ORIGINAL ARTICLE

Presepsin as a Novel Diagnostic Marker in Neonatal Septicemia

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ABSTRACT

Key words: Presepsin, Pathfast, neonatal septicemia

Background: Neonatal septicemia is a serious life-threatening condition with high mortality. The accurate diagnosis of sepsis is one of the main challenges in emergency medicine. A great effort to reduce the neonatal mortality rate is put into looking for new reliable biomarkers. Among biomarkers, presepsin could be one of the most promising and reliable biomarker for early diagnosis of sepsis. Objective: We aimed to evaluate the diagnostic value of presepsin in the early diagnosis of neonatal sepsis. Methodology: By chemiluminescent enzyme immunoassay (CLEIA), the level of presepsin was assessed in 40 full term neonates with suspected sepsis (Proven sepsis group: 23 patients with +ve blood culture & Probable sepsis group: 17 patients with -ve blood culture) and 15 healthy full term neonates. Results: Presepsin level was found to be significantly higher in patient group than control group as well as in proven sepsis group than probable sepsis group. The cut off value for presepsin was 875pg/ml at which the sensitivity and specificity of presepsin were (95.7%, 87.5%) respectively. Presepsin level was found to be significantly higher in females than males. There was no significant difference in the presepsin level as regard mode of delivery nor onset of sepsis. Conclusion: Presepsin is a novel biomarker with high sensitivity and good specificity for sepsis and its measurement can be useful for early diagnosis of neonatal sepsis.

INTRODUCTION

Neonatal sepsis is a global problem and is a significant contributor to morbidity & mortality. Approximately one million deaths a year occurring in the neonatal period (0-28 days) are caused by infection, accounting for over 25% of global neonatal deaths and 10% of all mortality in infants.

The prognosis and outcome of neonatal sepsis depend on early diagnosis and on-time and efficient antibiotic therapy. The accurate and timely diagnosis of septicemia in the neonatal population is challenging and problematic because of nonspecific clinical presentation and poor diagnostic yield (sensitivity of 50% or less) and delay of the standard blood culture. As such, there is much interest in developing rapid and sensitive diagnostic assays for diagnosis of the infected neonate.

An approach that has gained particular attention is detection of soluble CD14 subtype (sCD14-ST: named as presepsin) which is a 13 kD, soluble form of CD14. This small polypeptide has been proposed as a novel, early and reliable biomarker for the diagnosis of sepsis.

Preliminary studies suggest that the level of presepsin significantly differs in healthy individuals and in patients with local infection, SIRS, sepsis or severe sepsis. Presepsin is currently under investigation in clinical practice as a reliable marker of adult and neonatal sepsis and for the postmortem diagnosis of sepsis-related death.

Recent studies confirmed presepsin as a promising biomarker in the diagnosis of sepsis, as well as for assessing the severity and predicting the outcome in septic patients. Furthermore, a rapid assay, based on the chemiluminescence enzyme immunoassay principle is now available and can be used on a point-of-care testing basis, thus allowing the emergency physician to get presepsin values in a short time from whole blood samples.

METHODOLOGY

Patients:

This study was carried out during the period from January 2013 to October 2013 on 55 full term neonates divided into:-

- Patient group: 40 cases with suspected sepsis admitted to the Neonatal Intensive Care Unit (NICU) in Benha University Hospital.
- Control group: 15 healthy neonates selected from the outpatient neonatal clinic coming for follow up.

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An informed consent was taken from the parents of each neonate before their enrollment in the study. Those with congenital infections were excluded. Suspicion of sepsis by the caring neonatologists was based on:

- The presence of one or more of the following clinical signs: tachypnea, respiratory distress, apnea, cyanosis, lethargy, tachycardia, bradycardia, hypotonia, seizures and irritability.
- Presence of one or more risk factors (PROM ≥ 18 hs, presence of central venous line, endotracheal intubation, parental nutrition, maternal diabetes, maternal fever).
- Laboratory criteria of sepsis (elevated C-reactive protein level >6 mg/dl and Rodwell’s hematological sepsis score above 3).

**Blood samples collection and storage**

Four ml blood samples were collected by standard techniques. The sample divided into two parts:

- 3 ml injected directly into blood culture bottle (only for patient group).
- 1 ml placed into EDTA tube and centrifuged for obtaining plasma sample and then stored at -80°C for presepsin measurement.

