



Evaluation of serum levels of CD14 in neonatal sepsis and its correlation with type of infection in Beni-Suef University Hospital

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Abstract:

The aim of the present study was to identify the incidence of sepsis among neonates admitted at Beni-Suef University Hospital during the period of the study, to identify different organisms responsible for sepsis and the serum level of CD14 (presepsin) was investigated as a biomarker of sepsis. All neonates suspected with sepsis during the time of the study admitted in neonatal intensive care unit at Beni-Suef University Hospital were screened for sepsis by blood culture and presepsin levels were investigated. The incidence of sepsis was 32.4%. Presepsin marker had a significant role in prediction of presence of sepsis, at a cut off value of 2300 or more of presepsin, it can predict the presence of sepsis with sensitivity of (94.29%), specificity of (88.6%), positive predictive value (89.2%) and negative predictive value (93.9%)

Keywords: Neonatal sepsis, CD14, presepsin.

1. Introduction:

Sepsis is considered a systemic inflammatory response syndrome (SIRS) to an infection. It is characterized by severe response of the body to bacteria (1). It is one of the commonest causes of mortality and morbidity among neonates, in particular hospitalized ones (2). It accounts for 30 - 50 % of total neonatal deaths annually in developing countries (3). In fact, there are a lot of factors

that can predispose neonates in developing countries to sepsis in comparison with neonates in developed countries. (4). Intrinsic factors in the developing world include higher rates of prematurity, intrauterine growth retardation, birth asphyxia, premature and prolonged rupture of membranes and maternal peripartum infections. Among the most important extrinsic factors contributing to the

high risk of sepsis are the lack of antenatal care and unhygienic birth practices (5).

Blood culture is still the gold standard diagnostic method for diagnosing neonatal sepsis (6). Presepsin, the soluble component of CD14, is considered a promising early marker for sepsis that might distinguish between bacterial and nonbacterial infectious diseases (7).

The aim of the study was to identify the incidence of sepsis among neonates admitted at Beni Suf University Hospital during the period of the study, identify different organisms responsible for sepsis and the serum level of CD14 (presepsin) was investigated as a biomarker of sepsis.

2. Patients and Methods:

The current work was case-control study, it was carried out at the neonatal intensive care unit (NICU) of Beni-Suef University Hospital over a period of three months (December 2019 to February 2020).

1- All neonates suspected with sepsis during the time of the study admitted in neonatal intensive care unit at Beni Suf University Hospital were screened for sepsis by blood culture.

2- Neonates were divided into two groups as follows:

Group 1 (cases): comprising all neonates proven to have sepsis.

Group 2 (controls): healthy neonates; same number as cases with matched age and sex.

2.1 Inclusion criteria:

All neonates admitted in neonatal intensive care unit (NICU) of Beni-Suef University Hospital over the period from December 2019 to February 2020 were screened for sepsis by blood culture.

2.2 All patients were subjected to:

Detailed data collection, including History taking (Gestational age, mode of delivery, age, sex), probable risk factors of neonatal sepsis e.g. (IV cannula insertion, device attachment), broad spectrum antibiotic intake, cause of admission and relevant lab investigations e.g. CRP.

Blood samples were collected for blood culture and serum CD14 level detection.

Blood samples collected were inoculated into aerobic BacT/ALERT (BioMérieux) bottle which were incubated in the BacT/ALERT®3D instrument (BioMérieux) at 35°C for 5 days or until microbial growth was detected. Positive bottles were removed from the BACTEC blood culture system, and then sub-cultured on MacConkey, blood and chocolate agar media.

The isolated organisms from pure cultures were subjected to further identification by standard conventional microbiological techniques.

A volume of 2 ml peripheral venous blood was withdrawn from the septic patients as well as the healthy controls by venipuncture under

complete aseptic conditions. Each tube was labeled by the patient's name and date of collection. The blood samples were centrifuged to separate the serum. Sera of culture positive patients and healthy controls were stored at -20°C till time of examination of CD14.

CD14 levels were measured in all cases using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human Cluster of Differentiation 14 (CD14) ELISA Kit, Cat. No. 95553, glory science co., Ltd).

Eithecal considerations :

-The goal of the study, the method, the potential benefits and inconveniences of all aspects of the study were described to all patients' guardians .

