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The Evaluation of Serum Procalcitonin Levels in Neonatal Infections

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Abstract

Background
Procalcitonin is known as one of the bacteremia and sepsis markers such as cytokines, interleukin and reactive proteins. This study was conducted to determine the procalcitonin levels in neonatal sepsis.

Materials and Methods
In a cross-sectional study, the serum procalcitonin levels in 50 term newborns with suspected sepsis was compared with 50 healthy newborns in Ghaem Hospital, Mashhad-Iran, from 2013 to 2015. The newborns were divided in two groups of clinical infection [two or more clinical symptoms (such as lethargy, apnea, respiratory distress, restlessness, seizures, need for mechanical ventilation, abdominal distention, hypotension and oral intolerance) as well as two positive laboratory symptoms], and control group (less than 2 clinical symptoms improved within 24 hours or healthy term newborns). Finally, the serum procalcitonin levels was compared with CBC, ESR and CRP. The results were analyzed using SPSS version 21.0.

Results
The mean leukocyte count and neutrophil percentage in clinical sepsis newborns were higher than in healthy newborns (P<0.001). The mean procalcitonin levels in highly suspected sepsis and control groups were 0.14±0.03 and 0.07±0.02, respectively (P=0.002). The Sensitivity, specificity, positive and negative predictive values of procalcitonin in all patients were 92, 89, 85 and 83 percent, respectively.

Conclusion
In current study, the serum procalcitonin levels in newborns with clinical sepsis were about twice the normal newborns. Therefore, it seems that this marker can be helpful in early diagnosis of neonatal infections.

Key Words: Neonatal sepsis, Newborn, Procalcitonin.

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1- INTRODUCTION

Neonatal sepsis is a serious disease associated with high mortality. Due to non-specific symptoms as well as the absence of a definitive early diagnostic test, it is a major challenge for pediatrician. Cultivation of body fluids, especially blood and cerebrospinal fluid is a golden standard for the diagnosis of neonatal infection. But it takes 48-72 hours to determine the culture results (1, 2). Therefore it was recommended that all symptomatic newborns are examined in terms of infection to receive empirical antimicrobial therapy. The majority of newborns without sepsis have received at least three to five days of antibiotic treatment (2, 3).

Neonatal sepsis increases the length of stay and cost of treatment particularly in developing countries with limited neonatal intensive care facilities and insufficient space and personnel leading to problems for families, community and health system. However, definitive diagnosis is confirmed by blood culture which is a time-consuming method. On the other hand, neonatal clinical symptoms such as reduced primitive reflexes, bradycardia, hypothermia, hypotonia, seizures, respiratory distress and so on lack sufficient sensitivity for definitive diagnosis of neonatal sepsis. Furthermore, laboratory findings including laboratory and immunological tests and CBC have less sensitivity and specificity than the blood culture.

For this reason, researchers tested a number of other biochemical markers for accurate diagnosis of sepsis in the shortest time (1). In the last two decades, some biochemical markers such as interleukin and calcitonin have received much attention (1, 4, 5). Procalcitonin is a kind of prohormone which is produced by C cells of the thyroid gland. According to literature, procalcitonin in the blood increases in sepsis and is associated with sepsis severity. Therefore, procalcitonin is known as one of the bacteremia and sepsis markers such as cytokines, interleukin and reactive proteins. The aim of this study was to determine the diagnostic value of procalcitonin in the diagnosis of neonatal infection.

2- MATERIALS AND METHODS

2-1. Study design and population

In a cross-sectional study, the diagnostic value of procalcitonin in the diagnosis of neonatal infections was examined and compared with other diagnostic criteria. One hundred newborns were enrolled in the study. Of these, 50 were suspected of infection (case group) and 50 were healthy newborns (control group).

2-2. Methods

Demographic data including birth weight, recent weight, age, gender, Apgar score, clinical symptoms and laboratory results, were recorded in a questionnaire by pediatric residents. Newborns with suspected sepsis were identified on the basis of clinical and laboratory symptoms. Indicative Clinical symptoms of sepsis include lethargy, apnea, respiratory distress, restlessness, seizures, need for mechanical ventilation, abdominal distention, hypotension and oral intolerance.

