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ORIGINAL ARTICLE

Soluble CD14-subtype (Prespsin) and Hepcidin as Diagnostic and Prognostic markers in Early Onset Neonatal Sepsis

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Presepsin, sCD14, EONS, CRP, Hepcidin, Treatment

ABSTRACT

Background: *The clinical picture and laboratory markers of Early-onset neonatal sepsis (EONS) are nonspecific, however a variety of different molecules have been suggested as clinical biomarkers in sepsis. Presepsin (soluble CD14-subtype) has been identified as a protein whose level increases significantly in the blood of septic patients. Hepcidin, an iron homeostasis regulator, it can be used in diagnosis of neonatal sepsis.* ***Objectives:*** *The aim of this study is to evaluate the role of new markers namely presepsin and hepcidin in diagnosis of EONS compared to CRP before and after antibiotic therapy.* ***Methodology:*** *The study enrolled 62 neonates, 28 of them fulfilled the criteria of EONS, and 34 healthy neonates as a control group. Serum levels of presepsin, hepcidin, CRP, complete blood picture, blood gases, and serum iron parameters for all neonates and blood cultures were done for 28 of neonates with clinical picture of sepsis.* ***Results:*** *Serum levels of presepsin, hepcidin, and CRP were significantly higher in neonates with sepsis than in healthy neonates. The presepsin was more sensitive and specific than hepcidin and CRP for diagnosis of EONS. After antibiotic therapy, the serum level of presepsin was dramatically decreased as compared to its pretreatment level. The same results was noted, but to a lesser degree for hepcidin and CRP. Additionally, the presepsin level was significantly correlated to blood culture results and CRP levels.* ***Conclusion:*** *Presepsin is considered a promising biomarker for early diagnosis of EONS with higher sensitivity and specificity rather than hepcidin and CRP. Its correlation to sepsis markers and response to treatment is more informative. Future large scale studies are needed to understand the role of hepcidin and presepsin in development of sepsis in other pediatric age groups.*

INTRODUCTION

Sepsis is a major cause of morbidity and mortality in neonates. Early-onset neonatal sepsis (EONS) continues to be a severe condition associated with high morbidity and mortality. However, symptoms and laboratory markers of this serious condition are nonspecific and currently there are no available standard tests to provide perfect diagnostic accuracy. An early recognition and initiation of antimicrobial therapy is essential in order to prevent morbidity and mortality[1].

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Reliance on blood culture as a 'gold standard' presents several challenges in neonates because of long turn-around time for results and frequent falsely negative culture results secondary to low inoculums of bacteria in the small volume of blood sample collections [2]. Consequently, various adjunctive diagnostic tests, including biochemical markers, hematological indices, and scoring systems are used to aid decision-making in antibiotic therapy in infants suspected of sepsis[3,4]. The deleterious consequences of neonatal sepsis are particularly pronounced in low birth weight (LBW) infants, adversely impacting growth, neurodevelopment pulmonary function, and prolonging hospital stay [5].

Although procalciton in has an established role as a biomarker in septic patients and has been shown to correlate closely with infection, it has some limitations. It rises transiently in patients with non-septic conditions and systemic inflammatory response syndromes (for example, trauma, surgery, heatstroke) and is not

detectable in certain cases of sepsis [6]. Furthermore, biological predictors of mortality are absent, clinical scores appear to be of limited value and the role of procalcitonin as a poor prognostic factor in patients admitted to the emergency department because of sepsis remains to be proved [7, 8]. C-reactive protein (CRP) has been used for many years but its specificity has been challenged. It remains difficult to differentiate sepsis from other non-infectious causes of systemic inflammatory response syndrome, and studies are being continued to define a reliable biomarker [9].

The ideal biomarker should retain high sensitivity and specificity is cost-effective and promptly available. Cluster of differentiation 14 (CD14), the high-affinity receptor for lipopolysaccharide/lipopolysaccharide binding protein complexes, is a glycoprotein expressed in macrophage, monocyte, and granulocytecells and their cell membranes [10]. During inflammationplasma protease activity generates soluble CD14 (sCD14) fragments. One of them, called sCD14 subtype (sCD14-ST), or presepsin, has recently been identified[6].

