراءكمؤشر تنبؤللتسمم الوليدي

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نبذة مختصرة:

يتميز التسمم الوليدي بوجود تجرثم مع الدم والمظاهر السريرية التي تسببها الكائنات الحية الدقيقة ومنتجاتها السامة ، عندما يمكن تحديد التسمم الوليدي المبكّر وبدقة درجة شدته التي يمكن أن تساعد العلاج لذلك ، قد يساعد التعرف على علامة واحدة أو مجموعة من علامات تشخيص هذه المشكلة على تقليل تاثير التسمم الوليدى.

الهدف من العمل:

تقييم دور عرض توزيع الخلايا الحمراء كعلامة لتشخيص التسمم الوليدي وعلامة للتنبؤ بالنتائج السريرية للتسمم الوليدي.

طريقة البحث:

كانت الدراسة عبارة عن تحليل مستقبلي لمائة طفل حديثي الولادة (75 حالة تسمم وليديو 25 مجموعة) الذين تم

قبولهم في وحدة العناية المركزة لحديثي الولادة في مستشفى الحسين الجامعي خلال الفترة من يونيو 2018 إلى أبريل 2019. تم الحصول على موافقة كتابية من أولياء الأمور.

النتائج:

معدل التغيير فى احجام كريات الدم الحمراء كان أعلى بكثير في حالات التسمم الوليدى مقارنة مع المجموعة الضابطة (16.55 ± 2.56 و 14.96 ± 1.63 على التوالي) (عامل الفرق <0.006). فرق كبير فيما يتعلق بقيمة عامل توزيع الخلايا الحمراء التي زادت بالتوازي مع شدة المرض من تسمم الدم إلى تسمم الدم الحاد والصدمة الإنتانية (14.80 ٪ ، 16.50 ٪ و 19.25 ٪ على التوالى؛بعامل فرق <0.0001). كان عامل عرض توزيع الخلايا الحمراء متساويًا تقريبًا في الحالات والضوابط ، ولم يكن الاختلاف ذا دلالة إحصائية ، وكان مستوى عرض توزيع الخلايا الحمراء طبيعيًا في 80٪ من عناصر التحكم ، وكان مرتفعًا في جميع الحالات مع وجود فرق ذي دلالة إحصائية (<0.001). أظهر عامل توزيعالخلايا الحمراء علاقة ذات دلالة إحصائية (<0.001) مع شدة المرض ، أعلى في الصدمة الإنتانية من الحالات الشديدة من في الحالات الخفيفة (83.03 ± 34.41 و 33.68 ± 9.47 و 20.39 ± 10.40 على التوالي). كانت الصفائح الدموية أقل بين الصدمة الإنتانية من الإنتان الوليدي الوخيم مقارنة بالأشكال الخفيفة للمرض (120.7 ± 41.28 و 172.1 ± 48.88 و 241.4 ± 94.95 على التوالي) وتظهر علاقة ذات دلالة إحصائية مع شدة المرض (<0.001).

الكلمـــات المفتاحيـــة: حـــديثي الـــولادة؛ تعفـــن الـــدم. عـــرض توزيـــع الخلايا الحمراء.