

Procalcitonin and C- Reactive Protein as Diagnostic Markers of Neonatal Sepsis

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Abstract: Neonatal sepsis is a serious problem associated with significant neonatal morbidity and mortality. Diagnosis of neonatal sepsis may be difficult because the clinical presentations are often non-specific, bacterial cultures are time-consuming and other laboratory tests lack sensitivity and specificity. This study aimed to evaluate procalcitonin (PCT) and C-reactive protein (CRP) as diagnostic markers of neonatal sepsis. Sixty neonates with suspected neonatal sepsis were recruited from the neonatal intensive care unit (NICU), Sohag university hospital, Egypt. They were allocated into two groups; early-onset neonatal sepsis (EONS; n=32) and late-onset neonatal sepsis (LONS; n=28). Blood samples were obtained from the participants for complete blood count (CBC), blood cultures, serum CRP and PCT analysis. Area under the receiver operating characteristic (ROC) curve (AUC), predictive values and diagnostic cut off values of CRP and PCT were evaluated. In total, 42 (70%) neonates were confirmed to have sepsis based on positive blood culture results. Serum levels of CRP and PCT were significantly higher in neonates with EONS than those with LONS ($p < 0.05$). However, there was no significant statistical difference between the area under the curve (AUC) values of PCT and CRP in all studied cases, EONS or LONS cases ($p=0.32$, $p=0.29$, $p=0.28$ respectively). In conclusion, PCT and CRP are reliable diagnostic markers of neonatal sepsis, which have the same diagnostic accuracy. CRP; being easily measurable and more affordable can be conveniently used as a good marker for the diagnosis of neonatal sepsis, especially in developing communities with poor resources.

Key words: Neonatal sepsis; Procalcitonin; C-reactive protein

INTRODUCTION

Neonatal sepsis is one of the important causes of neonatal morbidity and mortality particularly in the developing countries (Osrin *et al.*, 2004). Neonatal sepsis is classified into early or late according to the different ages at onset of infection during the neonatal period (Stoll, 2011). The clinical relevance of this distinction is that early-onset disease is often due to organisms acquired during delivery while, late-onset disease is more frequently caused by organisms acquired from nosocomial or community sources (Robinson *et al.*, 2008).

The diagnosis of neonatal sepsis is difficult, because clinical signs of sepsis often overlap with other non-infectious causes of systemic inflammation (Baruti-Gafurri *et al.*, 2010). Although, microbiological culture can be used to distinguish sepsis from non-infectious conditions, this method lacks sensitivity and specificity, and often there is a substantial time delay (Afroza, 2006). Inflammatory markers including C- reactive protein, serum amyloid A, interleukins (IL-6, IL-8) and procalcitonin were evaluated as markers for neonatal sepsis with varying success. Although, these markers aid in the diagnosis, no single laboratory test has provided rapid and reliable identification of early infection and the best prediction is obtained using a combination of markers (Abdollahi *et al.*, 2012).

C- reactive protein (CRP) has been widely used as a diagnostic tool for infection identification (Povoa *et al.*, 1998 and Khoshdel *et al.*, 2008). Although, a high level of CRP was shown to be a sensitive and classical marker of inflammation, it can not differentiate between bacterial infections and other infections (Jaye and Waites, 1997). Moreover, non-infectious conditions, as perinatal asphyxia, respiratory distress syndrome, meconium aspiration syndrome and post surgical period can induce abnormal values of CRP (Zahedpasha *et al.*, 2009).

Procalcitonin (PCT) is an acute phase protein which has been reported as a measurable laboratory marker in inflammatory response that increases during bacterial, fungal, and parasitic infections (Reinhart *et al.*, 2000). In contrast to CRP, localized bacterial infections, severe viral infections and inflammatory reactions of non-infectious origin do not or only slightly change the PCT level (Eberhard *et al.*, 1997). The data about the diagnostic value of PCT is controversial. While some studies reported that PCT is more reliable than CRP for the diagnosis of neonatal sepsis (Guibourdenche *et al.*, 2002, Chiesa *et al.*, 2003 Joram *et al.*, 2006 and Naher *et al.*, 2011), others did not find any advantage of PCT over CRP (Franz *et al.*, 1999, Blommendahl *et al.*, 2002, Koskenvuo *et al.*, 2003 and Pérez Solis *et al.*, 2006). Thus, the aim of this study was to evaluate CRP and PCT as diagnostic markers for neonatal sepsis.