-A written consent was signed by every participant's guardians in this study.

- The study was approved by Local Ethical Research Committee at Beni-Suef Faculty of medicine .

Approval No:

FMBSUREC/03112019/Mahmoud

Statistical methodology:

- Analysis of data was performed using SPSS v. 25 (Statistical Package for Social science) for Windows.

Description of variables was presented as follows:

- Description of quantitative variables was in the form of mean, standard deviation (SD.)

- Description of qualitative variables was in the form of numbers (No.) and percent's(%)

Chi-squared test was used to detect the association between categorical variables

- Data was explored for normality using shapiro wilk/kolomogrove test.

- Independent t-test was used to compare between three independent groups regarding the normally distributed scale variable.

Receiver operating characteristic curve was used to detect the optimal cut-off for the best sensitivity, specificity, PPV and NPV .

The significance of the results was assessed in the form of P-value that was differentiated into:

- Non-significant when $P\text{-value} > 0.05$
- Significant when $P\text{-value} \leq 0.05$
- Highly significant when $P\text{-value} \leq 0.001$

3. Results:

The present study was conducted on all cases admitted at Beni-Suef University hospital NICU from December 2019 to February 2020. The total number of admitted neonates at NICU during the study period was 108 (47 Male & 61 Female) with average age of 19.7 ± 6.8 days .

By blood culture, the positive cases were 35 cases representing (32.4%).

Table (1): Characteristics of the studied groups:

Characteristics	cases n=35 (%)	controls n=35(%)
Age (days) mean±SD	19.7±6.8	19.4±3.1
Sex		
Males	13 (37.1)	14 (40)
Females	22 (62.9)	21 (60)
Mode of delivery		
Vaginal	14 (40)	14 (40)
Section	21 (60)	21 (60)
Maturity		
Preterm	10 (28.6)	8 (22.9)
Full term	25 (71.4)	27 (77.1)

The mean age of cases was 19.7±6.8 days, 37.1% of cases were males and 62.9% were females.

The vaginal delivery had the same prevalence in cases and controls 40% versus 60% were delivered by section.

Table (2): Causes of admission among cases and control groups:

Cause of admission	Cases n=35 (%)	Controls n=35(%)	P-value
Respiratory distress	25 (71.4)	17 (48.6)	0.05
Pneumonia	1 (2.9)	0 (0)	0.313
Refusal of oral intake	7 (20)	0 (0)	0.005*
Convulsions	2(5.7)	0 (0)	0.154
Jaundice	0 (0)	18 (51.4)	<0.001

The most common causes of admission among cases were respiratory distress followed by refusal of oral intake which were significant when compared to control group (p value 0.05 & 0.005* respectively).

Table (3): Risk factors among cases and controls:

Risk factors	Cases n=35 (%)	Controls n=35(%)	P-value
Cannula	35 (100)	16 (45.7)	<0.001**

Mechanical Ventilator (MV)	8 (22.9)	0 (0)	0.002*
Central venous line	2 (5.7)	0 (0)	0.154
Nasal O ₂	0 (0)	10 (28.6)	0.0007**
Continous Positive Airway Pressure (CPAP)	14 (40)	6 (17.1)	0.035*
Broad spectrum antibiotic intake	35(100)	20(57.1)	<0.001**

The most common risk factors among cases were using broad spectrum antibiotics and device attachment e.g. (cannula, MV, CPAP) with p value of <0.001, <0.001, 0.002, 0.035 respectively.

Table (4): The isolated organisms from blood cultures:

Culture	Cases n=35 (%)
Gram stain:	
Gram negative	22 (62.9)
Gram positive	13 (37.1)
Organisms:	
<i>Klebsiella</i>	18 (51.4)
<i>Pseudomonas</i>	3 (8.6)
<i>Enterobacter</i>	1(2.9)
CoNS	8 (22.9)
MRSA	2 (5.7)
<i>Enterococci</i>	3 (8.6)

The blood culture isolates showed predominance of Gram negative organisms (62.9%), most prevelant of which was *Klebsiella* (51.4%) followed by *Pseudomonas* (8.6%). While, Gram positive organisms were found to be (37.1%) where; the most prevalent of which was CoNS (22.9%) respectively.

