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ABSTRACT

In a trial for evaluation the role of serum procalcitonin (PCT) level in the diagnosis of neonatal sepsis, 39 neonates were included in this study. They were admitted to NICU of Benha university Hospital. They were divided into two groups, 20 newborns with sepsis (group I) and 19 high risk symptomatic newborns (group II). Also 15 healthy neonates were taken as control group (group III). All neonates were subjected to full history taking, clinical examination and clinical sepsis score. Laboratory investigations were done including CBC, CRP, ESR, blood culture to septic neonates and serum procalcitonin (PCT) level.

The mean PCT levels were significantly elevated in septic group than in the other two groups. PCT sensitivity for diagnosing sepsis was 90% and specificity was 100%. Also C-reactive protein (CRP) showed high statistical significant difference between all groups. Its sensitivity was 70% and specificity was 89.5%. In conclusion: PCT is superior to CRP for the diagnosis of neonatal sepsis. One of the most important indication for PCT determination is the monitoring of patients at risk of infection. Because of high specificity of PCT, it can be used for rapid decision between bacterial and non bacterial etiology of infection.

INTRODUCTION

Sepsis is an infection induced syndrome characterized by a number of symptoms and clinical signs[1]. *Mesters and Helterbrand*[2] mentioned that sepsis is the most common cause of death in NICUS, with a mortality rate of about 40%.

Bernard[3] demonstrated the variability in clinical presentation associated with sepsis making its clinical diagnosis is difficult and the laboratory diagnosis is an important adjunct. The diagnosis of sepsis could be made by recovery of the organism from blood culture[4].

However time of positivity of blood culture depends upon number of factors, including type of bacteria, the size of inoculum and the presence of antibiotics prior to culturing[5]. With the launch of procalcitonin (PCT) in 1996, a diagnostic tool became available for identifying severe bacterial infections. PCT facilitate a reliable follow up of the clinical course of these conditions[6].

Ferriere[7] concluded that PCT is a good predictor of early and late onset sepsis in neonates at high risk for infection. Procalcitonin is a 116 aminoacids, it is synthesized by leukocytes[8], and neurocrine cells of internal organs such as the lung and the intestine[9]. It has been proposed that the targeted proteolytic cleavage of PCT in Golgi apparatus is suppressed by the actions of cytokines and endotoxins. So procalcitonin are released into the circulating plasma[10]. At the same time, transcription of PCT mRNA is increased by inflammatory stimuli[8].

PCT is probably degraded by proteolysis. Renal excretion of it plays a minor role, therefore it can be used for the diagnostic purposes in patients with severe renal failure[11].

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PCT is very stable in whole blood, plasma or serum even at room temperature, therefore it can be easily used in routine diagnosis[12]. Therefore we conducted this work to study the role of procalcitonin for the diagnosis of neonatal sepsis.

SUBJECTS AND METHODS

This study was carried out on 39 neonates from neonatal intensive care unit (NICU) of Benha University Hospital during the period from July 2000 to March 2001. They were divided into two groups.

* *Group I (septic group):* It included 20 newborns with sepsis. 14 cases of them with culture proved sepsis and the other (6) cases with negative blood culture but with definite clinical signs of sepsis and positive other laboratory studies. Their ages ranged from 1-25 days and they were 11 males and 9 females.

* *Group II (high risk group):* It included 19 newborns presented with different causes of respiratory distress, birth asphyxia (8), hyaline membrane disease (6), transient tachypnea (2) and meconium aspiration syndrome (3). Their ages ranged from 1-15 days and they were 6 males and 13 females.

Exclusion criteria:

Infants of diabetic mothers were excluded because maternal diabetes was the only variable identified to induce a significant deviation from PCT reference range[6]. Also 15 healthy neonates coming for routine follow up after delivery or circumcision were taken as control group (group III). They were 6 males and 9 females and their ages ranged from 1-25 days.

All neonates were subjected to the following:

1- Full clinical evaluation including:

- History taking with special reference to history of maternal diseases (diabetes, maternal infection, drug intake) mode of delivery, premature rupture of membrane (PROM).
- Full clinical examination including assessment of gestational age, anthropometric measurements, Apgar score at one and five minutes to newborns delivered at the hospital only, general and systemic examination.

2- Clinical sepsis score to group I and group II only:

- Apnea/ tachypnea, cyanosis, respiratory distress.
- Bradycardia/ tachycardia.
- Hypotonia, seizures.
- Poor perfusion or hypotension
- Irritability, lethargy or poor feeding.
- Hepatosplenomegaly, jaundice, abdominal distension.

Clinical signs of infection were defined as the presence of three or more of these categories of clinical signs[10].

II- Laboratory investigations:

1-Complete blood count (CBC) by coulter.

2-C-reactive protein (CRP) by latex.

