

Study of Serum Presepsin as Diagnostic &Prognostic Marker in Early Onset Neonatal Sepsis

Thesis

Submitted for partial fulfillment of master degree in
pediatrics

By

Hoda Mostafa Nagi El-sayed

M.B.B.Ch

Ain Shams University

Under supervision of

Prof. Dr. Ghada Ibrahim Gad

Professor of Pediatrics

Faculty of medicine – Ain Shams University

Dr. Dina Mohamed Shinkar

Lecturer of Pediatrics

Faculty of medicine – Ain Shams University

Dr. Manal Mohsen M. Kamel El-Din

Lecturer of Clinical Pathology

Faculty of medicine – Ain Shams University

Faculty of medicine

Ain Shams University

2017



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا

عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

سورة البقرة آية (32)



Acknowledgement

First of all thanks to Allah the most kind and the most merciful.

I wish to express my deepest thanks, gratitude and profound respect to my honored Prof. Dr. Ghada Ibrahim Gad, Professor of Pediatrics, Faculty of Medicine, Ain Shams University. I consider myself fortunate to work under her supervision. Her constant encouragement and constructive guidance were of paramount importance for the initiation, progress and completion of this work.

I would like to express my sincere thanks to Dr. Dina Mohamed Shinkar, Lecturer of Pediatrics, Faculty of Medicine, Ain Shams University for her great support, facilities, careful supervision, continuous advice and guidance which were the cornerstone for this work.

I am also deeply grateful and would like to express my sincere thanks and gratitude to Dr. Manal Mohsen M. Kamel El-Din, lecturer of Chemical and Clinical Pathology, Faculty of Medicine, Ain Shams University for dedicating much of her precious time to accomplish this work and for her keen advices throughout the study.





*I would like to thank my family especially my
parents who encouraged and supported me all the time, to
them I dedicate this work.*

Abstract

Background: Sepsis is a complex clinical condition caused by dysregulated immune response to an infection resulting in multiorgan failure with a fatal outcome. More than 90% of neonatal deaths occur in the poorest countries of Asia and Africa. There is no gold standard for diagnosing sepsis as clinical and laboratory signs are neither sensitive enough nor specific enough. Biomarkers have shown great promise in diagnosis of sepsis and guiding appropriate treatment of neonates. Among these, soluble CD 14 sub-type (presepsin) has been suggested as a novel diagnostic and prognostic marker to identify high-risk patients, and may be useful for the clinical management of serious infectious diseases. However, few studies addressed the value of presepsin as a sepsis biomarker in neonates and data on its prognostic value in septic neonates are still lacking. **Aim:** We assessed presepsin levels as a potential early diagnostic and prognostic marker for early onset neonatal sepsis. **Methods:** A total of 60 full term neonates was enrolled; 40 neonates with clinical and laboratory signs of EONS and 20 healthy controls. Complete blood count, high sensitivity C-reactive protein (hs-CRP) and sepsis score including clinical and laboratory indicators were assessed. The diagnosis was verified thereafter by blood culture. According to the results of blood cultures, the studied septic neonates were classified into 2 groups: the culture-proven septic group and culture-negative septic one. presepsin levels were measured on initial sepsis evaluation and at 72 hours after antibiotic therapy. For ethical reasons, cord blood samples were collected from control neonates and only samples from neonates that proved to be healthy by clinical examination and laboratory analysis were further analyzed for presepsin. **Results:** Initial presepsin levels were significantly elevated in all neonates with EONS compared with healthy controls. Patients with culture-proven and

culture-negative sepsis had higher presepsin compared with controls while no significant difference was found between both septic groups. Elevated initial presepsin levels were associated with the occurrence of septic shock, high mortality rate and a worsening clinical course of the disease. Presepsin decreases significantly after 72 hours after antibiotic therapy in neonates. Presepsin was positively correlated to hs-CRP and sepsis score. ROC curve analysis revealed that presepsin cutoff value of 1400 pg/mL could be predictive of mortality among neonates with sepsis with 100% sensitivity and 88.5% specificity while the cutoff value of 480 pg/mL could be predictive of clinical course of sepsis with 100% sensitivity and 95% specificity. Neither baseline hs-CRP levels nor sepsis score had a prognostic utility in relation to mortality or clinical course of the disease. **Conclusions:** presepsin could be considered an early diagnostic marker for EONS before culture results. Elevated presepsin levels reflect sepsis severity and poor prognosis more accurately than CRP or sepsis score. Thus, it could be a valuable biomarker to monitor the disease course.