Indicative Laboratory symptoms of sepsis include leukocytosis (WBC $\geq$ 20,000 or leukopenia $\leq$ 5,000), thrombocytopenia (PLT $\leq$ 150,000), and positive CRP ($\geq$6 mg / dl). Newborns with clinical sepsis should have more than two clinical symptoms with two positive laboratory symptoms along with positive or negative blood cultures (1, 3). The control group consists of healthy term newborns and 3-5 day newborns referred for thyroid screening or due to neonatal jaundice.

2-3. Laboratory measurements

Blood culture, CBC, platelet count, and procalcitonin levels test were performed for all newborns enrolled in the study. Blood samples were centrifuged for 30 min after sampling for measuring procalcitonin levels. The serum was stored at a temperature below -20 ° C. Procalcitonin levels was measured by an immunometric method using 2 ml of serum within 20 min.

2-4. Ethical consideration

After the approval of the study by the Ethical Committee of Mashhad University of Medical Sciences, parental consent was obtained for each infant.

2-5. Inclusion and exclusion criteria

Inclusion criteria were: 2-28 day term newborns suspected of neonatal infection and exclusion criteria included an Apgar score below 7 at five minutes, congenital malformations, congenital TORCH infections [Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections], insufficient blood sample, newborns with underlying diseases (cardiovascular, renal, gastrointestinal, etc.), and those who received antibiotics before taking a blood sample.

2-6. Data Analyses

Data analysis was performed using SPSS version 16.0. A significance level of less than 5% was considered. In this study, statistical tests including Mann-Whitney, student t-test and Chi-square and logistic regression tests were used to evaluate the diagnostic value, sensitivity and specificity of procalciton in differentiating healthy and sick newborns and for the diagnosis of neonatal infection.

The Receiver operating characteristic (ROC) curve was plotted for estimating procalcitonin borderlines. P-value less than 0.05 was considered statistically significant.

3- RESULTS

In this study, 31.8% of newborns were female and 68% were male; of these, 49 percent were born by normal vaginal delivery and 51% by caesarean section. The average age of newborns was 7.8 ± 6.7 days with an average weight of 3,067 ±0. 422 grams. The maternal age was 26.4±5 years with a parity of 1.43 ± 0.7 and an Apgar score of 7.8±0.7.

The newborn's temperature at admission was 37.9 ± 0.6 °C with a heart rate of 125.9 ± 19.6 bits per minute and a respiratory rate of 44.1±16.01 per minute. The most common clinical symptoms in infected newborns include poor feeding (27%), fever, seizures and respiratory distress (10%), lethargy (9%), apnea (8%), weakness (7%), vomiting (5%), jaundice (4%) and agitation (2%) (**Figure.1**).

The average white blood cells were 12,866±5,869/mcl and the mean percentage of neutrophils was 46.5 ± 13.9. The average platelet count was 191,115 ± 42,854/mcl. The average procalcitonin levels were0.08±0.06 ng/dl. The mean serum ESR level for newborns in the study group was about 7.5±5.2 mm per hour. 12% of newborns in the study group had a negative CRP, 32.7% showed a CRP+, 28.6 percent showed CRP++ and 26.5 showed CRP+++.

The mean age of newborns with high clinical suspicion of sepsis was 10.6± 1.5 days. The mean age of newborns in the control group was 11.5±1.6 days (P =0.715). Platelet count was not significantly different in newborns with high clinical suspicion of sepsis and in controls (P =0.987).

The number of white blood cells in newborns with high clinical suspicion of sepsis was significantly higher than that in the control group (P=0.000). The percentage of neutrophils was significantly different in newborns with high clinical suspicion of sepsis and in controls

(P =0.000). The mean procalcitonin levels in newborns with high clinical suspicion of sepsis and in control were 0.14±0.03 and 0.07± 0.02, respectively showing a statistically significant difference (P=0.002). The procalcitonin levels in both gender with high clinical suspicion of sepsis was not significantly different (P =0.984).