3-Erythrocyte sedimentation rate (ESR) by the Westergren method recorded in mm/hr.

4-Blood culture using BACTE C-9050.

5-Measurement of procalcitonin, we used the LUMI test procalcitonin kit (Brahms Diagnostica, Gmb H, Berlin) by radioimmunoassay for the specific measurement of procalcitonin in the serum.

Specimen collection:

Ten millimeters of blood were collected by sterile venipuncture from the anticubital area. The specimen was divided as follows: -2ml on purple-topped EDTA tubes mixed (used for CBC & ESR). 1 millimeter was added to broth media for blood culture. Three ml were put in a plain red-topped tube, left to clot then centrifuged to separate serum for CRP. 4 ml were transferred into a polypropylene tube containing EDTA

(1mg/ml of blood) and Aprotinin (500 kIU/ml of blood), then centrifuged at 0°C to separate plasma which stored at -70°C.

Extraction of peptide from plasma was done using C18 columm, 1% trifluroacetic acid and 60% acetonitrile. The eluants were evaporated to dryness using centrifugal concentrator. Blow N2 gas to get rid of acetonitrite, then dissolve the residue in RIA buffer for radioimmunoassay.

Detection of procalcitonin level by RIA[13]:

The assay is based upon the competition between labeled ^{125}I-peptide and unlabeled peptide (either standard or unknown) binding to a limited quantity of specific antibody. As the concentration of standard or unknown in the reaction increases, the amount of ^{125}I peptide able to bind to the antibody decreases. By measuring the amount of ^{125}I peptide bound as a function of the concentration of the unlabelled peptide in standard reaction mixtures, a standard curve was constructed from which the concentration of the peptide in unknown samples was determined.

Statistical methods:

All results are presented as mean and standard deviation, Scheffe tests, student t-test, Chi-square, sensitivity, specificity, positive predictive and negative predictive values and accuracy were used.

RESULTS

Results are presented in tables from (1-8).

Table (1): Statistical comparison of the studied groups as regards weight (kg).

Weight in kg	Group I (sepsis) No = 20	Group II (high risk) No= 19	Group III (control) No = 15
Mean ± SD	2.29 ± 0.73	2.71 ± 0.62	3.00 ± 0.30
F	4.58		
P	< 0.05*		
Scheffe test	III > I		

Table (2): Distribution of the septic neonates according to type of the organisms in blood culture.

Organisms	No.	%
Gram +ve cocci	10	50.0
Proteus	1	5.0
Klebsiella	2	10.0
Citrobacter	1	5.0
-ve culture	6	30.0
Total	20	100.0

Table (3): Statistical comparison between the studied groups as regards complete blood count (CBC).

	Group I (no = 20)	Group II (no = 19)	Group III (no = 15)
RBC's x10^6/dl			
Mean ± SD	3.69 0.98	4.28 1.09	4.51 0.23
F	4.06		
P	<0.05*		
Scheffe test	II = III > I		
Hb (gm/dl)			
Mean ± SD	12.78 2.80	15.13 2.87	15.97 1.06
F	8.11		
P	<0.001*		
Scheffe test	II = III > I		
Haematocrite % (Ht)			
Mean ± SD	36.78 9.16	43.18 9.22	48.40 2.72
F	9.35		
P	<0.001*		
Scheffe test	II = III > I		
Platelets x 10^3/dl			
Mean ± SD	136.25 91.19	221.79 59.17	267.33 47.43
F	15.94		
P	<0.001*		
Scheffe test	II = III > I		
WBC's x 10^3/dl			
Mean ± SD	12.44 8.34	8.67 2.69	7.64 1.06
F	4.05		
P	<0.05*		
Scheffe test	II = III < I		

Table (4): Statistical comparison of the mean ESR (1st hour) and CRP (mg/l) among the studied groups.

	Group I (no = 20)	Group II (no = 19)	Group III (no = 15)
ESR 1st hour			
Mean ± SD	69.50 19.80	30.53 13.63	4.53 1.46
F	88.86		
P	<0.001*		
Scheffe test	All are significant		
CRP (mg/l)			
Mean ± SD	50.40 30.80	30.80 15.44	4.08 1.96
F	28.27		
P	<0.001*		
Scheffe test	All are significant		

Table (5): Statistical comparison between the studied groups as regards PCT level (pg/ml).

	Group I (sepsis) no = 20	Group II (high risk) no = 19	Group III (control) no = 15
Mean ± SD	3705.30 2660.31	390.21 382.33	84.93 73.38
Kruskal Wallis test	38.7		
P	<0.001*		
Dunn test	II = III < I		

Table (6): Sensitivity, specificity, PPV, NPV and accuracy of PCT pg/ml in detection of sepsis.

