

ORIGINAL ARTICLE

Serum Leptin versus C- reactive protein in the Early Diagnosis of Neonatal Sepsis

¹Mai M. Malek*, ²Enas A.H. Hussein, ²Ahmed G. Siam, ²Mohamed A. Arafa

¹Department of Medical Microbiology & Immunology- Faculty of Medicine- Zagazig University

²Department of Pediatrics, Faculty of Medicine- Zagazig University

ABSTRACT

Key words:

Leptin, neonatal sepsis, inflammatory, mediators

*Corresponding Author:

Mai M. Malek, MD.

Department of Medical

Microbiology & Immunology-

Faculty of Medicine- Zagazig

University-Egypt

Tel.:01000711138

Dr_mai281@yahoo.com

Background: When sepsis process starts, a variety of inflammatory cytokines pass into the circulation. These cytokines include tumor necrosis factor- α (TNF- α), leptin, interleukin-6 (IL-6, C-reactive protein (CRP) and procalcitonin (PCT). These cytokines are crucial inflammatory mediators. Measurement of the levels of inflammatory cytokines is an obvious marker for sepsis. Detectable serum cytokine levels can be obtained at a significant time before other laboratory indicators like leukocytosis or changes in absolute neutrophil count. This suggests their important role as diagnostic marker in sepsis. **Objectives:** To compare between sensitivity of serum leptin and C-reactive protein in early diagnosis of neonatal sepsis. **Methodology:** Prospective cohort study which carried out over 12 months from April 2017 to March 2018 at pediatrics department and Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University. All neonates were subjected to complete history taking, detailed clinical examination, basic laboratory investigations which include (C-reactive protein), Complete blood picture, Bacteriological blood culture for isolation of the causative organisms and bacteria were identified by Vitec2, Serum leptin assay by sandwich enzyme-linked immunosorbent assay (ELISA). **Results:** 34 neonates that were classified regarding culture results into: Group 1 included newborns whose blood culture was negative. Group 2 included newborns whose blood culture was positive. 76.5% of the studied group had positive blood culture results. There was statistical significance increase in WBCs, CRP 1st and CRP 2nd day among positive blood culture cases. There was statistical significance increase in Leptin among positive blood culture cases compared to negative blood culture cases. **Conclusion:** Leptin is a reliable diagnostic marker for neonatal sepsis and more accurate when compared with CRP.

INTRODUCTION

Neonatal sepsis is as a syndrome of bacteremia with clinical systemic signs and symptoms of infection in the first 28 days of life¹. When pathogenic bacteria pass to the blood, they may cause serious infection without apparent localization or may be localize to the lung, urinary tract or the meninges.²⁻⁴

Sepsis is the main cause of neonatal mortality and is responsible for around half of total neonatal deaths each year in developing countries. In neonatal ICU up to 20% of neonates develop sepsis and about 1% die from causes related to sepsis.⁵⁻⁷

In developed countries *Group B Streptococcus* and *E.coli* are the dominant pathogens involved in early onset neonatal sepsis while in late onset neonatal sepsis *coagulase- negative staphylococci* is the dominant pathogen followed by *GBS* and *Staph aureus*.^{8,9}

In developing countries, Gram negative organisms are more predominant *Klebsiella*, *E. coli* and *Pseudomonas* are the most common detected organisms.

Regarding Gram positive organisms, *Staph aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* are most commonly isolated.¹⁰⁻¹²

It is known that Procalcitonin and CRP are useful in the diagnosis of bacterial sepsis, but with limitations. The most important limitation is the time factor.¹³

Concentrations of CRP fail to allow immediate diagnosis and prognosis due to the time taken to produce a series of reactions and the duration taken for increased serum concentration.¹⁴⁻¹⁵

Human leptin is a protein formed of 167 amino acids, it is pleiotropic hormone which is released from adipose tissues and has a role in energy balance.¹⁶

Leptin receptors, which present on neutrophils, monocytes and lymphocytes, are classified as class I cytokine receptors¹⁷. Leptin in sepsis has stimulatory actions on hematopoietic, immunomodulatory and hepatocyte functions and acts as an acute phase reactant. It stimulates monocyte activation and proliferation in addition to release of IL-6 ve TNF- α from monocytes^{18,19}.

Leptin plays a role in immune system activation and serves as mediator of inflammation²⁰.

Many studies suggest that serum leptin has a significant and early correlation with neonatal septicemia and that it can be used as a useful biomarker^{21,22}.

METHODOLOGY

Study design:

A prospective cohort study was conducted over 12 months from April 2017 to March 2018 at Pediatrics Department and Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University.

Ethical considerations

The study was approved by Institutional Review Board (IRB) Committee

Subjects:

This study included 34 neonates. Classified based on culture results into:

- **Group 1** included newborns whose blood culture was negative
- **Group 2** included newborns whose blood culture was positive

Inclusion criteria:

Infants born to mothers having at least one of these risk factors, Premature rupture of membranes (PROM) >18 hours, Intrapartum fever (oral temperature >38°C), Foul-smelling liquor and untreated or partially treated urinary tract infection in the antenatal period.

