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COMPARISON OF THE ACCURACY OF NEUTROPHIL CD 64 VERSUS C-REACTIVE PROTEIN AS A TEST FOR THE EARLY DETECTION OF NEONATAL SEPSIS

By

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ABSTRACT

Background: Early identification of neonatal infection is, however, a major diagnostic problem due to presence of non specific clinical signs and limitation of current diagnostic procedures.

Objective: Making a comparison of the accuracy of neutrophil cluster of differentiation 64 (CD 64) versus C - reactive protein (CRP) as a single test for the early detection of neonatal sepsis.

Patients and Methods: A prospective study enrolled newborns with proved sepsis (n=64) and control newborn (no=32). CRP, neutrophil CD64, complete blood count and blood culture were taken at the time of suspected sepsis for proved sepsis and at the time of venipuncture for labortary tests in control newborns. Neutrophil CD64 was analyzed by flowcytometry.

Results: CD64 significantly elevated in the groups with proved sepsis ,whereas CRP did not significantly increase in comparison with controls. CD64 and CRP have sensitivity 85.9 % and 48.7 %, specificity 78.1% and 71.9%, positive predictive value of 88.7%and 77.5% and negative predictive value 73.5% and 41.1 % respectively.

The area under the receiver operating characteristic curves for CD64 and CRP were 0.83 and 0.48 respectively .

Conclusion: CD64 expression on neutrophils increased significantly in neonates with neonatal sepsis. CD64 was a more specific marker for neonatal sepsis than CRP as shown by area under the curve (AUC)

Key words : Neutrophil CD64, C-reactive protein, neonatal sepsis.

INTRODUCTION

Infection is one of most important causes of neonatal mortality**.** Early identification of neonatal infection is however a major diagnostic problem particularly in preterm and low-birth weight infants due to presence of non specific clinical signs and limitation of current diagnostic procedures ***(Prashant et al., 2013).***

Neonatal sepsis is classified into early - onset form (EONS) within the first 72 hours of life and late – onest form (LONS) afterwards ***(Stoll et al., 2011).***

The current gold standard for confirming diagnosis of neonatal sepsis is blood culture. However, blood culture

results are not available for 48 hours after starting the culture, and if blood cultures are drawn after administration of antibiotics, growth of microorganisms can be suppressed. Hence, a reliable inflammatory marker or set of markers is required for prompt and accurate identification of neonatal sepsis ***(Icardi et al., 2009).***

C-reactive protein (CRP) is a conventional inflammatory marker as a kind of acute phase protein. Concentrations of CRP increase at around 24 hours after onset of infection, peak between 36 and 50 hours and remain elevated throughout infection ***(Batlivala et al ., 2009).***

Neutrophil CD64, know as fc - gamma receptor 1(FcγRI), neutrophil CD64 has high affinity to the Fc portion of immunoglobulin G (IgG) and is expressed and upregulated by bacterial or endotoxin interaction. Upregulation of CD64 is considered to be very early step of the host's immune response to bacterial infection, increasing approximately one hour after invasion ***(Livaditi et al ., 2010).***

The present work aimed to compare between accuracy of neutrophil CD 64 and C – reactive protein as a test for the early detection of neonatal sepsis.

PATIENTS AND METHODS

The study was carried on ninety six newborns with the approval of the ethical committee of neonatal intensive care unit and parents of the neonates. They met all inclusion and exclusion criteria for enrollment into this study. They were divided into 2 groups.

Group 1 included 64 neonates with proved sepsis

Group 2 included 32 apparent healthy neonates

Inclusion criteria: Gestational age (30-42) weeks, and birth weight > 1. 2 kg

Exclusion criteria: Presence of any congenital anomaly and presence of surgical problem

All newborns were subjected to comprehensive history taking, clinical examination and laboratory investigations (CBC and analysis of neutrophil CD64)

Statistical analysis: Data were collected and tabulated. Mean and standard deviation (SD) or median and interquartile range (IQR) were estimates of quantitative data, while frequency and percentage were estimates of qualitative data. Differences in clinical and biochemical characteristics were tested by Student's *t* test for quantitative data and by chi-square test for non-parametric (qualitative) data. A two-sided *P* value <0.05 was considered statistically significant.

RESULTS

There was a highly significant difference between case and control as regard prematurity and low birth weight. No significant difference between case and control as regard sex and mode of the delivery (Table 1).

Table (1): Distribution of the studied groups according to sex, gestational age, birth weight and mode of delivery.