**Blood culture**

Using BD BACTEC Ped Plus/ F Culture vials, Soybean-Casein Digest Broth with Resins (BD BactecPed Plus /F bottles) Becton Dickinson, USA. Procedure was carried out according to manufacturer:

Three ml of blood were injected into the Bactec culture vial under complete aseptic conditions. The inoculated Bactec culture vials were placed in the Bactec 9050 fluorescent series instrument (Becton Dickinson, USA) as soon as possible for incubation and monitoring. Vials entered into the instrument will be automatically tested every 10 minutes for a difference in monitoring. Vials inoculated Bactec culture vials were placed in the Bactec 9050 fluorescent series instrument (Becton Dickinson, USA) as soon as possible for incubation and monitoring. Vials entered into the instrument will be automatically tested every 10 minutes for a difference in monitoring.

**Assay for PATHFAST™ Presepsin (PF1201-K)**

PATHFAST Presepsin is a chemiluminescent enzyme immunoassay (CLEIA) for the quantitative measurement of presepsin (sCD14-ST) concentration in whole blood or plasma within 17 minutes.

The test based on non-competitive CLEIA combined with *MAGTRATION® technology. During incubation of the sample with alkaline phosphatase labeled anti-presepsin polyclonal antibody and anti presepsin monoclonal antibody coated magnetic particles, the presepsin of the sample binds to the anti presepsin antibodies forming an immunocomplex with enzyme labeled antibody and antibody coated magnetic particles. After removing the unbound substances by *MAGTRATION® technology, a chemiluminescent substrate is added. After a short incubation, the luminescence intensity generated by the enzyme reaction is measured. The luminescence intensity is related to the presepsin concentration of the sample which is calculated by means of a standard curve.

**Statistical analysis**

The collected data were tabulated and analyzed using SPSS version 16 software (SpssInc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean and standard deviation. Student “t” test was used as tests of significance. ROC curve was used to determine cutoff values of presepsin with optimum sensitivity and specificity in early diagnosis of sepsis. The accepted level of significance in this work was stated at 0.05 (P < 0.05 was considered significant).

**RESULTS**

This study was carried out on 55 fullterm neonates divided into two main groups:

- **Patient group**: included 40 fullterm neonates with suspected sepsis. They were 23 females & 17 males. Mean ± SD of birth weight (gm) = 2518.0 ± 532.41. Mean ± SD of gestational age (weeks) = 37.5 ± 1.23. Mean ± SD of postnatal age (days) = 4.35 ± 3.53.
- **Control group**: included 15 healthy neonates. They were 9 males & 6 females. Mean ± SD of birth weight (gm) = 2581.0 ± 513.12. Mean ± SD of gestational age (weeks) = 37.77 ± 1.62. Mean ± SD of postnatal age (days) = 9.91 ± 5.12.

According to the results of blood culture, patient group was subdivided into two subgroups:

- **Proven sepsis group**: included 23 patients who gave positive blood culture results.
- **Probable sepsis group**: included 17 patients who gave negative blood culture results.

Blood cultures were positive in only 41.8% of our cases. Coagulase negative Staphylococci was the most common causative organism of sepsis followed by Staphylococcus aureus (17.5% and 12.5% respectively) (Table 1).

Presepsin level was found to be significantly higher in patient group than control group as well as in proven sepsis group than probable sepsis group (P < 0.001*) (Table 2).

The cut off value for presepsin was 875 pg/ml at which the sensitivity and specificity of presepsin were (95.7%, 87.5%) respectively. ROC curve analysis shows that presepsin is good test in early diagnosis of neonatal sepsis (AUCs of presepsin = 0.95) (P < 0.001*) (Figure 1).

Presepsin level was found to be significantly higher in females than males (P = 0.007*) (Table 3). There was no significant difference in the presepsin level as regard mode of delivery nor onset of sepsis (P = 0.77 and 0.42 respectively) (Table 3).
Table 1: Frequency distribution of patient group according to results of blood culture.