Exclusion criteria included Parent's refusal, premature delivery, newborn babies weighing less than 1,000g, newborn babies with lethal congenital anomalies.

Methods

All neonates were subjected to:

Full history taking including:

Weight, Gestational age in weeks, Mode of delivery, Maternal disease or medication, History of premature rupture of membranes.

Full clinical examination:

The clinical symptoms of infection were divided into 6 categories: General, Neurological, Respiratory, Cardiovascular, Gastrointestinal

Routine laboratory investigations: Complete blood cell count (CBC) and C-reactive protein.

Specimen collection: Blood for culture was collected and dispensed under complete aseptic conditions with great care as to avoid contaminating the specimen and culture medium.

Blood culture:

The blood was mixed with the broth without delay, and the bottles were labeled and incubated at 37°C.

Subculture was done after the first night incubation on blood agar MacConkey agar that was incubated aerobically for 24 hours at 37°C.

blood culture bottles were checked every other day for evidence of microbial growth (hemolysis, turbidity, gas production, or presence of visible growth). If there is no evidence of microbial growth after 10 days of incubation, Gram stains and last subcultures were performed before considering the culture as negative. Positive blood cultures were identified using Vitec 2 with subsequent antibiotic sensitivity.

Serum leptin assay:

The Leptin Sandwich ELISA is a solid phase (ELISA) based on the sandwich technique.

The microtiter plate was coated by biotinylated monoclonal Ab specific to epitope on leptin molecule.

Patient serum samples were incubated in the coated wells, followed by adding anti sera so that sandwich complex is created, after washing, enzyme complex was added. Color intensity is directly proportional to leptin concentration.

Calculation of Results

Standard curve was used, where its vertical axis represent absorbance values of standards, and horizontal axis represents the corresponding concentrations.

The absorbance value for patient sample was used to determine the corresponding concentration from the standard curve.

Very high concentrations (more than 100 ng/ml) should be diluted, and concentration is calculated considering the dilution factor.

Statistical analysis

Statistical analysis was performed by the Statistical Package for Social Science (SPSS) version 11.0 (IBM, USA). Chi-square test was used where appropriate. P values of (<0.05) were considered significant.

RESULTS

This study showed that, 76.5% of the studied group had positive culture results. The most frequent isolated organism was *S. aureus* 12 (35.3%) while *Klebsiella pneumoniae* was present among 10 (29.4%)

Table 1: Culture results among the studied group:

Variable	(n=34)	
	No	%
Culture:		
<i>Negative culture</i>	8	23.5
<i>Positive culture</i>	26	76.5
<i>Candida albicans</i>	4	11.8
<i>Klebsiella pneumoniae</i>	10	29.4
<i>Staphylococcus aureus</i>	12	35.3

Table 2: Relation between lab findings in both culture positive and negative

Variable		-ve (n=8)	+ve (n=26)	Test	P
WBCs (x10 ³ /mm ³)	Mean ± SD	7.83 ± 5.73	14.65 ± 8.25	MW	0.04*
	Median	7.5	12.4	2.01	
Platelets (x10 ³ /mm ³)	Mean ± SD	191.25 ± 122.6	221.69 ± 95.85	MW	0.29
	Median	138.5	188	1.06	
CRP 1 st day: (mg/dl)	Mean ± SD	22.5 ± 17.20	30.92 ± 25.62	MW	0.042*
	Median	18	24	0.80	
CRP 2 nd day (mg/dl)	Mean ± SD	22.5 ± 17.2	32.75 ± 15.11	MW	0.010*
	Median	18	24	1.66	
CRP 3 rd day (mg/dl)	Mean ± SD	18 ± 9.30	15.71 ± 6.14	MW	0.61 NS
	Median	18	12	0.52	

SD: Standard deviation MW: Mann Whitney test t: Independent t test
 NS: Non significant (P>0.05) *: Significant (P<0.05)

Table 2 shows there was statistically significance increase in WBCs, CRP 1st and CRP 2nd day among positive cases. The leptin among the studied group ranged from 0.02 to 7.3 with mean ± 0.97.

Table 3: Relation between Leptin level and culture results among the studied group:

Variable	Negative (n=8)	Positive (n=26)	MW	P
Leptin: Mean ± SD	0.31 ± 0.76	1.37 ± 2.40	2.01	0.04*
Median	0.04	0.22		

SD: Standard deviation MW: Mann Whitney test *: Significant (P<0.05)

Table 3 shows that there was a statistical significant increase in Leptin among positive cases compared to negative cases.