Table (5) Presepsin level among cases and control groups:

Presepsin	Cases n=35 (%)	Controls n=35(%)	P-value
Mean±SD	3060±459	1820±642.5	<0.001**

Range (min-max)	(1300-3800)	(100-3000)	
Median	3100	1900	

There was a statistically significant increase of the presepsin levels among cases when compared with control (P -value $<0.001^{**}$) as the mean presepsin among cases was 3060 ± 459 while among controls was 1820 ± 642.5 (table-5) (figure-1).

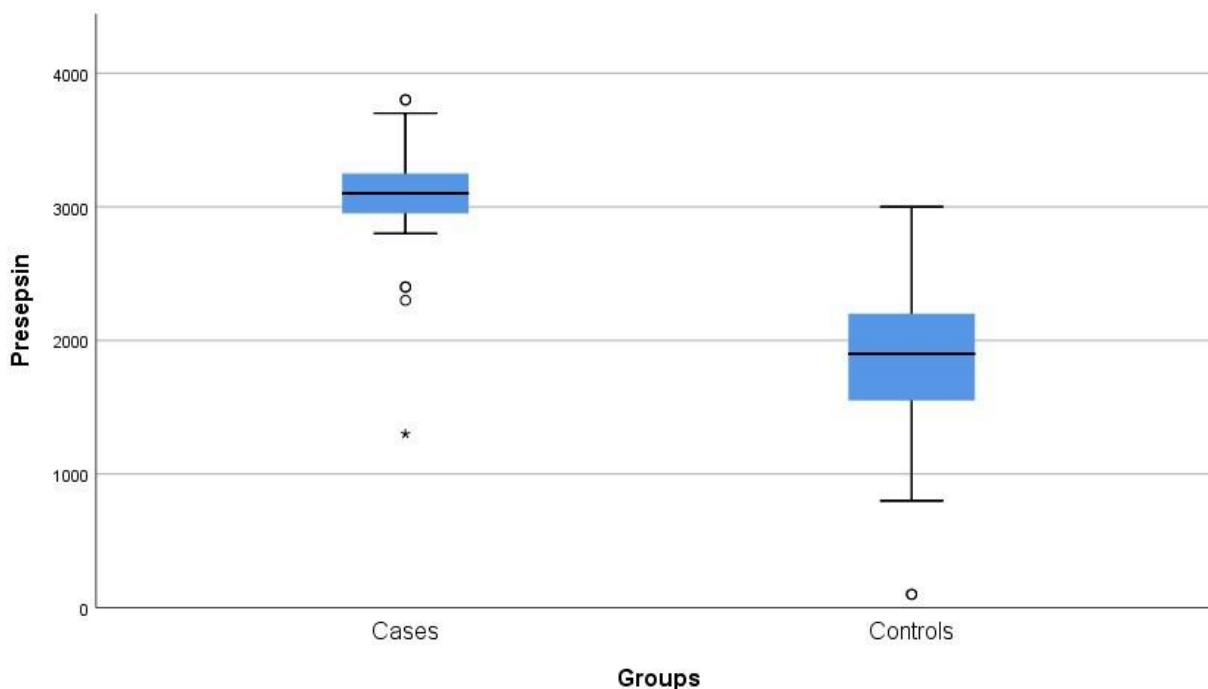


Figure (1) Description of presepsin levels among cases and controls.

Table (6) Comparison between Gram positive and Gram negative organisms regarding the presepsin level:

Organisms	Mean \pm SD	P-value
Gram negative	3077 \pm 349	0.777
Gram positive	3030 \pm 618	

There was no statistically significant difference between different Gram positive and Gram negative organisms regarding the presepsin levels among cases (P -value=0.777) (Table-11).