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Patients and Methods:

Study Design and Patients:

This cross sectional study was conducted over a 6-month period from September 2011 to February 2012 at the neonatal intensive care unit (NICU), Sohag university hospital in collaboration with departments of Medical Microbiology & Immunology and Clinical Pathology, Sohag University hospital, Egypt. Sixty neonates who were admitted at NICU with signs suggestive for sepsis or who developed signs of sepsis while in the ward were enrolled into the study. Neonatal sepsis was suspected according to the international criteria for bacterial sepsis (Vergnano *et al.*, 2005 and Stoll, 2011). The exclusion criteria were premature neonates, neonates to mothers with gestational diabetes and current antibiotic therapy. Written informed consents were obtained from parents for participation of their neonates in the study.

The eligible neonates were allocated into two groups; those with sepsis that developed within the first 7 days of life; early-onset sepsis group (EONS; n=32) and those with sepsis that developed after 7 days of life; late-onset sepsis (LONS; n=28).

Detailed history was obtained from the parents and a complete physical examination was undergone. Under complete aseptic conditions, blood samples were obtained from the studied patients for the following laboratory investigations: complete blood count (CBC), differential leucocytic count, blood cultures, serum CRP and PCT analysis.

Blood Culture:

Blood cultures were examined using the BACTECT™ 9050 automation system (Becton Dickinson, Ireland). The blood samples were inoculated into BACTEC Peds Plus/F blood culture bottles and were placed in the BACTECT™ 9050 blood culture instrument within 2 hours of collection. Positive cultures were detected by fluorescent sensors sensitive to an increase in carbon dioxide produced by growth of the organisms. Cases with positive blood cultures were considered as confirmed sepsis. Then, the cultures were centrifuged in serum separator vacutainer tubes (SSI) at 3000 RPM for 20 minutes and the deposit was subjected to subcultures onto blood agar plates and smears stained with Gram stain. Identification of the growing microorganisms was evaluated with automated Siemens Micro-Scan WalkAway®-96 plus system (USA).

CRP & PCT Assays:

Patients' blood samples were collected in SST, allowed to clot for 30 minutes then centrifuged at 3000 RPM for 15 minutes. Serum was separated and divided into 2 parts, one is tested immediately for CRP and the other stored at -80° C till PCT analysis.

Serum CRP level measurement was tested by the semi-quantitative latex agglutination test (Omega Diagnostics kits, UK) according to the manufacturer's guide.

Serum procalcitonin was measured quantitatively by ELISA technique (Enzyme-Linked Immunosorbent Assay) using RayBio® Human Procalcitonin ELISA kit (RayBiotech Company; USA). This assay employs an antibody specific for human procalcitonin coated on a 96-well plate.

The concentration of the provided standard was 55 ng/ml. Seven levels of dilution were done to gain the standard curve. Standards and samples were pipetted into the wells containing immobilized procalcitonin antibodies. The wells were washed and biotinylated anti-human procalcitonin antibody was then added. After washing away unbound biotinylated antibody: horse radish peroxidase (HRP)-conjugated streptavidin was added. Another wash was done then a tetramethylbenzidine (TMB) substrate solution was added. A color developed in proportion to the amount of procalcitonin bounded to antibodies. The stop Solution changed the color from blue to yellow, and the intensity of color was measured at 450 nm. These steps were performed by the fully automated Behring ELISA Processor III (BEP III) and the results were calculated automatically after the standard curve was gained by the internal software. Serum procalcitonin levels were measured in duplicates and the mean value of every sample is considered the final result.

Statistical Analysis:

Statistical analysis was performed using MedCalc® for Windows (version 11.0) and Statistica (STATA; version 9.0). The blood culture results were used as the gold standard, the sensitivity, specificity, positive (PPV) and negative predictive values (NPV) of the PCT and CRP for diagnosing sepsis were calculated using the receiver operating characteristic (ROC) curve. The diagnostic accuracy of PCT and CRP was expressed as the area under the ROC curve (AUC). Differences between groups were assessed by student t-test for normally distributed data and Mann-Whitney U test for data with uneven distribution. A p value <0.05 was considered significant for all statistical tests.

