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Research Article
Evaluation of Serum Leptin Level as Early Marker in Early Onset Neonatal Sepsis

[1]Magdy Ashmawy Sakr, [1]Hussein Metwally Abdel Maksoud, [2]Magdy Zaki EL-Ghanam and [3]Shaimaa Ateia Soliman

[1]Department of Pediatrics,
[2]Department of Clinical Pathology, Al Azhar University (New Damietta), Egypt
[3]Department of Pediatrics, Farskour Central Hospital, Damietta, Egypt

Abstract

Background: Despite improved neonatal care over the past decades, sepsis remains a common and life-threatening condition among neonates admitted to NICU. Human leptin plays a role in the activation of the immune system and act as a mediator of inflammation. Leptin deficiency is associated with increasing frequency of infections and it is also involved in the mediation of the systemic response to sepsis. **Objective:** The present study was designed to evaluate the level of serum leptin in cases of early onset neonatal sepsis and its importance in early diagnosis. **Methodology:** This case control study included 50 full term newborns ≤3 days suspected clinically as having EOS (cases group) and another 30 apparently healthy, age and sex matched neonates as control group. They were recruited from NICU of Al Azhar University Hospital (New Damietta) during the period from May, 2013-June, 2015. All cases with suspected neonatal sepsis according to clinical sepsis score were submitted to laboratory investigations including Complete Blood Count (CBC) that including red blood Cell counts (RBCs), hemoglobin (Hb) Total Leukocyte Count (TLC), Absolute Neutrophils Count (ANC), immature/total count ratio (I/T), C-Reactive Protein (CRP), blood culture (for cases) and serum leptin level by ELIZA. **Results:** Regarding demographic data, there was non-significant difference between cases and control as regard age, sex, gestational age, birth weight and delivery mode. As regard laboratory data, there was non-significant difference between cases and control as regard RBCs ($p = 0.097$), Hb ($p = 0.1$) and platelet count ($p = 0.396$), while cases had significant increase of TLC, ANC, I/T ratio, CRP and leptin levels ($p<0.001$). There was a significant positive correlation between leptin from one side and both CRP and ANC from the other side. As regard ability of serum leptin levels to predict neonatal sepsis it had a good sensitivity as denoted by the area under curve (0.89), the best cut-off value was selected to be 2.5 ng dL^{-1} (according to simultaneous best sensitivity and specificity). At this cut-off value, sensitivity was 98% and specificity was 74%. **Conclusion:** Study revealed that serum leptin level was elevated early in cases of EOS and it seems that it may have a role in its early diagnosis at cut-off point 2.5 ng dL^{-1} with sensitivity 98% and specificity 74%. Recommend measurement of serum leptin level early on suspicion of EOS which may aid in confirming the diagnosis. However, large, prospective multi-centre studies should be done to confirm the association between early high serum leptin level and EOS.

Key words: Neonatal care, sepsis remains, life-threatening condition, human leptin, immune system, mediators of inflammation

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Corresponding Author: Hussein Metwally Abdel Maksoud, Department of Pediatrics, Al Azhar University (New Damietta), Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Neonatal sepsis is one of the major causes of neonatal morbidity and mortality[1]. The non-specific clinical signs and absence of a good diagnostic test hinder an accurate early diagnosis[2]. Therefore, a rapid effective marker for early diagnosis and subsequent proper treatment are required[3]. Early-onset sepsis remains a common and serious problem for neonates in spite of recent advances in health care units[4].

Human leptin is a protein of 167 amino acids. It is a pleotropic hormone manufactured primarily in the adipocytes[5]. Leptin plays a role in the activation of the immune system and act as mediator of inflammation, leptin receptors belongs to the family of class I cytokine receptors and have been found in neutrophils, monocytes and lymphocytes[6]. Leptin is not only an adipostatic hormone but also a stress related hormone and serum leptin level increases significantly in sepsis[7]. Leptin has important roles in modulating innate and adaptive immunity, it stimulates neutrophil chemotaxis, promotes macrophage phagocytosis as well as production of pro-inflammatory cytokines[8].

So, the aim of this study was to evaluate the level of serum leptin in cases of early onset neonatal sepsis and its importance in early diagnosis.

