Pancreatic Stone Protein as a Novel Marker for Early Onset Neonatal Sepsis

Safaa ELMeneza1*, Rana Fouad1 and Iman El Bagoury2

Affiliation
1Pediatrics department, Faculty of medicine for girls, AL-Azhar University, Egypt
2Clinical pathology department, Faculty of medicine for girls, AL-Azhar University, Egypt

*Corresponding author: Safaa ELMeneza, Pediatrics department, Faculty of Medicine for Girls, AL-Azhar University, Egypt,
Email: safaa5@hotmail.com


Abstract

Background: Early-onset sepsis is one of the main causes for admission of newborns to the neonatal intensive care unit. Traditional markers are inadequate for identification of early-onset sepsis. Pancreatic stone protein is a promising sepsis marker in adults with limited studies in neonatal population. The aim of this study was to assess value of pancreatic stone protein as a novel sepsis biomarker in diagnosis of early onset neonatal sepsis in neonates.

Methods: This was case control study with 90 newborn infants were involved. They were admitted to Al-Zahraa University hospital with diagnosis of early onset sepsis. The cases were allocated into 2 groups; group (1) the early onset sepsis group and group (2) control group of normal newborn infants who had no sepsis.

Results: Pancreatic stone protein was significantly higher in early-onset sepsis group than control group with 100% sensitivity, specificity, positive predictive value, negative predictive value at cut off point 133.6 pg/ml and cut off value of 125.6 pg/ml for preterm infant. There was statistically significant increase of Pancreatic stone protein among non-survival cases. There was correlation between Pancreatic stone protein and weight, IT ratio, Immature myeloid series, Pco2, bicarbonate, urea, Töllner score and haematological score.

Conclusions: Our findings advocate that role of Pancreatic stone protein as a valuable marker in diagnosis of neonatal early onset sepsis. Pancreatic stone protein has high sensitivity and specificity; it may empower the neonatologist for safe care and judicious use of antibiotic. Pancreatic stone protein may assist in distinguishing the serious cases that may have bad prognosis.

Keywords: Early neonatal period, Neonatal sepsis, Newborn infant, Preterm, Neonatal Outcome, Pancreatic Stone Protein, Sepsis.

Introduction

Neonatal sepsis is a common, devastating, and overwhelming disease that contributes to morbidity and death. It has life-long impact plagued by a lack of accurate diagnostic and prognostic testing [1]. The clinical signs are non-specific and indistinguishable from those caused by a diversity of neonatal noninfectious disorders. Early detection and diagnosis of neonatal sepsis is difficult but extremely important because prompt institution of antimicrobial therapy improves outcomes.

Early-Onset Sepsis (EOS) is one of the major reasons for the neonatal admission in Neonatal Intensive Care Unit (NICU). EOS has been variably defined based on the age at onset, with bacteremia or bacterial meningitis occurring during 72 hours in infants hospitalized in the NICU [2]. Neonatal early-onset sepsis occurs in the following criteria newborns with early-onset sepsis, 85% present within 24 hours, 5% present at 24-48 hours, and a smaller percentage presents within 48-72 hours. Onset is most rapid in premature neonates [3]. Although of the constant efforts to diagnose neonatal sepsis still it persist a perplexing topic for neonatologists due to multiple factors including the lack of ideal diagnostic markers that fit both full- term and preterm infants. Pancreatic Stone Protein (PSP) is a protein secreted by the pancreas recognized by varied scale of functions, as adhesion and signaling receptors in homeostasis and innate immunity, it is vital in inflammatory process and leukocyte and platelet trafficking.

Moreover, the observation that PSP level rose in response to septic insults in mice and rats encouraged its clinical evaluation as biomarker of sepsis in various settings and conditions [4]. PSP has recently emerged as a promising sepsis marker in adults, with high PSP levels predicting sepsis, sepsis associated multiple-organ failure, in patients with ventilator-associated pneumonia, post-traumatic sepsis and mortality. Few consistent data are available for PSP in the clinical setting of neonatal sepsis [5-7].

Our research question was can Pancreatic stone protein be used as novel marker for diagnosis of early onset sepsis in neonatal population?
Objective

The objective of this study was to assess the value of PSP as a novel sepsis biomarker in diagnosis of early onset neonatal sepsis.

Patient and Methods

Study design

This study was a case-control study. It was conducted in the NICU of Al-Zahraa University Hospital. The study included 90 newborn infants, who were allocated into 2 groups, group 1 with EOS (n=60) and group 2 control, apparently healthy newborn infants with no risk factors or clinical manifestations of sepsis (n=30). Inclusion criteria for the study group were diagnosis of early onset sepsis in the first 72 hours after birth, with the “presence of at least two clinical symptoms and at least two laboratory signs in presence of or as a result of suspected or proven infection (positive culture, or microscopy polymerase chain reaction)” as suggested by report on the expert meeting on neonatal and pediatric sepsis 2010 [8]. Diagnosis of cases with negative blood culture was based upon the clinical sepsis and hematological scores.