Results:

The study group included 60 neonates with suspected neonatal sepsis, 32 females (53.3%) and 28 males (46.7%). The mean age of the participants was 9.7±7.1 days and the mean weight was 2.38± 0.53 Kg. Forty two

patients (70%) were proved to have sepsis by positive blood cultures. The identified bacteria included Gram positive cocci; *Staphylococcus epidermidis* (n=10), *Staphylococcus aureus* (n=8), *Streptococci agalacti* (n=6), and Gram negative bacilli; *Escherichia coli* (n=8), *Klebsiella pneumoniae* (n=4), *Enterobacter aerogens* (n=3), *Pseudomonas aeruginosa* (n=3).

When newborns with early-onset neonatal sepsis (age range: 1-6 days) were compared with those with late-onset neonatal sepsis (age range: 9-28 days), there was no significant difference regarding the gender, mean weight, clinical and laboratory findings. On the other hand, mean serum CRP and PCT values were significantly higher in EONS than those with LONS ($p=0.009$ and $p=0.002$ respectively) (Table 1).

Table 1: Laboratory and clinical findings of the studied patients.

Characteristic	EONS (n=32)	LONS (n=28)	P value
Age (days)	4.2 ±1.6	16 ± 5.3	<0.0001
Gender			
Female	16 (50%)	16 (57.1%)	0.58
Male	16 (50%)	12 (42.9%)	
Weight (Kg)	2.5 ±0.5	2.3 ±0.5	0.25
Clinical manifestations			
General	25 (78.1%)	22 (78.6%)	0.38
Respiratory	21 (65.6%)	24 (85.7%)	
Cardiovascular	12 (37.5%)	13 (46.4%)	
Neurologic	26 (81.3%)	20 (71.4%)	
Gastrointestinal	24 (75%)	23 (82.1%)	
Laboratory findings			
a- Complete blood count			0.42
Leucocytosis ($> 20 \times 10^9/L$)	14 (43.8%)	13 (46.4%)	
Leucopenia ($< 5 \times 10^9/L$)	7 (21.9%)	6 (21.4%)	
Thrombocytopenia ($< 150 \times 10^9/L$)	12 (37.5%)	10 (35.7%)	
b- Blood culture			0.02
Negative culture	6 (18.8%)	12 (42.9%)	
Positive culture	26 (81.2%)	16 (57.1%)	
c- Serum Procalcitonin (PCT) (ng/ml)	3.1 ± 2.0	1.8 ±1.0	0.002
d- Serum C-reactive protein (CRP) (mg/dl)	40.5 ±30.4	21.86 ±14.9	0.009

Variables are expressed as Mean ± Standard deviation, or number (percentage).

The optimum diagnostic cutoff values, AUC values, sensitivity, specificity, PPV and NPV of PCT and CRP in the different groups were shown in Table (2). No significant statistical difference was found between the AUC values of PCT and CRP in all studied cases, EONS or LONS cases ($p=0.32$, $p=0.29$, $p=0.28$ respectively) (Figures; 1-3).

Table 2: AUC, sensitivity, specificity, PPV, and NPV of PCT and CRP at optimum diagnostic cut-off values for neonatal sepsis.

Cut-off value	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV
All cases (n=60)					
PCT >1.3 ng/ml	0.92 (0.81 - 0.97)	95.24	77.78	90.90	87.50
CRP >12 mg/dl	0.88 (0.77 - 0.95)	80.95	77.78	89.50	63.60
Early onset NS (n=42)					
PCT >2 ng/ml	0.94 (0.79 - 0.99)	76.92	100.00	100.00	50.00
CRP >6 mg/dl	0.91 (0.76 - 0.98)	100.00	66.67	92.90	100.00
Late onset NS (n=28)					
PCT >1.3 ng/ml	0.90 (0.72 - 0.98)	100.00	83.33	88.90	100.00
CRP >12 mg/dl	0.79 (0.60 - 0.92)	62.50	83.33	83.30	62.50

PCT: procalcitonin, CRP: C-reactive protein, AUC: area under the curve, PPV: positive predictive value, NPV: negative predictive value.