Presepsin, a novel biomarker for diagnosing sepsis, a subtype of soluble CD14 (soluble CD4-subtype), is a 13 kDa protein truncated N-terminal fragment of 64 amino acid residues [10]. Presepsin is said to be responsiblefor intracellular transduction of endotoxin signals. Presepsin is produced in association with infection and this occurrence is significantly expressed in sepsis [11].

Presepsin is normally present in very low concentrations in the serum of healthy individuals and has been shown to be increased in response to bacterial infections according to the severity of disease[10]. Presepsin is currently under investigation in clinical practice as a reliable marker of adult and neonatal sepsis[12].

Hepcidin, the key regulator of iron homeostasis, is also an acute-phase reactant, which has a critical role in inflammation and contributes to host defense by interfering with microorganism's access to iron[13,14]. Since hepcidin is expressed by macrophages and neutrophils in response to bacterial pathogens and its expression is induced by interleukin-6 (IL-6), it also plays a role in the innate immune system [15]. Hepcidin contributes to host defense by depriving microbes' access to iron and through direct antimicrobialactivity against bacteria and viruses [16]. According to several studies, presepsin and hepcidin could be seen as valuable biomarkers for early diagnosis for sepsis and to distinguish it from non-infectious diseases. But there is no data about presepsin levels on prognosis of sepsis or therapy modification.

The goal of this study is to evaluate the diagnostic and prognostic value of presepsin compared to hepcidin and CRP in predicting neonatal sepsis.

METHODOLOGY

1. Patients

The current study included 62 neonates, born between October 2013 and August 2014 at Neonatology Unit of Menoufiya University Hospital and Ahmed Maher Teaching Hospital. Twenty eight newborn infants met the criteria of EONS and 34 newborn infants were healthy with no clinical picture suggestive of neonatal sepsis were included as controls.

Neonatal sepsis is considered as early-onset if it is diagnosed in the first 72 h of life. Inclusion criteria: were postnatal age 72 h, presence of nonspecific signs of sepsis (temperature instability, apneic spells, need for supplemented oxygen, need for ventilation, tachycardia/bradycardia, hypotension, feeding intolerance, abdominal distension and necrotizing enterocolitis), and clinical deterioration considered to be due to sepsis [17].

Blood samples were collected from all 62 newborn infants (after parents' consent).

Exclusion criteria:

Neonates, born with hydrops fetalis, congenital hypoplastic anemia, placentalabruption and twin to twin transfusion syndrome were excludeddue to the central role of hepcidin in patients with anemia. Infants of mothers with anemia were also excluded from the study.

All neonates were evaluated with:

- **Complete clinical examination:** (for signs of sepsis, including lethargy, feeding intolerance, fever or hypothermia, tachypnea, cardio-respiratory instability, or hypotonia).
- **Complete blood count:** performed on 3 part differential automated cell counter Sysmex KX-21N (Sysmex, Kobe, Japan).
- **Quantitative measurement of the level of C-reactive protein** (CRP) using automatic auto-analyzer Integra 400 (Roche Diagnostics, Mannheim, Germany).
- **Iron parameters:** Serum ferritin, and iron, were assessed using Integra 400 (Roche Diagnostics, Mannheim, Germany), and total iron binding capacity (TIBC) using Dimension autoanalyser (Simens –Germany).
- **Blood gases,** on AVL (Roche Diagnostics, Mannheim, Germany).
- **Serum hepcidin:** by capture enzyme-linked immunosorbent assay, this detects the 25-amino acid mature form of hepcidin. A commercial kit from Peninsula Laboratories (Bachem, Torrance, CA, USA) was used according to the manufacturer instructions. The intra-assay coefficientof variation (CV) was 5%-19% and the inter-assay reproducibility had an average CV of 12% [18].
- **Serum presepsin:** Blood samples were collected in ethylenediaminetetraacetate (EDTA) tubes,