The mean procalcitonin levels in newborns older than 7 days was not significantly different from newborns younger than 7 days (P =0.402). CRP was significantly different in newborns with high clinical suspicion of sepsis and in controls (P=0.000). Sensitivity, specificity, positive and negative predictive values of procalcitonin in patients was 92, 89, 85 and 83 percent, respectively. Sensitivity, specificity, positive and negative predictive values of procalcitonin in patients with positive CRP was 93, 90, 89 and 89 percent, respectively.

Sensitivity, specificity, positive and negative predictive values of procalcitonin in patients with CRP+++ was 100, 86, 86 and 89 percent, respectively.

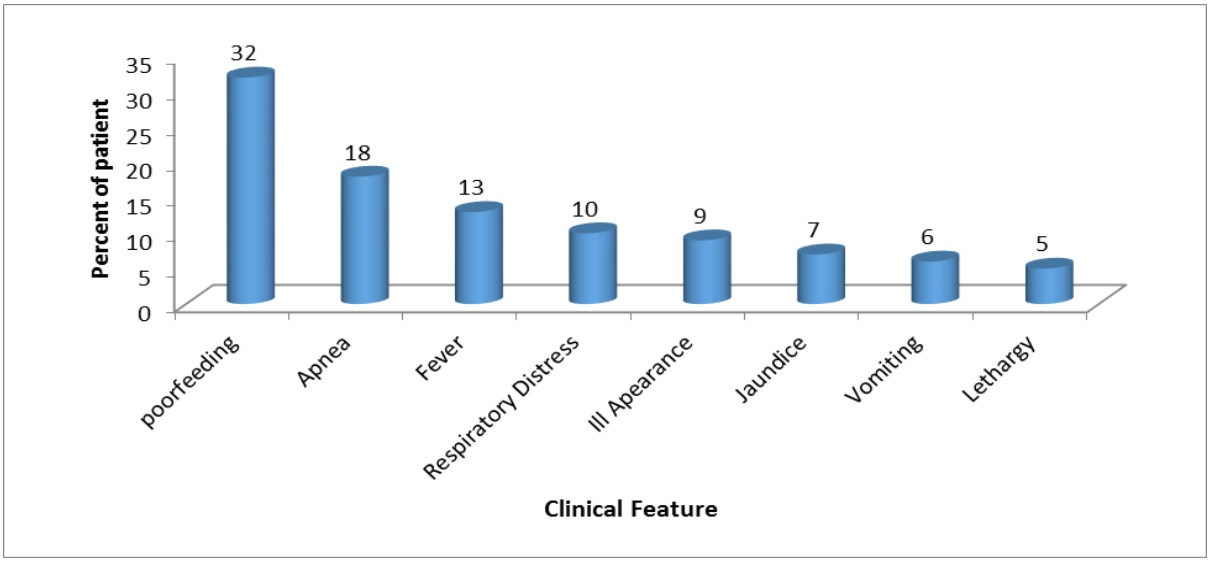

Fig1: The percentage of clinical sign and symptoms in newborn

Table-1: Comparison Laboratory characteristic between newborns with high clinical suspicion of infection and control group

Variables	Mean ± SD		P- value
	Control	Clinical infection	
White blood cells (/mcl)	9973±5503	4640±17022±	0.000
Neutrophils (%)	38.1±4.9	65.3±7.9	0.000
Platelet count (/mcl)	178465±37187	199733±41613	0.987
Procalcitonin (ng/dl)	0.07±0.02	0.14±0.03	0.002
ESR(mm/h)	7.9±6.2	9.4±5.6	0.678

ESR: Erythrocyte Sedimentation Rate; SD: Standard Deviation.

4- DISCUSSION

In this study, the number of white blood cells and neutrophil percentage in clinical infections were significantly higher than those in the control group (P=0.000). But, leukocytosis increased in most neonatal patients nonspecifically and is not specific to infection. In the present study, 88% of newborns in the study group had a positive CRP. The positive CRP cases in the clinical infection group were significantly more than those in the control group (P=0.000). However, increased acute phase reactants (APRs) cannot differentiate infectious and non-infectious causes of inflammation (6).