MATERIALS AND METHODS

The present study is a case control study which was conducted at Al Azhar University Hospital (New Damietta) during the period from May, 2013-June, 2015. All full term newborns (58 newborns) ≤3 days with suspected neonatal sepsis according to clinical sepsis score[9] were initially included in the study. Newborns presenting with major congenital malformations, suspected chromosomal abnormalities, infants of diabetic mothers and those whose mothers had received corticosteroids or regular hormonal therapy during pregnancy were excluded from the study. Further assessment for diagnosis of sepsis according to the Egyptian Neonatal Network (EGNN) as a presence of at least 2 out of the following criteria: (1) Presence of risk factors of sepsis (e.g., PROM), (2) Presence of two or more clinical signs of sepsis (poor reflexes, lethargy, respiratory distress, apnea, convulsions and bleeding) and (3) Abnormal CBC, positive CRP and positive culture[10]. Neonates who did not meet the diagnosis of sepsis (5 newborns) or had sampling problems (2 newborns) or refused to participate (1 newborn) were excluded from the study. The total number of cases was 50 newborns with early onset sepsis. Another 30 healthy age and sex matched neonates were chosen as a control group.

Laboratory investigations: within the first 24 h of clinical sepsis suspicion, about 3 mL were collected from a peripheral vein. Approximately, 1 mL of blood was inoculated directly into blood culture medium vials and sent to microbiology laboratory for cultivation and subsequent processing. Second portion (1 mL) was put in a sterile EDTA for CBC. Third portion (1 mL) was put in plain tube, left to clot for 15 min then centrifuged. Serum was separated in 2 plastic tubes for determination of CRP and leptin. Tubes for leptin were stored at -20°C till the time of the assay.

The blood cultures were incubated aerobically at 37°C and observed daily for the 1st three days for the presence of visible microbial growth by one of the following: Haemolysis, air bubbles (gas production) and coagulation of broth. The CRP was detected using latex agglutination test with multiple titers to detect highest sample dilution that will still show clearly visible agglutination. Complete blood count was determined by Sysmex-SF-3000 automated hematology Analyzer. Serum leptin assay by immunospec corporation NO; E18-073 which is an ELISA format, performed in microwells coated with anti-Leptin antibody (monoclonal).

Ethical consent: The study was approved by the Hospital Ethics Committee in accordance with local study governance requirements and it was explained to the prospective participants. All participating parents signed an informed consent form.

Statistical analysis: Data collected were reviewed, coded and entered PC, where statistical analysis were done using statistical package for social science (SPSS) version 16. For descriptive statistics, frequency number and percentage are used for qualitative data. Mean and Standard Deviation (SD) for quantitative data were used. Student t-test was done for 2 independent groups. Spearman correlation coefficient test (r) was used to test a positive or negative relationship between two variables. A Receiver Operating Curve (ROC) analysis was performed to define a cut-off value of serum leptin for the risk of neonatal sepsis and the associated specificity and sensitivity levels. The level $p<0.05$ was considered the cut-off value for significance[11].

RESULTS

Regarding demographic data, there was non-significant difference between cases and control as regard age, sex, gestational age, birth weight and delivery mode (Table 1). As regard laboratory data, there was non-significant difference between cases and control as regard RBCs ($p = 0.097$), Hb ($p = 0.1$) and platelet count ($p = 0.396$), while cases had significant increase of TLC, ANC, I/T ratio, CRP and leptin levels ($p<0.001$) (Table 2), there was a significant positive correlation between leptin from one side and both CRP and ANC from the other side (Table 3). As regard ability of serum leptin levels to predict neonatal sepsis, it had a good sensitivity as denoted by the area under curve (0.89), the best cut-off value was selected to be 2.5 (according to simultaneous best sensitivity and specificity). At this cut-off value, sensitivity was 98% and specificity was 74% (Table 4, Fig. 1).