centrifuged at 3,000 g for 10 min and then plasma were stored at -70°C. The entire procedure for presepsin was automatically performed using the PATHFAST kit (Mitsubishi Chemical Europe GmbH, Worrstadt-Germany). Principle: It is based on a non-competitive chemiluminescent enzyme immunoassay (CLEIA) combined with MAGTRATION technology. The presepsin binds to (ALP) labelled polyclonal Abs and monoclonal Abs coated magnetic particles. Briefly, 100µl whole blood were dispensed into the wells of the reagent cartridge, then 25µl dilution solution, 50 µl magnetic latex reagent and 50 µl labelled Ab reagent were added and allowed to react at 37˚C for 5 min. Then washing was done three times with the wash solution provided. After that 100 µl luminescent substrates were added. The luminescence intensity generated was related to presepsin concentration in the sample. The presepsin concentration was measured by comparison with the amount of luminescence of calibration agents (CAL 1 & 2) that has been subjected to the same procedure as the sample. Method precision was tested on plasma, yielding within-series coefficients of variation (CVs) of 12.3%, 3.9% and 2.8%, respectively, as well as between-series CVs of 15.0%, 7.7% and 4.2%, respectively [19].

- **Blood cultures:** It was done for 28 neonates with suspected sepsis under both aerobic and anaerobic condition. One ml of blood was collected under aseptic precautions, for the aerobic and anaerobic blood culture bottles, incubated at 37C for 48 h. Subcultures were done every 48 h under both aerobic and anaerobic conditions. Identification of the growing colony was done using Gram staining film and biochemical characters and automatic microorganism identification was done by using of Vitek2 compact system (The Vitek 2, BioMérieux, Inc. Hazelwood, MO, USA).
- **Post therapeutic assessment:** As per neonatal intensive care unit protocol, neonates suspected to have sepsis were evaluated routinely with complete blood count, blood culture, and CRP prior to initiation of empiric antibiotic therapy. Then another blood sample was taken to reevaluate CRP, hepcidin and presepsin.

Statistical analysis:

The data were collected, tabulated, and analyzed by SPSS (statistical package for social science) version 17.0 on IBM compatible computer. Chi-square test (χ^2): to study association between two qualitative variables. Student t-test used for comparison between means of two groups (with quantitative and normally distributed variables). Mann Whitney U test: a nonparametric test of significance was used for comparison between two groups not normally distributed and having quantitative variables. Correlation analysis used as a statistical process for estimating the relationships among variables. The significance level was chosen at $p<0.05$.

RESULTS

Table 1: Comparison between the studied groups regarding descriptive data

Parameters	*Neonates with sepsis (n=28)*	*Control neonates (n=34)*	*P value*
Gestational age (week)	37.6±1.7	38.3±1.3	>0.05
Birth Weight (gm)	3242±269	3198±235	>0.05
Sex (M/F)	11/17	14/20	>0.05
Age at sampling (days), M± SD	2.6±0.42	2.1±0.74	>0.05
Mode of delivery, Cesarean delivery, n (%)	10 (35.7%)	15 (44%)	>0.05
Apgar Score	7.6±1.4	8.2±1.7	>0.05

p >0.05= non significant.

Data in Table 1 show that there were no significant differences between both groups regarding gestational age, birth weight, sex, sampling age, mode of delivery or Apgar score, (p >0.05) for all parameters.

Table 2: Isolated bacteria from culture positive neonates with sepsis

Organisms	*Neonates with positive blood culture (n=16)*
Escherichia coli	7 (43.75%)
Coagulase-negative S*taphylococci*	5 (31.25%)
Group B *Streptococcus*	3 (18.75%)
Klebsiella pneumonia	1 (6.25%)

The culture results (Table 2) revealed that, 16 (57%) out of 28 newborn with sepsis were culture positive. The isolated bacteria were E-coli, 7 cases (43.75%), coagulase negative *Staphylococci* were 5 cases (31.25%), group B beta hemolytic *Streptococci* 3 cases (18.75%) and *Klebsiella pneumonia* one case (6.25%).