Groups / Parameters	Control group		Cases group		P-value
	No.	%	No.	%	
Sex					
Female	18	56.2%	23	35.9%	0.058
Male	14	43.8%	41	64.1%	
Gestational age					
Preterm	8	25.0%	58	90.6%	<0.001
Full term	24	75.0%	6	9.4%	
Mean ± SD	38.41 ± 1.27		34.00 ± 2.25		<0.001
Range	36 – 41		31 – 38		
Birth weight					
<1.5	0	0.0%	23	35.9%	<0.001
1.5-2.5	0	0.0%	30	46.9%	
>2.5	32	100.0%	11	17.2%	
Mean ± SD	3.26 ± 0.38		1.85 ± 0.76		<0.001
Range	2.6 – 4		0.22 – 4.3		
Mode of delivery					
Spontaneous vaginal delivery	18	56.2%	24	37.5%	0.081
Cesarean section	14	43.8%	40	62.5%	

There was a significant comparison between neutrophil CD64 and CRP with gestational age and birth weight. No significant comparison between CD64 and CRP with sex (Table 2).

Table(2): Comparison between CD64 and CRP with sex, gestational age and birth weight in our studied cases**.**

Groups / Parameters	Neutrophil CD 64	CRP
	Mean ± SD	**Mean ± SD**
Sex		
Female	51.53 ± 12.8	13.69 ± 16.32
Male	55.48 ± 13.43	18.32 ± 20.49
T	1.456	0.967
P-value	0.149	0.337
Gestational age		
Preterm	56.57 ± 12.62	21.86 ± 20.13
Full term	47.68 ± 12.71	8.96 ± 14.13
t	0.454	2.843
P-value	0.002	0.006
Birth weight		
<1.5	60.69 ± 10.91	30 ± 24.27
1.5-2.5	55.92 ± 12.02	29 ± 17.32
>2.5	48.62±13.34	8.23 ± 11.96
F	7.757	13.262
P-value	<0.001	<0.001

There was a highly significant comparison between CD64 and CRP with staff % , I: T ratio, lymphocyte, monocyte and platelet . No significant comparison between CD64 and CRP with WBCs, mature and esinophils (Table 3).

Table(3): Comparison between CD64 and CRP with complete blood count in our studied cases.

Groups / Parameters	Neutrophil CD 64		CRP	
	r	**P-value**	**r**	**P-value**
WBC (x 1000)	0.137	0.182	0.123	0.336
Staff (%)	0.733**	<0.001	0.697**	<0.001
Mature (%)	-0.016	0.877	-0.092	0.475
Immature to total	0.735**	<0.001	0.729**	<0.001
Lymphocyte (%)	-0.389**	<0.001	0.810**	<0.001
Monocyte (%)	0.964**	<0.001	0.876**	0.004
Esinophils(%)	0.042	0.681	0.118	0.356
Platelets (x 1000)	0.825**	<0.001	-0.263*	0.010

There were significant higher levels of CD64 and CRP than control group **(**Table 4**).**

Table (4): Differences between cases and control group in the mean level CD64 and CRP

Groups / Parameters		Control group	Cases group	Independent t-test	
		No=32	**No=64**	**t**	**P-value**
Neutrophil CD 64	Mean ± SD	42.14 ± 12.51	58.12 ± 11.42	6.232	<0.001
CRP	Mean ± SD	4.03 ± 1.52	29.03 ± 20.03	7.043	<0.001

There was a highly significant higher sensitivity and specificity of CD64 than CRP (Table 5).

Table (5): Sensitivity and specificity of CD64 and CRP

Variables	Cut off point	AUC	Sensitivity	Specificity	+PV	-PV	P-value
Neutrophil CD 64	>43.7	0.839	85.9	78.1	88.7	73.5	< 0.001
CRP	>6	0.488	48.7	71.9	77.5	41.1	

DISCUSSION

The diagnosis of sepsis remains one of the most difficult tasks for physicians and other medical staff. Blood cultures often remain negative in the presence of pneumonia, meningitis and even fulminant blood born septicemia. A rapid laboratory test with high specificity for neonatal sepsis would be a valuable tool in therapeutic decision making ***(Baranda et al., 2010)***.

This rapid upregulation of CD64 expression on the PMNs is a true indication of the current status of neonatal sepsis. Thus, neutrophil CD64 expression should provide improved diagnostic detection of sepsis compared with the standard diagnostic tests ***(Dilli and Dilmen, 2010).***

In this work, ninty six cases of neonates were admitted to the NICU of Al Glaa teaching Hospital and subgrouped into sixty four cases proven as neonatal sepsis and thirty two cases as a control group. All neonatal sepsis patients were diagnosed according to clinical signs, laboratory and radiological criteria.