Table 1: Demographic data of case and control groups

Groups	Case (n = 50)	Control (n = 30)	p-value
Sex			
Male	31 (62.0%)	19 (63.3%)	0.91
Female	19 (38.0%)	11 (36.7%)	
Age	2.120±0.48	2.230±0.65	0.76
Gestational age	38.96±0.87	39.12±0.72	0.4
Birth weight (kg)	3.060±0.58	3.210±0.42	0.22
Delivery mode			
NVD	28 (56.0%)	14 (46.7%)	0.18
CS	22 (44.0%)	16 (53.3%)	

NVD: Natural vaginal delivery and CS: Cesarean section

Table 2: Laboratory data of case and control groups

Parameters	Case (n = 50)	Control (n = 30)	p-value
RBCs ($\times 10^6$)/cc	4.032±0.58	4.75±0.55	0.097
Hb (g dL^{-1})	13.270±1.22	13.79±1.59	0.104
TLC ($\times 10^3$)/cc	23.830±3.66	12.91±1.83	<0.001*
ANC ($\times 10^3$)/cc	11.960±6.74	7.80±6.12	<0.001*
Platelets ($\times 10^3$)/cc	288.460±39.25	301.36±94.43	0.396
Immature/total ratio	0.253±0.05	0.13±0.03	<0.001*
CRP	20.340±14.72	5.12±2.4	<0.001*
Leptin (ng dL^{-1})	8.640±4.11	3.02±0.9	<0.001*

*Significant at $p<0.05$, RBCs: Red blood cell count, Hb: Hemoglobin, TLC: Total leukocyte count, ANC: Absolute neutrophils count and CRP: C-reactive protein

Table 3: Correlation between leptin and other studied variables

	Leptin	
Parameters	r	p
CRP	0.600	0.000*
RBCs	-0.006	0.964
Hb	0.004	0.979
TLC	0.206	0.151
Platelets	-0.195	0.175
ANC	0.341	0.015*

*Significant at $p<0.05$, CRP: C-reactive protein, RBCs: Red blood cell count, Hb: Hemoglobin, TLC: Total leukocyte count and ANC: Absolute neutrophils count

DISCUSSION

The study of leptin has provided a robust framework upon which current understanding of the mechanisms involved in energy homeostasis has been built[5]. Leptin stimulates inflammatory responses, T-lymphocyte proliferation and Th1 cytokine production, indicating that it is an important link between nutrition and the immune system. Recent studies have shown that leptin is also involved in the mediation of the systemic response to sepsis[12]. Furthermore, serum leptin is related to tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1 (IL-1) release in septic newborn infants[13]. The IL-6 increases early during infection probably stimulated by TNF. High levels of IL-6 have been observed in neonates with sepsis and measurement of IL-6 may be a sensitive diagnostic test[4]. The IL-6 and TNF induce the acute phase response that includes the increase of CRP and TNF enhances the shedding of adhesion molecules from cell membranes[14].

In this study, there was statistically significant increase in TLC, ANC and I/T ratio among cases groups, which were in agreement with the reviewed literature where I/T neutrophil ratio of ≥0.2 suggests bacterial infection. Thrombocytopenia is a non-specific indicator of infection[1].

Table 4: Sensitivity and specificity of leptin in diagnosis of neonatal sepsis

Cut-off	Sensitivity	Specificity (%)
2.00 (ng dL^{-1})	98%	66
2.25 (ng dL^{-1})	98%	70
2.50 (ng dL^{-1})	98%	74
2.75 (ng dL^{-1})	94%	76
Area under the curve	0.89	
Standard error	0.034	
CI 95%	0.82-0.96	

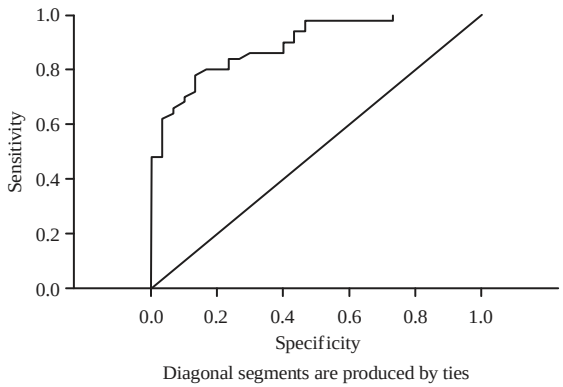

Fig. 1: ROC curve for sensitivity and specificity of leptin in diagnosis of neonatal sepsis

On the other hand, in this study there was no statistically significant difference as regard Hb level or platelet count between cases and control groups. This disagree with Shehab El-Din *et al.*[10] who found that there was statistically significant difference in Hb level and platelet count between cases and control groups.