Table 4: Validity of Leptin & CRP in diagnosis of sepsis among the studied group:

Marker	Cutoff	AUC	CI	Sens.	Spec.	+PV	-PV	Accuracy	p-value
CRP	≥ 18	0.58	0.36 – 0.80	65.4	50	81	30.8	61.8	0.52 NS
Leptin	≥ 0.07	0.83	0.65 – 1.02	84.6	87.5	95.6	63.6	85.3	0.005**

Table 4 shows that the sensitivity of CRP in diagnosis of sepsis at cut off 18 was 65.4%, specificity was 50% and the accuracy was 61.8%. While the sensitivity of Leptin in diagnosis of sepsis at cut off 0.07 was 84.6%, specificity was 87.5% and the accuracy was 85.3% (Fig. 1).

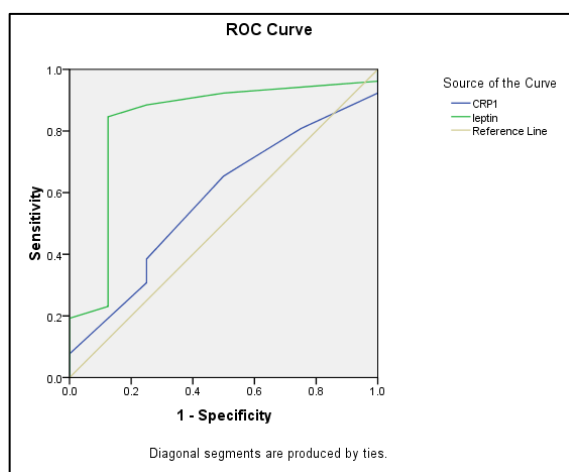


Fig. 1: ROC curve for Leptin & CRP in diagnosis of sepsis.

DISCUSSION

Sepsis is a systemic inflammatory reaction that is triggered by an infective agent (such as bacteria, viruses, fungi or parasites)^{23,24} in which immune with coagulation factors are incorporated leading to cascade of interactions between host and microorganism which is crucial in clinical course and prognosis of sepsis. To improve the prognostic issue associated with sepsis, there is an urgent requirement for sensitive and specific markers for the diagnosis and assessment of sepsis.^{25,26}

This study showed that 76.5% of the studied group had positive culture results.

The most frequent organism was *S. aureus* 12 (35.3%) while *Klebsiella pneumoniae* was present among 10 patients (29.4%). This is quite similar to finding of Mokhtar et al.²⁷ who found The commonest isolated organisms were *Coagulase negative staph (CONS)* in 10 cases (34.4%), followed by *Klebsiella pneumoniae* (24.1%). The percentage of culture positive cases in our study was in agreement with El-Mashad et al.²⁸ but in their study *Klebsiella pneumoniae* was the most common isolate followed by *S. aureus*.

This study showed that there was a significant increase in WBCs, CRP level among culture positive cases which is in agreement with Adib et al.²⁹, Ahmed and Salah³⁰, and Hisamuddin et al.³¹.

This study showed that, there was a significant increase in Leptin among culture positive cases compared with negative cases. This agreed with El-Mashad et al.²⁸ who found a significant higher level of serum leptin in patients with positive blood cultures compared with those with negative blood cultures.

This is in agreement also with the study of Orbak et al.³² and Sakr et al.³³ who found that serum leptin level in newborns with septicemia was significantly higher than those found in their control group.

Many studies as Diez et. al.³⁴ and Chen et al.³⁵ who included adult septic patients in their studies, they demonstrated a significance association of leptin with septicemia.

In the current study, it was found that there was positive statistical significance correlation between serum leptin levels and CRP 1st day.

This is in agreement with the results on the studies of Somech et al.³⁶, who reported a positive correlation between level of leptin in serum and (CRP).

Our study showed that the sensitivity of CRP for diagnosis of sepsis at cut off 18 was 65.4%, specificity was 50% and the accuracy was 61.8%. While the sensitivity of Leptin in diagnosis of sepsis at cut off 0.07 was 84.6%, specificity was 87.5% and the accuracy was 85.3%.

This agreed with El-Mashad et al.²⁸ study who showed that the serum leptin for the detection of sepsis with a 76.1% sensitivity and a 71.4% specificity.

Also Zhang et al.³⁷ who concluded that leptin is a good diagnostic marker in sepsis when compared with classical biomarkers.

Leptin has great advantage over other markers of organ dysfunction in predicting prognosis and monitor the treatment of sepsis³⁸.

Concentrations of CRP have been monitored in septic patients, but these concentrations fail to allow the desired immediate diagnosis and to monitor treatment due to the lag of the body to produce a reaction in response to sepsis dilemma³⁹. However, leptin plasma concentration increased rapidly by action of inflammatory mediators⁴⁰.

CONCLUSION & RECOMMENDATIONS

Immediate diagnosis of sepsis needs dependable biomarkers that are properly correlated to culture results. Sensitivity and specificity of CRP do not match the desired clinical expectations. Serum leptin measurement can be considered of reasonable sensitivity and specificity and is superior to CRP as a diagnostic and prognostic marker. Serum leptin measurement can bridge the lag met with other

diagnostic biomarkers. Based on the results of this study serum leptin measurement should be added to sepsis diagnostic and prognostic panel.

Conflicts of interest:

- The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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