In this study full term infants were those >37 weeks of gestation and preterm infants (32-34) weeks. Exclusion criteria included newborn infants with congenital anomalies, chromosomal abnormalities or inborn errors of metabolism; confirmed intrauterine viral infection, perinatal asphyxia. Neonates receiving parenteral antibiotic at the time of sepsis evaluation or newborn had just undergone surgery.

Procedure

All neonates were subjected to history taking, clinical examination, laboratory investigations including: Complete Blood Count (CBC), liver and kidney function tests, blood culture, C-Reactive Protein (CRP), and serum pancreatic stone protein using Enzyme-Linked Immunosorbent Assay (ELISA), kits from Kono Biotech Co.,Ltd. catalogue number: KN 2065Hu (My Biosource/MBS285689, San Diego, California, USA). Clinical sepsis score according to Töllner score was done, as well as the hematological score [9,10]. Samples were collected at the time of diagnosis of sepsis. The study was approved by the ethics committee of the scientific research, Faculty of Medicine for girls, AL-Azhar University. An informed consent was obtained from the parents or caregivers of each neonate before enrollment in the study.

Statistical analysis

Data were collected, coded, revised and entered to the statistical package for social science (IBM SPSS) version 20.

Results

The results are shown in Tables 1- 4.

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>X/ t/ Z</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOS group (N=60)</td>
<td>38.8 ± 2.35</td>
<td>38.5 ± 1.98</td>
<td>-0.600 **</td>
<td>0.549</td>
</tr>
<tr>
<td>Control group (N=30)</td>
<td>38.5 ± 2.04</td>
<td>38.3 ± 1.94</td>
<td>0.013</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weight (kg)</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>X/ t/ Z</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large for GA</td>
<td>9 ± 15.0%</td>
<td>10 ± 20.0%</td>
<td>0.092*</td>
<td>0.762</td>
</tr>
<tr>
<td>Appropriate for GA</td>
<td>30 ± 60.0%</td>
<td>24 ± 80.0%</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Small for GA</td>
<td>15 ± 25.0%</td>
<td>6 ± 20.0%</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

| Gender | Female | 24 ± 40.0% | 13 ± 43.30% | 0.092* | 0.762 |
|        | Male   | 36 ± 60.0% | 17 ± 56.70% | 0.001 |

| Apgar score at 1 min | Median (IQR) | 6 (6-7) | 7 (6-7) | -3.871*** | 0.001 |
| Apgar score at 5 min | Median (IQR) | 9 (8-10) | 9 (9-10) | -4.982*** | 0.001 |
| Apgar score at 10 min | Median (IQR) | 9 (8-10) | 10 (9-10) | -3.866*** | 0.887 |
| Töllner score | Median (IQR) | 6 (5-6) | 0.5 (0-1) | -7.850*** | 0.001 |
| Postnatal age (days) | Median (IQR) | 1 (1-2) | 27 ± 90.00% | 8.541* | 0.013 |

Discussion

PSP has been studied in adults and shown to accurately predict multi-organ failure and mortality in patients with ventilator-associated pneumonia and post traumatic sepsis [5,6]. This study analyzed the role of PSP as a novel sepsis biomarker in diagnosis of early onset neonatal sepsis in newborn infants. There was no significant difference in gestational age between EOS group and control group. There was predominance of male gender (60%) among the sepsis group. The present study showed significant decrease in Apgar score among sepsis group at 1-5 minutes. Töllner score was higher among sepsis group than the control group. The current study results showed the importance of PSP as novel sepsis marker in diagnosis of early onset neonatal sepsis as well as its prognostic values. The mean values PSP was increased statistically in EOS group in comparison to control group (P<0.001). This increase among the sepsis group may be due to promoting cellular proliferative responses in the pancreas by PSP and activation of polymorph nuclear cells, PSP/reg binds and activates neutrophils behaving as an acute-phase protein that responds to injury during the early phase of infection [5].