Table 3: Comparison between studied groups regarding routine laboratory data

	Neonates with sepsis (n=28)	*Healthy neonates(n=34)*	*P value*
Hb (g/dl)	14.9±2.4	15.1±2.7	>0.05
MCV (fl)	94.5±4.3	96.2±3.1	>0.05
RDW(fl)	17.2±3.5	15.8±2.1	>0.05
Ht (%)	39.8±1.3	42.1±2.4	>0.05
TLC (x10^3 /uL)	12.3±5.7	11.2±3.9	>0.05
PMN count (x10^3 /uL)	6.73±2.24	5.62±1.88	>0.05
Platelet count (x10^3 /uL)	158±72	312±59	<0.001**
S. Iron (ug/dl)	92.4± 25.1	112.4±22.7	>0.05
TIBC (ug/dl)	`87.6 ± 43.1	97.2±54.2	>0.05
S. ferritin (ng/ml)	416.2 ± 183	397.2±143	>0.05

*P>0.05= non- significant, **p<0.001=highly significant*

Table 3 shows full blood counts, and iron profile (Serum ferritin, TIBC, and serum iron). There was no statistically significant difference for all parameters except for platelets count, the platelet was reduced in neonates with sepsis compared to control neonates (p<0.001).

Table (4) Comparison between studied groups regarding marker of sepsis

Parameters	*Neonates with sepsis (n=28)*	*Control neonates (n=34)*	*P value*
CRP (mg/L)	58.3± 49.2	2.6± 1.7	<0.001**
S. Hepcidin (ng/ml)	206.5 ±99.7	145.1± 42.8	<0.05*
S. Presepsin (pg/ml)	872.6 ± 234.1	379.8 ± 127.3	<0.001**

**P<0.05= significant, **p<0.001=highly significant*

The sepsis markers were significantly higher in neonates with sepsis than in healthy control neonates as shown in table (4).

Table 5: The sensitivity and specificity of sepsis markers

	CRP	*Hepcidin*	*Presepsin*
Cutoff	8.12 mg/L	105 ng/ml	672 pg/ml
AUR	0.67	0.79	0.95
Sensitivity	58%	92%	97%
Specificity	92%	83%	98%
PPV	84%	87%	96%
NPV	72%	88%	92 %

Table 5 shows the sensitivity and specificity of the tested sepsis markers. At a cutoff 672 pg/ml, the presepsin can discriminate between neonates with sepsis and control neonates with a sensitivity of 97%, specificity of 98%, PPV of 96% and NPV of 92%, area under ROC curve (AUR) was 0.95 which are higher than hepcidin and CRP.

Table 6: Correlation between sepsis markers and patient data

	Serum hepcidin		*Serum presepsin*	
	`r	P	r	P
Gestational age	0.13	>0.05	0.24	>0.05
Birth Weight	0.22	>0.05	0.18	>0.05
Blood culture results	0.38	<0.05*	0.51	<0.01**
CRP	0.43	<0.01**	0.64	<0.001**
Hb.	0.19	>0.05	0.09	>0.05
Ht	0.16	>0.05	0.11	>0.05
TLC	0.24	>0.05	0.20	>0.05
S. Iron	0.06	>0.05	0.17	>0.05
S. Ferritin	0.14	>0.05	0.08	>0.05

*P>0.05= non- significant *p<0.05= significant, **p<0.01 and <0.001=highly significant*

Table 6 shows a positive correlation between serum levels of hepcidin and presepsin, and each of the blood culture results as well as CRP level.