Discussion:

Despite major advances in the field of neonatology, bacterial sepsis is still one of the most important causes of neonatal morbidity and mortality worldwide, especially in developing countries (Osrin *et al.*, 2004). Definitive diagnosis of neonatal sepsis is based on blood culture which takes at least 24 to 48 hrs and is often falsely negative. This leads to the over treatment of large number of neonates who present with clinical suspicion of sepsis (Naher *et al.*, 2011). Several markers of infection have been studied in critically ill neonates aiming to differentiate between sepsis and noninfectious conditions, among them are CRP and PCT (Hatherill *et al.*, 1999; Enguix *et al.*, 2001). The aim of this study was to evaluate CRP and PCT as diagnostic markers for neonatal sepsis.

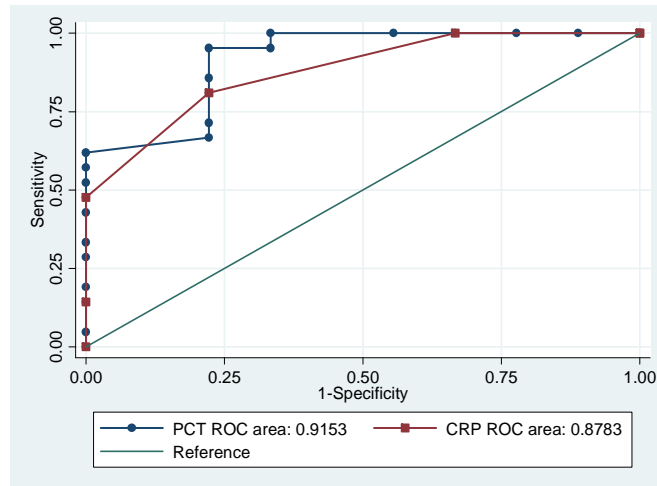


Fig. 1: ROC curve comparing the sensitivity and specificity of serum PCT and CRP in all studied patients ($p = 0.32$).

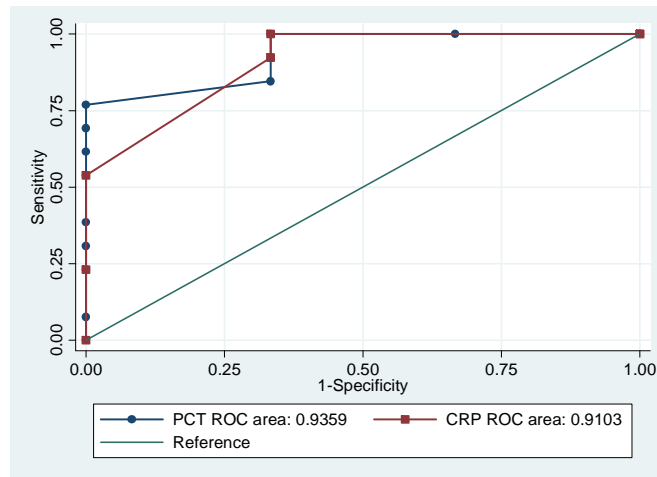


Fig. 2: ROC curve comparing the sensitivity and specificity of serum PCT and CRP in neonates with EONS ($p = 0.29$).

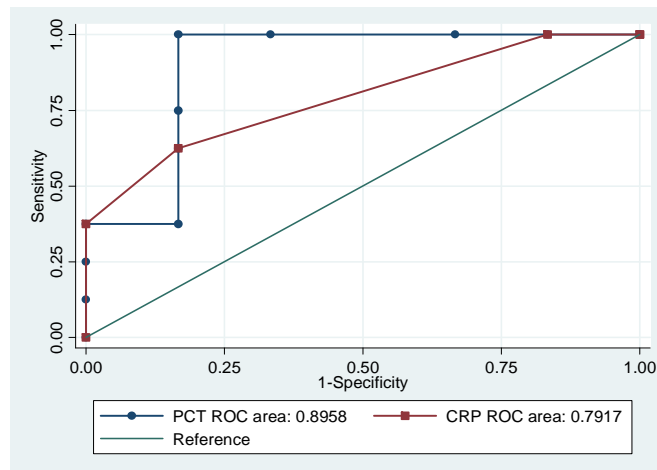


Fig. 3: ROC curve comparing the sensitivity and specificity of serum PCT and CRP in neonates with LONS ($p = 0.28$).