Procalcitonin level	Group I (sepsis)		Group II (high risk)		Total	
	No.	%	No.	%	No.	%
> 1200 pg/ml	18	90.0	0	0.0	18	46.2
≤ 1200 pg/ml	2	10.0	19	100.0	21	53.8
Total	20	100.0	19	100.0	39	100.0

Sensitivity = 90.0% Specificity = 100.0%
Positive predictive value (PPV) = 100.0%
Negative predictive value (NPV) = 90.5%
Accuracy = 94.9%

Table (7): Sensitivity, specificity, PPV, NPV and accuracy of CRP mg/l in detection of sepsis.

CRP level	Group I (sepsis)		Group II (high risk)		Total	
	No.	%	No.	%	No.	%
> 24	14	70.0	2	10.5	16	41.0
≤ 24	6	30.0	17	89.5	23	59.0
Total	20	100.0	19	100.0	39	100.0

Sensitivity = 70.0% Specificity = 89.5%
PPV = 87.5% NPV = 73.9%
Accuracy = 79.5%

DISCUSSION

This study was carried on 54 newborn infants, they were classified into three groups, group I (septic group) were 20 newborns, group II (high risk group) were 19 newborns and group III (control group) were 15 newborns. This study showed that the mean birth weight in septic group was 2.29 ± 0.73 kg and in control group was 3.00 ±0.30 kg and the difference between both groups was statistically significant (Table 1). This was in agreement with[14].

The most common organisms isolated from blood culture of our septic neonates were gram +ve cocci (50%), proteus (5%), klebsiella (10%), citrobacter (5%) and negative blood culture (30%) (Table 2). Similar findings were reported by Opal and Cohen[15], who found that the epidemiology of sepsis has changed in the last 2 decades with the predominance of gram positive organisms.

As regard RBC's count, HB level and Ht% in this study, there were significant statistical differences of them among the studied groups, with group I (septic) much lower than the other two groups (Table 3). This can be secondary to hemolytic process in sepsis as recorded by Vervloet et al.[16]. In this study platelet count showed statistical significant difference between the studied groups, with group I lower than the other groups (Table 3). This can be attributed to many factors, the most important of them are DIC and the direct effect of bacteria or its products on platelets and/or endothelium with platelets aggregation and adhesion to the damaged endothelium[17]. Also WBC's count showed statistical significant difference between the studied groups with group I much higher than the other two groups (Table 3). This was in agreement with Gazy[18] who stated that changes in leucocytic count is useful in direct identification of bacterial infection. On the other hand[19] found that the total WBC's count was of poor predictive value in neonatal sepsis.

In this study CRP mean value was 50.40 ± 30.80 mg/l in septic neonates, was 30.80 ± 15.44 mg/l in the high risk group and 4.08 ± 1.96 mg/dl in control group with statistical significant difference between them (Table 4). Also CRP gives sensitivity of 70%, specificity of 89.5%, positive predictive value of 87.5% and accuracy of 79.5% in detection of sepsis between group I and group II (Table 7). This was in agreement with Lacour et al.[20] who stated that CRP sensitivity was 89% and specificity was 75% in detecting neonatal sepsis. Abd El-Maksoud[21] recorded CRP sensitivity of 75% in septic newborn, Abd El-Hady[22] found its sensitivity 85% in detection of sepsis, while Amara[23] recorded CRP sensitivity 100% in septic cases. The difference may be due to that the time of sampling varies being lower in first 24 hours and being higher with increasing time.

Procalcitonin (PCT) mean levels was 3705.30 ± 2660.31 pg/ml in group I, was 390.21 ± 382.33 pg/ml in group II and was 84.93 ± 73.38 pg/ml in group III and the difference between them was highly statistically significant with group II = group III < GI (Table 5). PCT showed sensitivity of 90%, specificity was 100% in detection of neonatal sepsis, positive predictive value of 100%, negative predictive value 90.5% and accuracy was 94.9% (Table 6). Lacour et al.[20] found that the sensitivity of PCT was 93% and specificity of 78% meanwhile[24] found that PCT sensitivity was 57% and specificity was 66%. In another study done by Enguix et al.[25] they compared the serum PCT level to those of CRP in suspected bacterial sepsis. They found that PCT was of

diagnostic efficiency 93.8% and was 89.7% for CRP. PCT concentration increases in response to infection and because of its long plasma half life, PCT determination might be a reliable alternative in prediction of neonatal sepsis[26].

When PCT values were compared at the corresponding time with CRP values, the sensitivities of PCT and CRP were 85.7% and 46.4% respectively in initial determination. These sensitivities rose to 100% and 89.2% respectively by the time of the next determination[6]. In conclusion PCT is a sensitive marker of bacterial sepsis.

RECOMMENDATION

We recommend routine measurement of PCT in the neonates with a risk factors for infection to reduce the need for invasive collection of samples for bacteriological testing and for reducing the use/abuse of antibiotics.

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