The results of a systematic review showed that there is not sufficient evidence supporting the CRP as a factor in neonatal infection (7). Acute phase reactants are proteins produced by hepatocytes in response to inflammation. Inflammation can be secondary to infection, trauma or destruction process. There is a variety of APRs including C reactive protein (CRP), fibronectin, complement 3, serum amyloid A (SAA), Lipopolysaccharide bonding protein (LBP), alpha-1-acid glycoprotein (AGP) and alpha-1 antitrypsin.

Progress was made in the determination of APRs with the development of quantitative and automatic rapid immunoassay techniques. In several studies, APRs have been studied as an early indicator of septicemia (6). CRP is one of the markers used for the diagnosis of neonatal sepsis. In some studies, the sensitivity, specificity, and positive and negative predictive value of CPR were 50%, 97%, 67% and 94%, respectively (18). On the other hand, decrease in CRP concentration was observed in the first 12-24 hours of life in newborns with sepsis, especially in those infected with Group B streptococcus (GBS). Increased levels of CRP can be identified usually within 6-18 hours so that maximum CRP level is observed within 8-60 hours from the onset of infectious process (8). The mean serum ESR levels in newborns with clinical infections and in controls were 9.4±5.6 and 7.9±6.2 ml/hr, respectively (P =0.687). In a study conducted by Imani in Shahr-e Kord, ESR, PLT, CRP, C3 and WBC during sepsis were significantly higher than those in the pre and post-sepsis phases (P<0.05); in this study given the significant differences between ESR, CRP and WBC during sepsis in comparison with the beginning and the end of sepsis reveal that these tests may be used as simple and inexpensive tests instead of PCT and C3 tests for the diagnosis of sepsis in intensive care unit (9). In the present study, the mean procalcitonin levels in newborns with clinical sepsis were about twice the normal newborns. Procalcitonin level is not affected by age and gender of newborns. Procalcitonin levels in this study, was lower than other studies which can be attributed to the absence of positive culture (10). The results of a study on Iranian newborns with suspected sepsis showed that procalcitonin levels less than 5.0 ng/ml, between 0.5 and 2 ng/ml, between 2 and 10 ng/ml and higher than 10 ng/ml are respectively associated with systemic inflammatory response syndrome (SIRS) sepsis, septic syndrome and septic shock (10); no case of septic shock or syndrome was observed in this study.

In another study, the serum procalcitonin levels in newborns with hyaline membrane disease, sepsis and pneumonia were 0.19±0.1, 0.6 ± 1.77 and 2.22±0.7 ng/ml, respectively (P<0.01). In the present study, patients were divided into two groups. Some were suspected of sepsis or improved within 24 hours after the onset of symptoms and some were healthy term newborns. The change in the procalcitonin levels in patients with sepsis symptoms may be a more reliable index in neonates. Therefore, determining the serum procalcitonin levels before and after the onset of symptoms, may be more helpful

in identifying patients, but is more expensive. Procalcitonin is a polypeptide procalcitonin precursor and its secretion is increased during bacterial infection. PCT increase within 2 hours of infection is more important than next CPR rise. It is clear that PCT has a diagnostic application similar to CRP and Interleukin-6 (IL – 6). PCT is the more sensitive index for early diagnosis of neonatal sepsis in the first 12 hours of life. Nevertheless, PCT with a cut-off point of 6ng/ml has a moderate sensitivity and specificity (77% and 91%, respectively). Procalcitonin is a kind of prohormone which is produced by the thyroid C cells. According to literature, procalcitonin in sepsis increases in the blood and is associated with sepsis severity. Therefore, procalcitonin is known as a bacteremia and sepsis marker such as cytokines, interleukin and reactive protein. It has been suggested that PCT is increasingly known as a good marker for bacterial infections and sepsis and is considered as an important tool in clinical studies (11, 12).