In this study, forty two (70%) neonates were proved to have bacterial sepsis based on positive blood culture results. Although culture-proved sepsis ranged from 20-30% of clinically suspected patients in some studies (Pavcnik-Arnol *et al.*, 2004; Naher *et al.*, 2011; Abdollahi *et al.*, 2012), Ucar and his coworkers (2008) reported a ratio of 72.2% which is consistent with our result.

Higher serum levels of PCT and CRP were observed in neonates with EONS than those in neonates with LONS with significant statistical difference. ($p=0.002$ and 0.009 respectively). Similar results were reported by Lopez-Sastre and associates (2006 and 2007) who concluded that PCT is a useful marker of bacterial sepsis of vertical transmission, but is not sufficiently reliable to be the sole marker of neonatal sepsis of nosocomial origin.

This study showed that sensitivity of PCT for the diagnosis of neonatal sepsis was higher (95%) than that of CRP (81%) using a cut off value of 1.3 ng/ml and 6 mg/L for PCT and CRP respectively. The higher sensitivity of PCT in comparison to CRP was reported by some researchers (Kawczynski & Piotrowski, 2004; Vazzalwar *et al.*, 2005; Naher *et al.*, 2011). At the same cut off values, we found that specificity of PCT is similar to that of CRP (78%). In contrast, the specificity of PCT was found to be lower than that of CRP in different studies (Janota *et al.*, 2001; Naher *et al.*, 2011), and higher in others (Vazzalwar *et al.*, 2005).

Both PCT and CRP were found to have the same PPV of 90%, while NPV of PCT was higher (87.5%) than that of CRP (63.3%). These results were consistent with those reported by some studies (Kocabas *et al.*, 2007; Naher *et al.*, 2011; Abdollahi *et al.*, 2012).

In this study, the cut off values of PCT & CRP were 1.3 ng/ml and 12 mg/l respectively. Area under the curve (AUC) values were 0.92 and 0.88 for PCT and CRP respectively without any significant statistical difference ($P=0.32$). So the two markers seemed to be equally accurate for the diagnosis of neonatal sepsis. This result was supported with the experience of Enguix *et al.* (2001) and Naher *et al.* (2011).

Among neonates with suspected early-onset sepsis, sensitivity, specificity, PPV, NPV and AUC values of PCT were 76.9%, 100%, 100%, 50% and 0.94 respectively. These values were obtained at a cut-off value of > 2 ng/ml which was found to be the most appropriate cut-off value by using ROC curves. These results were consistent with Abdollahi *et al.* (2012). However, Enguix and his colleagues (2001) reported higher sensitivity and NPV of PCT at a cut-off value of 1.7 ng/ml.

At a cut-off value of > 6 mg/L, CRP had sensitivity, specificity, PPV, NPV and AUC values of 100%, 66.7%, 92.9%, 100% and 0.91 respectively. These results were comparable to those reported by Enguix *et al.* (2001). On the other hand, Abdollahi *et al.* (2012) reported lower sensitivity and NPV and higher specificity of CRP in detecting sepsis among their study group.

However, using ROC curves for sensitivity and specificity of PCT and CRP to detect early-onset sepsis; no significant statistical differences were found between both markers ($p=0.29$), consistently with Franz *et al.* (1999) and Pérez Solis *et al.* (2006).

When evaluating neonates with suspected late-onset sepsis, we found that PCT had sensitivity, specificity, PPV, NPV and AUC values of 100%, 83.3%, 88.9%, 100% and 0.9 respectively at a cut-off point of > 1.3 ng/ml. Contrary to our result, Lopez-Sastre *et al.* (2006) reported 73.7% sensitivity of PCT to detect late-onset neonatal sepsis. Among the same group, CRP was found to have sensitivity, specificity, PPV, NPV and AUC values of 62.5%, 83.3%, 83.3%, 62.5% and 0.79 respectively at a cut off point of >12 mg/dl. Again, there was no significant statistical difference found between CRP and PCT sensitivity and specificity for detecting LONS, according to the ROC curves ($p=0.28$).