Table (7) Cut off, sensitivity and specificity of the presepsin level for prediction of presence of sepsis:

Area under the curve(95%CI)	0.953 (0.874 to 0.989)
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Optimal Cut off point(95%CI)	2300
Sensitivity (95%CI)	94.29 % (80.8 - 99.3)
Specificity (95%CI)	88.57% (73.3 - 96.8)
Positive predictive value (PPV)	89.2% (76.6 - 95.4)
Negative predictive value (NPV)	93.9% (80.1 - 98.4)
P-value	<0.001

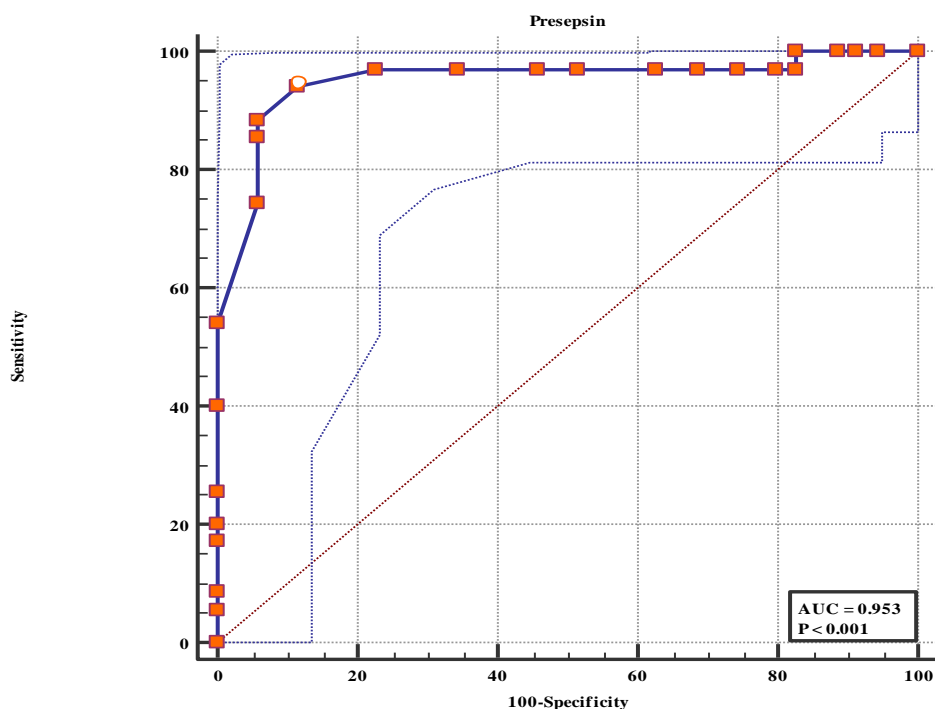


Figure (2) Receiver operating characteristic curve for prediction of presence of sepsis using the presepsin marker

Presepsin marker had a significant role in prediction of presence of sepsis, at a cut off value of 2300 or more of presepsin, it can predict the presence of sepsis with sensitivity of (94.29%), specificity of (88.6%), PPV (89.2%) and NPV (93.9%) (Table-7) (figure-2).

4. Discussion:

Neonatal sepsis is an invasive bacterial infection that happens during the initial month of life and is one of the most common causes of neonatal morbidity and mortality (8).

Prompt diagnosis and treatment with appropriate antimicrobial chemotherapy is an important measure in reducing morbidity and mortality associated with sepsis (9).

Blood culture is the gold standard method for detecting of sepsis (10). In the current study, the aim of the work was to evaluate the incidence of sepsis in NICU of Beni-Suef University Hospital, the causative organisms. The present study is a nested case- control study that was conducted on all cases admitted at Beni-Suef University hospital NICU from December 2019 to February 2020. The total number of admitted neonates at NICU during the study period was 108 (47 Male & 61 Female) with average age of 19.7 ± 6.8 days.

In the present investigation, the incidence of septicemia was 32.4%. This finding was compared to the work of Lamichhane, (2019) and Hammam et al., (2019) (11,12) studies who showed that the incidence of sepsis at NICU was (43, 94% and 44% respectively). However, it contradicates with the result of Medhat et al., (2017) (13) and Abdellatif et al., (2019) (14) studies that showed low incidence of sepsis (8.6%, 7.6% and 3.5%) respectively. These variations may be attributed to geographical variations, preventive strategies adopted in each country, clinical criteria for sepsis diagnosis, differential sensitivity and specificity of the culture methods in different laboratories, health status of mothers during pregnancy, sanitary status in delivery section, and social-economic status in different countries.

This study showed that there were no significant differences between the 2 groups as

regard to gestational age, sex and mode of delivery (p -value = 0.673, 0.874, 0.999 respectively) and this agreed with Lakhey et al., (2017) study (15) who found that there was no significant difference between sepsis group and non sepsis group with respect to their gestational age, sex and mode of delivery.