The difference in results might be due to the difference in timing of sampling which was earlier in this study (within the first 24 h of sepsis suspicion). Anemia and decreased platelet count is usually a late sign and is very non-specific[15].

In this study, there was a significant difference in CRP level between cases and control groups. This comes in agreement with the results of Hisamuddin *et al.*[2].

In this study, it was found that leptin level was significantly higher in cases group compared to the control group. This comes in agreement with the study of Saleh *et al.*[13] whose study included 14 cases diagnosed as neonatal sepsis and 14 healthy neonates as controls, serum leptin was measured as soon as diagnosis of neonatal sepsis and they reported that there was a highly significant increase in serum leptin levels between septic and control neonates ($p<0.001$). Furthermore, Orbak *et al.*[16] studied 16 neonates with bacterial septicemia and 15 controls, found that serum leptin levels in newborns with septicemia were significantly higher than those of control group ($p<0.01$).

In contrast, Cesur *et al.*[17] investigated the prognostic value of leptin and CRP levels in newborn sepsis. A total of 57 newborns with nosocomial sepsis and 30 healthy newborns were included to the study. Initial leptin levels were found to be high in the control group ($p = 0.00$) and CRP levels were found to be high in the patient group ($p = 0.00$). However, on the 5th day of the therapy CRP ($p = 0.023$) and leptin ($p = 0.00$) levels were significantly high in the patient group. Also, Koc *et al.*[18] whose study included 20 newborns with neonatal sepsis and 15 healthy newborns, they demonstrated that there was non-significant difference in serum leptin levels between septic and controls (0.47 vs 0.37 ng mL^{-1}, $p>0.05$). However, this study was limited to early onset sepsis, while in the study of Koc *et al.*[18], EOS and LOS were considered in addition to the difference in sample size, technique of sampling and the racial difference.

On the other hand, many studies included adult septic patients demonstrated the significance of leptin association with septicemia. Chen *et al.*[19] in a retrospective study conducted on 331 patients from an intensive care unit. Septic patients displayed significantly higher serum leptin concentrations compared with those of the non-sepsis group (mean concentration, 11.67 vs 4.824 mg dL^{-1}, $p<0.001$).

Such variation reflects the variable role of leptin in the process of infection and sepsis and probably points to the influence of other factors affecting serum leptin level.

In this study, it was also found that there was no correlation between serum leptin levels in cases group and birth weight. These results disagree with the study of Christou *et al.*[20] who found that body weight had a positive correlation to leptin levels in in 142 cord blood samples from full-term deliveries.

This study reported positive correlation between serum leptin levels and CRP. This comes in agreement with the study of Cesur *et al.*[17]. The positive correlation between leptin level and CRP could imply that leptin stimulates proliferation of lymphohematopoietic cells and phagocytic activity of macrophages[16].

In this study, there were no correlations between serum leptin level and all other hematological parameters (Hb level, TLC, platelets count). On the other hand, these results disagree with the study of Somech *et al.*[21] who found that there were positive correlation between serum leptin and TLC. This difference may be due to the difference in timing of sampling as this study aimed to measure leptin level very early which was not considered in the other study.

Several factors have been suggested to influence the serum leptin level in cord blood and early neonatal period. The relation between these factors and serum leptin has shown variable results. These factors include gender of the patients[22], mode of delivery, duration of labor[23], maternal weight[24], neonatal anthropometric measures[25]. The effect of those factors on serum leptin level in the present study might be a limitation of the study.

CONCLUSION AND RECOMMENDATION

This study revealed that serum leptin level was elevated early in cases of EOS and it seems that it may have a role in its early diagnosis at cut-off point 2.5 ng dL^{-1} with sensitivity 98% and specificity 74%.

Measurement of serum leptin level early on suspicion of EOS may aid in confirming the diagnosis. However, large prospective multi-centre studies should be done to confirm the association between early high serum leptin level and EOS.

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