The considerable heterogeneity of the results among the studies evaluating different markers for detection of neonatal sepsis can be explained by the lack of a universally acceptable definition of neonatal sepsis, different cutoff values incorporated in the studies and physiologic alterations of some markers such as PCT that occur in healthy neonates.

Conclusion:

Procalcitonin and CRP are reliable diagnostic markers of neonatal sepsis, which have the same diagnostic accuracy. High cost of PCT precludes its clinical and routine application. Therefore, CRP; being easily measurable and more affordable, can be conveniently used as a good marker for the diagnosis of neonatal sepsis, especially in developing communities with poor resources.

REFERENCES

- Abdollahi, A., S. Shoar, F. Nayyeri, M. Shariat, 2012. Diagnostic value of simultaneous measurement of Procalcitonin, Interleukin-6 and hs-CRP in prediction of early-onset neonatal sepsis. *Mediterr J Hematol Infect Dis.*; 4; Open Journal System.
- Afroza, S., 2006. Neonatal sepsis-a global problem: an overview. *Mymensingh Med J.*, 15(1): 108-14.
- Baruti-Gafurri, Z., H. Paçarizi, B. Zhubi, *et al.*, 2010. The importance of determining procalcitonin and C-reactive protein in different stages of sepsis. *Bosnian Journal of basic medical sciences*, 10(1): 60-64.