Our study showed that the percentage of sepsis was high in neonates born by cesarean section than by vaginal delivery with no statistically significant difference. This may be due to increased number of cesarean section than vaginal delivery. This result was in agreement with (Kayiga et al., 2018) (16) who revealed that the mode of delivery did not have a statistically significant impact on perinatal morbidity.

As regards gestational age, we found predominance of sepsis among term infants (71.4%) than preterm one (28.6%). This results agree with with Adatara et al., (2019) (17) and Awad et al., (2020) (18). Nevertheless, contrary to this observation, Al-Mayah et al., (2017) (19) and Reddy et al., (2017) (20) found sepsis was more prevalence in preterm babies than full term. This may be justified by their larger sample size.

Regarding the cause of admission, respiratory causes (RD and pneumonia) represented (74.3%) followed by refusal of oral intake (20%) and convulsion (5.7%). Similar observations were reached by Shehab et al., (2015) (21) who showed that dyspnea and respiratory distress was the most common

presentation of cases with neonatal sepsis (54.6%) followed by jaundice (4.9%), temperature instability (2.3%) and convulsions (1.6%).

These variations in clinical manifestations of the affected neonates are due to variations in the involved organs, the pathogen, gestational age, age of the neonate and several other conditions.

Of the assessed variables, length of hospital stay, using broad spectrum antibiotics and attached medical devices showed significant association with presence of septicemia in the present study with p value < 0.001.

The blood culture finding in the current study showed predominance of Gram negative organisms (62.9%), most prevalence of which was *Klebsiella* (51.4%) followed by *Pseudomonas* (8.6%). While Gram positive organisms were found to be (37.1%) where the most prevalent was CoNS (22.9%).

This was found in agreement with Atif et al., (2021) (22) and Kumari et al., (2021) (23) who demonstrated that Gram negative organisms were predominant in neonatal sepsis in their study and *Klebsiella* was the most common organism.

The predominance of *Klebsiella* among the causative Gram-negative pathogens was also reported in another study in Egypt (Fahmey, 2013) (24).

On the contrary, Umemura et al., (2021)(25) demonstrated that other Gram-negative bacteria such as *Escherichia coli* could be

identified as the most common Gram-negative isolate associated with neonatal sepsis.

On the other hand, Osman et al., (2015) (26) found that 63% of neonatal sepsis was caused by Gram-positive bacteria.

These differences in the causative organisms of neonatal sepsis may be justified by variations in geographic regions and facilities. Also, composition of these organisms have changed over the last century because of changing trend of antibiotic use and life style (27).

Moreover, the present results showed that presepsin had 94.29 % sensitivity and 88.57% specificity with a cut-off value of 2300 pg/ml for culture-proven sepsis. This coincided with findings of Khater et al., (2020) (28); it demonstrated that presepsin was a successful marker for neonatal sepsis with a cut off value of 500 pg/ml, having 95% specificity and 81% sensitivity.

However, due to the lack of information about presepsin reference range in neonates, many studies reported different cut-off values (539 to 1800 pg/ml) (29) and(30). Relatively different cut-off values may be due to the use of different measurement methods.

In this study, the presepsin levels were significantly higher among both Gram-positive and Gram-negative infected cases. there was slight elevation in presepsin levels among Gram-negative infected patients compared with than in Gram-positive infected ones. However, it was not statistically significant.

This agreed with Zou et al., (2014) (31) who showed no significant difference between presepsin level in Gram positive and Gram negative bacteria.

5. Conclusion and Recommendations:

Neonatal sepsis is a common cause of admission to NICU with significant morbidity and mortality. Incidence of sepsis in NICU of Beni-Suef university hospital was 32.4 %

Frequency of Gram negative organisms was much more common in comparison with Gram positive organisms. *Klebsiella* was the commonest isolated organism in the study.

Presepsin was proved to increase significantly in neonates with neonatal sepsis so it can be used as a biomarker for disease diagnosis.

We recommend developing effective antibiotic stewardship programs in each healthcare facility. Also we need more effective infection control measures.

More work is needed to define the association between presepsin and the response to antimicrobial therapy in the clinical setting.

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