Prematurity and PROM more than 18 hours, were the most important risk factor of neonatal sepsis in which study accounted for (79.5%) and (45.3%) of studied cases. This agreed with (***Ng et al,. 2009)*** who have reported that PROM more than 18 hours is a strong risk factor for neonatal sepsis.

In this study, we found that positive history of maternal fever was present in about 10.9% of studied cases due to maternal infections. This agreed with ***(Fjaertoft et al., 2010)*** who had noted that maternal fever due to maternal infections like chorioamnionitis and urinary tract infection accounts for 20% of neonatal sepsis cases.

In this study, the most common organisms isolated from proven septic neonates were staph. auerus (46.88%), E.coli (28.13%), strept epidermidis (6.25%), pseudomonus (12.5%), and Klebsilla (6.25%). ***Stoll et al. (2011)*** showed that 25% of all episodes of EONS were caused by Klebsiella and E coli (17.5%), Staphylococcus aureus (18%), GBS (7%), Acinetobacter and pseudomonas (12%).

Abd El Haleim et al. (2012) found that the most commonly isolated microorganisms from blood were CONS (45%), where gram negative bacilli including E.coli, Klebsiella and Enterobacter spp represented 18% of isolated microorganisms.

In this study, about 90.6% of cases were preterm with GA less than 37 weeks, and about 82.8% of cases were LBW with birth weight < 2500 grams. This was agreement with ***(Stoll, 2010)*** who stated that the majority of cases of early onset sepsis had a gestational age < 30 weeks, and a birth weight < 1500 g.

A high statistical significant difference was shown between studied cases and controls as regards staff cell, I/T ratio, CRP and platelets count. This agreed with a study done by **Khair *et al. (2010)*** who found that sepsis episodes were characterized by significantly higher white blood cell counts, and immature/total neutrophil ratios, compared with nonseptic episodes, as well as lower platelet counts.

In this study, the mean CD64 expression in neonates with sepsis was significantly higher than those in the control group. These findings were in agreement with a previous study done by ***Dilli and Dilmen (2010)*** and a study by ***Streimish et al. (2013)*** who showed raised percentages of CD64+ cells in proven sepsis groups.

In this work, ROC curve showed area under the curve (accuracy) for CD64 greater than area under the curve (accuracy) for CRP, AUC for CD64 =0.83, while for CRP =0.48. This implied the greater discriminating power for CD64 over CRP for early detection of neonatal sepsis.

In this study, we found 85.9% sensitivity and 78.1% specificity for CD64 expression. On the other hand, ***Ng and his Colleagues (2009)*** showed very high sensitivity and specificity for CD64 in neonatal sepsis (about 97% and 89%, respectively).

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المقارنة بين دقة نيتروفيل سى دى ٦٤ مقابل بروتين سى التفاعلى كإختبار للكشف المبكر للتسمم الوليدى

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خلفية البحث : يعتبر التشخيص المبكر لعدوى الأطفال حديثى الولادة من المشاكل الخطيرة نظرا لوجود أعراض غير متخصصة ونقص فى طرق التشخيص.

الهدف من البحث : عمل مقارنة بين دقة النتروفيل سى دى ٦٤ وبروتين سى التفاعلى كاختبار للكشف المبكر للتسمم الوليدى .

المرضى وطرق البحث: تضمنت هذه الدراسة مجموعتين: المجموعة الأولى تشمل ٦٤ طفلا مصابين بالعدوى المبكرة ، والمجموعة الثانية تشمل ٣٢من الأطفال الأصحاء كمجموعة ضابطة.

وقد تم اخذ بروتين سى التفاعلى ، ونتروفيل سى دى ٦٤ ، وصورة دم كاملة ، ومزرعة دم من المجموعة الأولى عند التوقع بحدوث العدوى، وتم أخذ عينة من المجموعة الثانية عند سحب المعامل العادية

النتائج : نسبة ظهور سى دى٦٤ لكرات الدم البيضاء متعددة النواه أعلى فى الأطفال المصابين بالعدوى عن نسبته فى أقرانهم الأصحاء وأعلى نسبة فى التشخيص من بروتين سى التفاعلى. وقد وجد أن التحديد والإحساس بإستخدام نتروفيل سى دى ٦٤ أعلى من بروتين سى التفاعلى .

الإستنتاج : نسبة ظهور السى دى ٦٤ أعلى فى الأطفال المصابين بالعدوى عن أقرانهم الأصحاء ويعتبر نتروفيل سى دى ٦٤ علامة تشخيصية للأطفال المصابين أكثر من بروتين سى التفاعلى.