PCT level is low in viral infections, chronic inflammatory disorders or autoimmune diseases. PCT level in sepsis is usually above 0.5-2 ng/ml and often reaches 10-100 ng/ml. PCL level in some patients is much higher than above levels leading to differential diagnosis between various clinical conditions and an acute bacterial infection (sepsis) (11). PCT concentration increases approximately 3 hours after bacterial infection and reaches its maximum level within 6-12 hours. The half-life is about 24 hours in the body and is stable in laboratory environment. It does not require any sample preparation and special storage conditions (13). In this study, sensitivity, specificity, positive and negative predictive values of procalcitonin in patients were 92, 89, 85 and 83 percent, respectively. Sensitivity, specificity, positive and negative predictive values of procalcitonin in patients with positive CRP was 93, 90, 89 and 89 percent, respectively. Sensitivity, specificity, positive and negative predictive values of procalcitonin in patients with CRP+++ was 100, 86, 86 and 89 percent, respectively. In our study, the highest sensitivity of procalcitonin was found in patients with CRP+++. In another study, sensitivity, specificity and positive predictive value was 100%, 65%, 67%, respectively. A cut-off point of 0.6ng/ml can increase the sensitivity to 100 percent (19). In another study, sensitivity, specificity, and positive and negative predictive values of PCT was 87.5%, 98.7%, 87.5%, and 98.7%, respectively. The corresponding values for CRP was 50%, 97%, 67% and 94%, respectively. This study showed that the cord PCT measurement can be effective in the diagnosis of neonatal sepsis (14). In a study by Charles et al. in France, the PCT cut-off point of 0.44 ng/ml showed a sensitivity and specificity of 62.5 and 83%, respectively (15).

In a study by Lopez in Spain, procalcitonin levels until 48 hours after the confirmation of the diagnosis of infection in newborns were significantly higher than other newborns. A procalcitonin level of 0.59 ng/ml on admission showed a sensitivity of 81.4%, and a specificity of 80.6%. A procalcitonin level of 1.34 ng/ml in the first 24 hours showed a sensitivity of 73%, and a specificity of 80%. A procalcitonin level of 0.69 ng/ml within 36 to 48 hours showed a sensitivity of 86.5% and a specificity of 72.7% (16). According to a study by Kordek in Poland, a PCT level of 1.22 ng/ml showed a sensitivity of 80.4%, a specificity of 71.6%, a negative and positive predictive value of 35.4% and 95.03%, respectively. This study showed that the use of laboratory tests including the number of neutrophils, red blood cells, PCT, CRP and Apgar can be effective in faster and more accurate diagnosis of neonatal sepsis (17). In this study, the combined results of PCT with CRP

improved diagnostic accuracy of PCT. It seems that a cut-off point of 0.6 ng/ml shows the highest diagnostic accuracy in neonates with sepsis. But the cut-off point was lower in our study due to lack of proven neonatal sepsis with blood cultures. In general, the use of procalcitonin levels or cut-off point along with various clinical and laboratory symptoms will improve the accuracy of this test. Our results showed that CRP improves the predictive value of this test. Due to the relatively high cost of both tests, given the clinical and laboratory conditions of newborns and existing facilities, the cost and effectiveness of tests should be investigated to take appropriate measures.

4-1. Limitations of the study

The main limitation of our study was the absence of definitive infection in our infants. The studies with larger sample size that compared PCT between healthy infants and definitive sepsis recommended.

5- CONCLUSION

Laboratory tests including leukocyte count, neutrophil count, PCT and CRP can be effective in faster and more accurate identification of newborns with clinical sepsis.

6- ABBREVIATION

CBC: Complete blood count,

ESR: Erythrocyte sedimentation rate,

CRP: C - reactive protein,

CMV: Cytomegalovirus,

ROC: Receiver operating characteristic,

APR: Acute phase reactant,

SIRS: systemic inflammatory response syndrome,

WBC: White blood cell,

PLT: Platelet,

PCT: Procalcitonin.

7- CONFLICT OF INTEREST: None.

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