Animal studies have shown induction of PSP expression in subset of intestinal and gastric cells by stress conditions in absence of direct pancreatic inflammation [8,11]. In this study, PSP at a cutoff value of >133.8 pg/mL, had the sensitivity, specificity, Positive Predictive Values (PPV) and Negative Predictive Values (NPV) of 100%, and the Area Under Curve (AUC) was 1.000 (p < 0.001) for the EOS group, this results are more or less the same as those of Rass et al. who reported that at a cutoff level of 12.96 mg/mL, the sensitivity was...
96.2%, the specificity was 88.5%, PPV value was 95.8%, NPV was 89.3%, and AUC was 0.87 and to Schlapbach, et al. who reported that PSP has high NPV of 90% and 79% sensitivity in newborn infants, also to results by Wu, et al. who reported sensitivity of 79.7% in pediatrics patients. Also Dima, et al. found that the diagnostic performance of PSP was superior to that of traditional markers [7,12-14]. Further analysis of the data for the preterm infants (32-34 weeks), showed 100% sensitivity, specificity as well as PPV and NPP at a cut off value of >125.6 pg/ml.

There was significant increase in PSP mean values in preterm infants with EOS in comparison to full term infants (P=0.006). It could be due to systemic exaggerated immune inflammatory response to invasive bacteria and significant stress among the studied preterm infants. Schlapbach, et al. reported that PSP has bell-shaped distribution from birth to adulthood in normal population; this is opposite to the results of current study [15]. On the other hand, Stoll et al. stated that the risk of early onset sepsis increases with decrease of gestational age because of the inability of white blood cells to carry out phagocytosis, immaturity of the immune system, low complement levels, and hypogammaglobulinemia [16]. As far as we know this study was first to look at PSP in full term and preterm infants. To understand the exact mechanism, it may need further larger study.

In practice, different thresholds for PSP may be needed to detect severity of illness in newborns compared to older age groups. So this study looked also at the differences of PSP between the survival and non-survival cases. There was statistically significant increase in PSP in non-survival EOS group weather preterm or full term than those survival cases of EOS group (P=0.046). The increase in PSP could be due to additional stress and severity of inflammation among severely sick dead cases that led to expression of more PSP, also metabolic disturbances and respiratory failure lead to acidosis, which can increase expression of PSP [15].

<table>
<thead>
<tr>
<th>Cut off point</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>All EOS group &gt;133.8 pg/ml</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100.001</td>
</tr>
<tr>
<td>Full term &gt;133.8 pg/ml</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100.001</td>
</tr>
<tr>
<td>Preterm &gt;125.6 pg/ml</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100.001</td>
</tr>
</tbody>
</table>

Table 4: Receiver-Operating Characteristic Curve (Roc) Analysis and Diagnostic Performance for Pancreatic Stone Protein in EOS Group.

Previous studies showed significant PSP increase in presence of significant stress and multi-organ dysfunction syndrome in critically ill children and patients who died [17]. These findings agree with those of Que, et al. who found that risk of mortality in adult population increased continuously for each ascending quartile of PSP in a prospective cohort of patients with sepsis requiring (Intensive care unit) ICU management [18].

on Myeloid Cells-1 (sTREM-1), suggesting PSP is independently increased in presence of infections. The PSP is a rapid laboratory test (<1.3h) and require minimal blood volume (<50 μL) [7]. PSP is more sensitive, specific and has a good negative predictive value compared to CRP confirming its value as marker to rule out early onset neonatal sepsis.

In the present study PSP did not increase from postnatal age day 1 to day 3 in the EOS groups (P=0.806), Schlapbach, et al. observed a slow increase in PSP concentrations over the first days of life [15]. Also there was no significant difference in mean values of PSP in relation to gender in the studied groups. This study showed significant correlation of PSP with clinical Töllner score and hematological score. This may strengthen the role of clinical sepsis score and hematological score in diagnosis of sepsis in limited resources countries. PSP can safely guide the decision to initiate empirical antibiotic treatment in infants with suspected EOS. Errors related to use of anti-infective drugs was reported in 83.4% of a study done by ELMeneza, et al [21]. Finally, the present study revealed that PSP is valuable in both diagnosis and prognosis of neonatal sepsis.

The limitations of this study include the relatively small sample size resulting inability to explain the significant increase of PSP in preterm infants than full term infants with EOS. In conclusion, our findings advocate that role of PSP as a valuable marker in diagnosis of neonatal EOS, PSP has high sensitivity and specificity; it may empower the neonatologist for safe care and judicious use of antibiotic. PSP may assist in distinguishing the serious cases that may have bad prognosis.

**Recommendation**

PSP can be used as a new marker for diagnosis of early onset sepsis. Further study should be conducted with larger number of neonates to confirm PSP values in early diagnosis and prognosis of neonatal sepsis especially among preterm and very low birth weight infants.

**Statement of Ethics**

The parents have given their informed consent and the study protocol was approved by the Faculty of Medicine for Girls ethical committee.

**References**