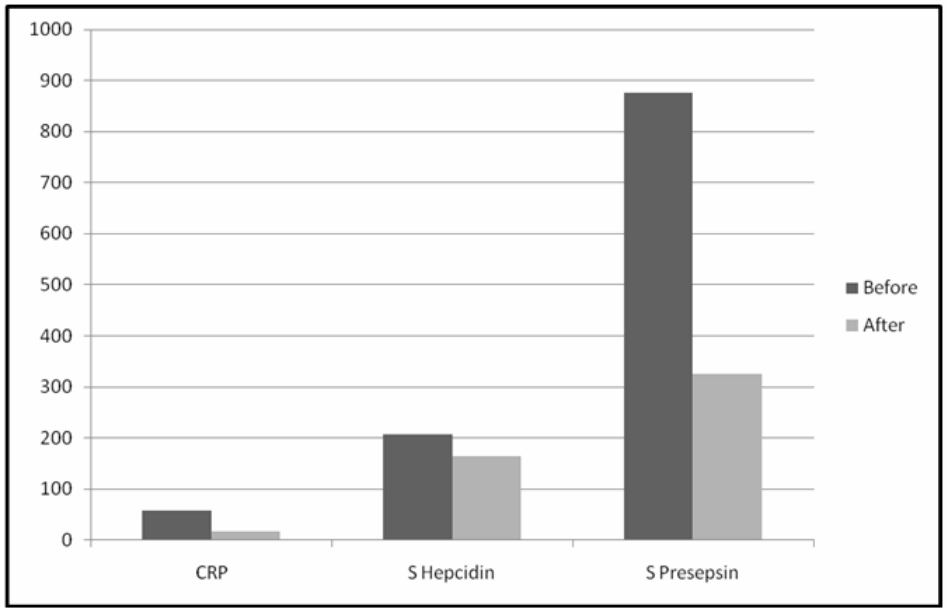

Fig. 1: Shows the comparison between sepsis biomarkers levels before & after therapy

The comparison between sepsis biomarkers levels before and after therapy is shown in figure (1). CRP decreased from 58.3± 49.2 mg/L to 17.2± 3.24 mg/L following therapy of sepsis. Serum hepcidin dropped from 206.5 ±99.7 ng/ml to 164.5±22.3 ng/ml following therapy of sepsis. The same also was noted for serum hepcidin which dropped from 872.6 ± 234 pg/ml to 325.1± 87.2 pg/ml following therapy of sepsis.

DISCUSSION

Neonatal sepsis remains a major problem associated with high morbidity and mortality in newborns, particularly amongst preterm infants [20]. However, symptoms and laboratory markers of this serious condition are nonspecific and currently there are no available standard tests to provide perfect diagnostic accuracy. An early recognition and initiation of antimicrobial therapy are essential in order to prevent morbidity and mortality [1].

In the present study, the cultures results revealed that, 16 (57%) out of 28 newborn with sepsis were culture positive. The isolated bacteria were *E-coli* 7 cases (43.72%), coagulase- negative *Staphylococci*. 5 cases (31%), group B beta hemolytic *Streptococc*i 3 cases (18%) and *Klebsiella pneumonia* 1 case (8%). These results were in agreement with the positive cultures in the study that carried out by Ali et al.[21] which were (52%) and the isolated organisms were E-coli (4o%), *Staph. aureus* (27%), *Staph. epidermidis* (0.09%), *ureplasma* and GBS one case for each (0.9%). Also, Hassan et al.[22] reported the positive yield of blood culture was 57% in neonatal sepsis, and gram negative organism as 97.8% of the total isolates**.** Ramesh et al. [23] reported a high incidence of gram negative neonatal sepsis (90.8% of culture isolates), with an overall positive cultures of 17.8% of neonatal sepsis.

Reports of total blood leukocytic counts, serum ferritin, iron, and TIBC were not statistically differed between the neonatal sepsis and healthy newborns, except for platelets which is decreased in neonatal sepsis group. Similar reports were concluded by Arif et al.[24]**,** who revealed that thrombocytopenia was seen frequently in early sepsis with or without laboratory evidence of overt DIC. That study was conducted on 85 neonates admitted in NICU with clinical diagnosis of septicemia. Thrombocytopenia was seen in 83.5% cases where as bacterial culture was positive in only 41.1% cases

Regarding sepsis biomarkers, in this study, the serum level of CRP, TLC and PMN counts were significantly higher in newborn with sepsis than in the healthy neonates. The same was reported by Ali et al. [21] and Lorrot et al.[25]**,** but most of these inflammatory indicators were non-specific parameters with changing reliabilities denoting that, both may not be enough to diagnose bacterial infection**.**

In the present study, the serum level of presepsin was significantly higher in neonatal sepsis than in the healthy newborn ($p<0.001$). This was in accordance with the results of the study that carried out by Yagashi et al.[26] and Liu et al. [11] who concluded that, the serum level of presepsin was higher in patients with sepsis than in healthy controls. Also, Agilli et al.[27]**;** Meraelli et

al. [28] and Vodnik et al. [29], stated that the serum level of presepsin was significantly higher in infectious sepsis patients than in non-infectious patients and healthy individuals.