- Blommendahl, J., M. Janas, S. Laine, A. Miettinen, P. Ashorn, 2002. Comparison of procalcitonin with CRP and differential white bloodcell count for diagnosis of culture-proven neonatal sepsis. *Scand J Infect Dis.*, 34: 620-622.
- Chiesa, C., G. Pellegrini, A. Panero, J.F. Osborn, 2003. C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. *Clin Chem.*, 49: 60-68.
- Eberhard, O.K., M. Haubitz, F.M. Brunkhorst, *et al.*, 1997. Usefulness of procalcitonin for differentiation between activity of systemic autoimmune disease (systemic lupus erythematosus/systemic anti neutrophil cytoplasmic antibody associated vasculitis) and invasive bacterial infection. *Arthritis Rheum.*, 40: 1250-56.
- Enguix, A., C. Rey, A. Concha, A. Medina, *et al.*, 2001. Comparison of procalcitonin with C-reactive protein and serum amyloid for the early diagnosis of bacterial sepsis in critically ill neonates and children. *Intensive Care Med.*, 27: 211-215.
- Franz, A.R., M. Kron, F. Pohlandt, G. Steinbach, 1999. Comparison of procalcitonin with interleukin 8, C-reactive protein and differential white blood cell count for the early diagnosis of bacterial infections in newborn infants. *Pediatr Infect Dis J.*, 18: 666-671.
- Guibourdenche, J., A. Bedu, L. Petzold, M. Marchand, *et al.*, 2002. Biochemical markers of neonatal sepsis: value of procalcitonin in the emergency setting. *Ann Clin Biochem.*, 39: 130-135.
- Hatherill, M., S.M. Tibby, K. Sykes, C. Turner, I.A. Murdoch, 1999. Diagnostic markers of infection: comparison of procalcitonin with C reactive protein and leucocyte count. *Arch Dis Child.*, 81: 417-421.
- Janota, J., Z. Stranak, S. Belohlavkova, K. Mudra, J. Simak, 2001. Postnatal increase of procalcitonin in premature newborns is enhanced by chorioamnionitis and neonatal sepsis. *Eur Clin Invest.*, 31: 978-83.
- Jaye, D.L., K.B. Waites, 1997. Clinical applications of C-reactive protein in pediatrics. *Pediatr. Infect. Dis. J.*, 16: 735-747.
- Joram, N., C. Boscher, S. Denizot, V. Loubersac, 2006. Umbilical cord blood procalcitonin and C reactive protein concentrations as markers for early diagnosis of very early onset neonatal infection. *Arch Dis Child Fetal Neonatal Ed*, 91: F65-F66.
- Kawczynski, P., A. Piotrowski, 2004. Prokalcytonina oraz bialko C-reaktywne w rozpoznawaniu posocznicy u noworodków. *Ginekol Pol.*, 75: 439-444.
- Khoshdel, A., M. Mahmoudzadeh, S. Kheiri, *et al.*, 2008. Sensitivity and specificity of procalcitonin in diagnosis of neonatal sepsis. *Iranian Journal of Pathology*, 3(4): 203-207.
- Kocabaş, E., A. Sarıkçıoğlu, N. Aksaray, G. Seydaoğlu, Y. Seyhun, A. Yaman, 2007. Role of procalcitonin, C-reactive protein, interleukin-6, interleukin-8 and tumor necrosis factor- α in the diagnosis of neonatal sepsis. *The Turkish Journal of Pediatrics*, 49: 7-20.
- Koskenvuo, M.M., K. Irjala, A. Kinnala, O. Ruuskanen, P. Kero, 2003. Value of monitoring serum procalcitonin in neonates at risk of infection. *Eur J Clin Microbiol Infect Dis.*, 22: 377-378.
- Lopez Sastre, J., D. Perez Solis, V. Roques Serradilla, *et al.*, 2006. Procalcitonin is not sufficiently reliable to be the sole marker of neonatal sepsis of nosocomial origin. *BMC Pediatric*, 6: 16.
- Lopez Sastre, J., D. Perez Solis, V. Roques Serradilla, *et al.*, 2007. Evaluation of procalcitonin for diagnosis of neonatal sepsis of vertical transmission. *BMC Pediatric*, 7: 9.
- Naher, B.S., M.A. Mannan, K. Noor, *et al.*, 2011. Role of serum procalcitonin and C-Reactive Protein in the diagnosis of neonatal sepsis. *Bangladesh Med. Res. Counc. Bull.*, 37: 40-46.
- Osrin, D., S. Vergnano, A. Costello, 2004. Serious bacterial infections in newborn infants in developing countries. *Curr. Opin. Infect. Dis.*, 17: 217-224.
- Pavcnik-Arnol, M., S. Hojker, M. Derganc, 2004. Lipopolysaccharide-binding protein in critically ill neonates and children with suspected infection: comparison with procalcitonin, interleukin-6, and C-reactive protein *Intensive Care Med.*, 30: 1454-1460.
- Pérez-Solis, D., J.B. Lopez-Sastre, G.D. Coto-Cotallo, M.A. Diéguez-Junquera, 2006. Procalcitonina para el diagnostico de sepsis neonatal de transmision vertical. *An Pediatr (Barc)*, 64: 341-348.
- Povoa, P., E. Almeida, P. Moreira, *et al.*, 1998. C-reactive protein as an indicator of sepsis. *Intensive Care Med.*, 24: 1052-1056.
- Reinhart, K., W. Karzai, M. Meisner, 2000. Procalcitonin as a marker of the systemic inflammatory response to infection. *Intensive Care Med.*, 26(9): 1193-2000.
- Robinson, D.T., P. Kumar, S.B. Cadichon, 2008. Neonatal Sepsis in the Emergency Department. *Clin Ped Emerg Med.*, 9: 160-168.
- Stoll, B., 2011. Clinical manifestations of transplacental intrauterine infection. In: Kliegman R, Stanton B, Geme J, et al.(eds). *Nelson textbook of pediatrics*; 19th ed. Elsevier, Saunders, 12, 103(6).
- Ucar, B., B. Yildiz, M.A. Aksit, C. Yazar, O. Colak, Y. Akbay and E. Colak, 2008. Serum Amyloid A, Procalcitonin, Tumor Necrosis Factor- α , and Interleukin-1 β Levels in Neonatal Late-Onset Sepsis. *Hindawi Publishing Corporation. Mediators of Inflammation*, Article ID 737141, 7 pages.

Vazzalwar, R., E. Pina-Rodrigues, B.L. Puppala, D.B. Angst, L. Schweig, 2005. Procalcitonin as a screening test for late-onset sepsis in preterm very low birth weight infants. *J Perinatol.*, 25: 397-402.

Vergnano, S., M. Sharland, P. Kazembe, *et al.*, 2005. Neonatal sepsis: an international perspective. *Arch Dis Child Fetal Neonatal Ed.*, 90: 220-224.

Zahedpasha, Y., M. Ahmadpour Kacho, M. Hajiahmadi, M. Haghshenas, 2009. Procalcitonin as a Marker of Neonatal Sepsis. *Iran J Pediatr*, 19(2): 117-122.