Keywords: early onset neonatal sepsis, presepsin, mortality, septic shock, CRP.

List of Contents

	Page No.
List of Abbreviations	I
List of Figures	VI
List of Tables	IX
Introduction	1
Aim of the Work	3
Review of Literature	4
• Chapter 1: Neonatal sepsis	4
• Chapter 2: Presepsin	52
Patients and Methods	64
Results	81
Discussion	115
Summary	130
Conclusion	133
Recommendations	134
References	135
Arabic Summary	—

List of abbreviations

• AAP	American academy of pediatrics
• APPs	Antimicrobial proteins and peptides
• AUC	The areas under the ROC curve
• BP	Blood pressure
• BSI	Blood stream infection
• CBC	Complete blood count
• CD	Cluster of differentiation
• CNS	Central nervous system
• CoNS	<i>Coagulase-negative Staphylococcus</i>
• CRP	C-reactive protein
• CS	cesarean section
• CSF	Cerebrospinal fluid
• DIC	Disseminated intravascular coagulation
• DM	Diabetes mellitus
• DNA	Deoxyribonucleic acid
• E coli	<i>Escherichia coli</i>
• ECG	Electrocardiography
• EDTA	Ethylenediaminetetraacetic acid
• ELISA	Enzyme-linked immuno assay

• EONS	Early Onset Neonatal Sepsis
• ESR	Erythrocyte Sedimentation Rate
• GA	Gestational age
• GBS	<i>Group B streptococcus</i>
• G-CSF	Recombinant Granulocyte-colony stimulating factor
• G-CSF	Granulocyte colony-stimulating factor
• GI	Gastrointestinal tract
• GM-CSF	Granulocyte-macrophage stimulating factor
• GPI	Glycosyl-phosphatidyl inositol
• HCT	Haematocrit
• HLA	Human Leukocyte Antigen
• HR	Heart rate
• HRC	Heart rate characteristics
• HRP	Horseradish peroxidase enzyme
• HSS	Hematological scoring system
• HSV	<i>Herpes simplex virus</i>
• I/M	Immature-to-mature neutrophil ratio
• I/T	Ratio of immature to total neutrophils
• IAP	Intrapartum antibiotic prophylaxis
• IFN	Interferon
• Ig	Immunoglobulin

• IL	Interlukin
• IMV	Invasive mechanical ventilation
• IV	Intravenous
• IVIG	Intravenous immunoglobulin
• K.pneumonia:	<i>Klebsiella pneumonia</i>
• KDa	kilodalton
• LBP	lipopolysaccharide-binding protein
• LFTs	Liver function tests
• LONS	Late Onset Neonatal Sepsis
• LPS	Lipopolysaccharide
• mCD14	Membrane-bound CD14
• MD2	Myeloid Differentiation Protein-2
• MODS	Multiple Organ Dysfunction Syndrome
• MW	Molecular weight
• nCD	Neutrophil expression of CD64
• NFκB	Nuclear Factor kappa B
• NICUs	Intensive Care Units
• NK	Natural Killer
• OD	Optical density
• P value	Probability value
• PAF	Platelets activating factor
• PAMP	Pathogen-Associated Molecular Patterns