The clear link between the hepcidin molecule and innate immunity may be used for the detection of EONS. In the present study, the serum level of hepcidin was significantly higher in neonates with sepsis than in neonates without sepsis ($P<0.05$), this was in agreement with the result of Cizmeci et al.[1] study, who stated that the serum level of hepcidin in cord blood of neonates with sepsis was higher than in healthy neonates. Also, Wu et al. [16] study concluded that, the level of hepcidin was 4-fold higher in neonates with sepsis than in healthy neonates. Similarly, Yapakci et al.[30] mentioned that the serum level of hepcidin in neonates (both pre and full term) with sepsis was significantly higher than in healthy neonates.

In the present study, at a cut off values of 672 pg/ml, the presepsin can discriminating the neonates with sepsis from healthy neonates with a sensitivity of 97%, specificity of 98%, PPV of 96% and NPV of 92% , area under ROC curve (AUR) was 0.95 which are higher than hepcidin and CRP. In agreement with our results, Zou et al.[31] study reported that presepsin seemed to have a better sensitivity and specificity in the diagnosis of sepsis compared with other biomarkers and is not only suitable for the early diagnosis of sepsis but also for assessment of its severity and prognosis.

In another study carried out by Shozushima et al. [32], at cut off value 415 pg/ml presepsin was found to be more useful in the diagnosis of sepsis, the sensitivity was 80.1%, specificity was 81%, AUR which was higher than that of PCT. The results of study carried out by Cizmeci et al. [11], revealed that, at a cut off values of 317 ng/ml for diagnosis of sepsis, the sensitivity was 70.8%, the specificity was 85.8%, the PPV was 93.2% and NPV was 51.6%.

The diagnostic and prognostic values of serum hepcidin as a biomarker in neonatal sepsis were also evaluated, and compared with other sepsis biomarkers namely prespsin and CRP. At cutoff value 105 ng/dl of hepcidin, the sensitivity was 92%, the specificity was 83%, PPV was 87%, and the NPV was 88%, which were higher than CRP, but lower than that of presepsin. However, at a cut off >8.12 mg/L, CRP gives a sensitivity of 58%, specificity of 92%, PPV of 84% and NPV of 72%. Ali et al. [21], reported that, at a cut off >12 mg/L, CRP gives a sensitivity of 88%, specificity of 77.8%, PPV of 77% and NPV of 70%. Also, Mehr and Doyle[33] stated that, the sensitivity of CRP for the detection of EONS infection ranged from 35%-65% and the specificity varies from 76-86% for CRP.

Regarding relationship with serum iron parameters, we found no significant differences between serum iron, serum ferritin and the serum level of hepcidin in neonates with and without sepsis. This was in accordance with results of Cizmeci et al.[1] who stated that no significant relationship between the serum level of hepcidin and iron parameters in neonates with and without sepsis, this indicates that hepcidin may indeed be non-functional with regards iron homeostasis in septic neonates, as stated by Yapakci et al. [30].

In contrast, there was a positive relationship between presepsin and hepcidin levels and each of blood culture results and CRP level, denoting their pivotal roles in development of neonatal sepsis. Presepsin activities were also measured among the major organ transplant patients for possible sepsis diagnosis and the study results revealed that activities of presepsin were significantly higher among transplanted patients which correlated with blood culture results [34].

Few data are available about presepsin levels on prognosis of sepsis or therapy modification. After antibiotic administration to neonates with sepsis, second blood sample was collected for reevaluation of CRP, hepcidin and presepsin after therapy. Their levels were significantly lower than that before treatment levels, denoting the diagnostic and prognostic volubility in neonatal sepsis. Kweon[35] and Ramana et al. [36] stated that, the presepsin has a potential role not only as a diagnostic marker of sepsis, but is also efficient in predicting the prognosis after antibiotic therapy and survival chances of sepsis patients. Agilli et al.[27] mentioned that presepsin can be used for diagnosis and specification of sepsis more than CRP or other inflammatory biomarkers.

CONCLUSION

Presepsin is considered an earlier, more sensitive and specific marker rather than hepcidin and CRP for diagnosis of neonatal sepsis, where it rises early after infection, and its correlation with sepsis severity and response to treatment is more informative compared with CRP and hepcidin. Further large scale studies may be needed to find the relationship between presepsin and other pediatric age.

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