<table>
<thead>
<tr>
<th>Blood culture result</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulate negative staphylococci</td>
<td>7</td>
<td>17.5</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>Pseudomonas aerogenosa</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Pneumococci</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>Candida</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>No growth</td>
<td>17</td>
<td>42.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Comparison between studied groups regarding presepsin level.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Presepsin(pg/ml) Mean ± SD</th>
<th>St&quot;t&quot;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>40</td>
<td>1176.20 ± 443.80593</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>15</td>
<td>549.60 ± 75.99699</td>
<td>5.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>proven sepsis group (+ve blood culture)</td>
<td>23</td>
<td>1453.78 ± 372.494</td>
<td></td>
<td></td>
</tr>
<tr>
<td>probable sepsis group (-ve blood culture)</td>
<td>17</td>
<td>800.64 ± 169.402</td>
<td>6.7</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Table 3: Variation of Presepsin level according to gender of patients, mode of delivery and onset of sepsis.

<table>
<thead>
<tr>
<th>Gender:</th>
<th>No. (40)</th>
<th>Presepsin(pg/ml) Mean ± SD</th>
<th>St&quot;t&quot;</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>17</td>
<td>963.23 ± 362.950</td>
<td>2.8</td>
<td>0.007*</td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>1333.60 ± 438.642</td>
<td>0.29</td>
<td>0.77</td>
</tr>
<tr>
<td>Mode of delivery:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NVD</td>
<td>26</td>
<td>1161.11 ± 448.16</td>
<td>0.81</td>
<td>0.42</td>
</tr>
<tr>
<td>CS</td>
<td>14</td>
<td>1204.21 ± 450.93</td>
<td>0.29</td>
<td>0.77</td>
</tr>
<tr>
<td>Onset of sepsis:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early onset sepsis</td>
<td>17</td>
<td>1109.76 ± 461.821</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>23</td>
<td>1225.30 ± 433.727</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Receiver Operating Characteristic (ROC) curve of presepsin in early diagnosis of sepsis.
DISCUSSION

Infections are responsible for significant mortality and long-term morbidity for infants in the neonatal intensive care units. Early diagnosis of neonatal sepsis is essential pre-requisite to improve survival and to improve therapeutic outcome. As such, there is much interest in developing rapid and sensitive diagnostic assays that can effectively predict and identify patients who are at risk of infection.

Among new biomarkers, presepsin (Soluble CD14) could be one of the most interesting and reliable candidates for sepsis management, specifically for early diagnosis, distinguishing it from non-infectious diseases and the classification into severity degrees.

Despite every year almost one million newborns dying from infections, there are very few studies about presepsin in the neonatology population. So, our study aimed to evaluate the diagnostic value of presepsin in neonatal sepsis which would help us in early and accurate diagnosis of sepsis.

The current study was carried out on forty neonates with suspected sepsis and fifteen healthy controls. Blood culture was done for the cases and according to its result, the cases were classified into proven sepsis group (+ve blood culture) and probable sepsis group (-ve blood culture). Levels of presepsin were assessed in these groups.

Although blood culture is the gold standard method for detecting the presence of microorganism in the bloodstream, it has limited usefulness for early detection of infection because it usually requires several days for results to be known. Also, the sensitivity of blood cultures in neonatal sepsis is low and depends on the timing of cultures taken, blood volume, culture medium, technique, temperature, organism density as well as antibiotic administration. Hsu et al. stated that the implementation of peripartum maternal antibiotic treatment makes the diagnostic value of neonatal blood cultures uncertain.

In this study, it was found that the percentage of positive blood cultures were (41.8%) in the cases group. Nearly similar results have been found in the study of Chacko and Sohi, who found that culture proven sepsis occurred in (41.6%) of cases with sepsis. In the study of Procionoy and Silveira, it was found that blood cultures were positive in only eighteen of eighty-eight cases (21%). Also, in a study by de Guadiana et al., out of 226 patients with SIRS, only 37 patients (16.4%) had a positive blood culture result.

In the present study, Coagulase negative Staphylococcus (17.5%) was the most common organism isolated in the positive blood cultures followed by Staphylococcus aureus (12.5%) and Klebsiella pneumonia (10%) (Table 1).

This comes in disagreement with the study of Dzwonek et al., in which nearly half of the positive blood cultures grew Klebsiella pneumonia, also in the study of De Benedetti et al., the isolated pathogens included Klebsiella pneumonia (47.5%), Pseudomonas aeruginosa (20%), Escherichia coli (10%), Candida albicans (10), Staphylococcus aureus (7.5%) and Enterococcus (5%).