• PCR	Polymerase chain reaction
• PCT	Procalcitonin
• PMNs	Polymorphonuclear leukocytes
• PPHN	Persistent pulmonary hypertension of the newborn
• PPV	Positive predictive value
• PROM	Premature rupture of membranes
• PRRs	Pattern-Recognition Receptors
• rG-CSF	Recombinant Granulocyte-colony stimulating factor
• rhG-CSF	Recombinant Human G-CSF
• ROC	Receiver operating characteristic curves
rRNA	Ribosomal ribonucleic acid
RSV	Respiratory syncytial virus
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SABC	HRP-Streptavidin Conjugate
sCD14	Soluble CD14
sCD14-ST	sCD14 subtype
SD	Standard deviation
SIRS	Systemic Inflammatory Response Syndrome
SST	Serum separator tube
SVD	Spontaneous vaginal delivery
Temp	Temperature

• THP1	Human cell line of monocytic cells
• TLC	Total leucocytic count
• TLRs	Toll-like receptors
• TMP	Tetramethylbenzidine substrate
• TNF- α	Tumor Necrosis Factor – α
• TPN	Total parenteral nutrition
• UNICEF	United Nation Children's Emergency Foundation
• UTI	Urinary tract infection
• VLBW	Very low birth weight infants
• WBCs	white blood cells

List of Figures

<i>Figure No.</i>	<i>Title</i>	<i>Page No.</i>
Figure (1):	Progression of neonatal sepsis	5
Figure (2):	Role of innate immunity in responses to in utero infection	8
Figure (3):	Pathogenesis and overview of clinical findings of neonatal sepsis	23
Figure (4):	Score card for combining HeRO and clinical findings for diagnosis of sepsis	25
Figure (5):	secondary prevention of early-onset group B streptococcal (GBS) disease among newborns	43
Figure (6):	Recommended regimens for intrapartum antibiotic prophylaxis for prevention of early-onset group B streptococcal (GBS) disease	44
Figure (7A):	Evaluation of asymptomatic infants <37 weeks' gestation with risk factors for sepsis	45
Figure (7B):	Evaluation of asymptomatic infants ≥ 37 weeks' gestation with risk factors for sepsis	46
Figure (7C):	Evaluation of asymptomatic infants ≥ 37 weeks' gestation with risk factors for sepsis (no chorioamnionitis)	46
Figure (8):	The mechanisms by which gram positive and gram negative bacteria cause sepsis.	54

Figure (9): Secretion mechanism of Presepsin	57
Figure (10): Presepsin elevation is caused by bacterial phagocytosis	59
Figure (11): Clinical types of sepsis among septic neonates	84
Figure (12): comparison between septic neonates and control group as regard initial serum presepsin level.	86
Figure (13): comparison between initial and after 72 hrs serum presepsin level among septic neonates	87
Figure (14): Initial Tollner score of neonates with septic shock and those without shock.	90
Figure (15): comparison between all septic patients as regard initial serum presepsin level	92
Figure (16): Bacteriological profile of septic neonates.	93
Figure (17): Blood culture results among septic neonates.	94
Figure (18): Inotropic support in neonates with positive blood culture and those with negative blood culture	97
Figure (19): Comparison between neonates with positive blood culture and those with negative blood culture as regard initial serum presepsin level	99
Figure (20): comparison between survivors and non survivors septic neonates as regard (A) initial and (B) after 72hrs serum presepsin level	100

Figure (21): comparison between initial and after 72 hrs presepsin level among (A) survivors and (B) non survivors septic neonates	103
Figure (22): initial serum presepsin level in relation to mechanical ventilation in septic neonates	105
Figure (23): correlation between initial serum presepsin level and Apgar score at 1 min.	108
Figure (24): Correlation between initial serum presepsin level and Tollner score.	108
Figure (25): Correlation between initial serum presepsin level and CRP.	109
Figure (26): ROC curve showing utility of initial serum presepsin level in early diagnosis of sepsis	110
Figure (27): ROC curve showing utility of initial serum presepsin level in prediction of septic shock.	111
Figure (28): ROC curve showing prognostic utility of initial serum presepsin level in predicting mortality	112