This variation may be due to differences in the environment, the microbial etiology of sepsis and supportive care practice between centers.

Despite there is much interest about presepsin, there are very few studies about presepsin in the neonatology population and so, there is lack of information about its reference range in this category of population. In this study, we found that the cut off value was 875pg/ml. This cut off value more or less close to that (781pg/ml) found by AbdElaziz, and to that (885pg/ml) found by Poggi et al.

In the current study, we found that mean level of presepsin (pg/ml) was significantly higher in patients group (1176.2 ± 443.81) than control group (549.60 ± 75.996) (P<0.001) and was significantly higher in proven sepsis group (1453.78 ± 372.49) than probable sepsis group (800.64 ± 169.4) (P<0.001)(Table 2) and area under the receiver operating characteristics (ROC) curve (AUC) for presepsin was 0.95 with sensitivity and specificity of 95.7% and 87%, respectively (Figure 1), indicating that the level of presepsin is a good marker for the diagnosis of neonatal sepsis.

In a study by AbdElaziz, that was carried out in University of Cairo, Egyptian 188 neonates suspected to be infected, blood samples were collected in 3 successive days to measure presepsin. He concluded that presepsin can effectively differentiate between bacterial and non–bacterial infections including SIRS and that presepsin is early, sensitive and specific sepsis marker where it rises soon in the 1st day of infection.

Also, in a work by Poggi et al., they studied presepsin in newborns with LOS (n = 19) and non-infected controls (n = 21) at enrollment, and 1, 3, and 5 days later. They reported that presepsin level at enrollment was higher in the LOS than the control group (median 1295 vs 562 pg/ml, P = .0001) and remained higher throughout the study period. The ROC of presepsin at enrollment showed an AUC of 0.972 and the best calculated cutoff value was 885 pg/ml, with 94% sensitivity and 100% specificity. They concluded that presepsin is an accurate biomarker for the diagnosis of possible LOS and may also provide useful information for monitoring the response to therapeutic interventions.

Also, Stubljar et al. in their work, compared diagnostic accuracy of presepsin, to predict bacterial infection in comparison to established biomarkers like biochemical analysis of CSF and they found that presepsin was significantly higher in children with clinically proven ventriculitis compared to those without meningitis/ventriculitis and AUC for presepsin,
leucocytes and proteins measured in CSF were 0.877, 0.798 and 0.857, respectively.

In contrast, Urbonas et al., 21 in their study on a pediatric oncology population with febrile neutropenia, they found that there was no significant difference between the level of presepsin in the sepsis/bacteremia group (where infections were further confirmed with positive blood cultures) compared to that in the fever of unknown origin group (with negative blood cultures).

Many different works studied the role of presepsin as a sepsis marker in adult population and all reported that presepsin is specifically expressed in sepsis, as elevated concentrations of presepsin were observed in septic patients compared to controls and its levels were significantly higher in patients with sepsis than the SIRS group indicating that the presepsin concentration is a significantly sensitive indicator of sepsis and useful marker for the rapid diagnosis of sepsis.8, 13, 23, 24, 25

In the current work, we studied the variations in the presepsin level according to different parameters and we found that there was no significant difference in the presepsin level as regard mode of delivery (P= 0.77), nor the onset of sepsis (P= 0.42) (Table 3). However, our results showed that the presepsin level was significantly higher in females than males (P= 0.007) (Table 3). The explanation of this result may need further studying, as there are no available data about other works that study these relations to support or deny our result or to explain it.

According to several studies, the authors tend to confirm that presepsin is a promising biomarker for early diagnosis of sepsis and distinguish it from non-infectious diseases. It is readily available and cost-effective. Preliminary findings provide a solid basis for its future application even if more insights are needed concerning the pathophysiological conditions associated with presepsin release and a more extensive evaluation of presepsin as a biomarker for severe sepsis and septic shock is advisable 26. The added value of this biomarker for clinical decision-making in terms of diagnosis, risk stratification and therapy monitoring should also be clarified 27.

CONCLUSION

In light of the results of the present study, presepsin is a novel biomarker with high sensitivity and good specificity for sepsis and consequently, we can conclude that measurement of presepsin can be useful for early diagnosis of neonatal sepsis.

REFERENCES


