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Serum Neopterin in Diagnosis of Early Onset Neonatal Sepsis

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ABSTRACT
Background: The systemic reaction to infection in infants younger than one month old is known as neonatal sepsis. Neopterin is one of the immune activity molecular markers that is valuable for inflammatory diseases detection. Conditions with cell-mediated immune response are associated with an elevated serum neopterin levels.
Objective: The current study was to assess the diagnostic value of serum neopterin level and risk factors in neonatal sepsis.
Patients and methods: A case control study was conducted at Pediatrics Department of Fakous General Hospital. Case group included 24 neonates in the first 72 hours had neonatal sepsis and control group included 24 healthy neonates in the first 72 hours. Blood culture for sensitivity and measurement of serum neopterin, complete blood count and C-reactive protein were performed. **Results:** Regarding neopterin, cases had significantly increased neopterin levels than controls. **Conclusion:** In newborns with early-onset sepsis, neopterin was identified as a valuable diagnostic marker.
Keywords: Neonatal sepsis, Neopterin, CRP, Case control study, Zagazig University.

INTRODUCTION

The systemic reaction to infection in infants younger than one month old is known as neonatal sepsis that is classified as early or late neonatal sepsis [(1)].

Globally, an estimated 4 million neonatal deaths happen annually. About 36% of these fatalities are due to infections & 40% of these fatalities occur within underdeveloped nations [(2)].

Early onset sepsis (EOS) and late onset sepsis (LOS) are the 2 clinical categories of sepsis, EOS manifests within the initial 72 hours of life. In severe circumstances, the newborn may develop symptoms at birth. Infants with EOS typically demonstrate pneumonia & respiratory distress. LOS becomes apparent after 72 hours of age. LOS infections are either community- or hospital-acquired, and commonly manifest as pneumonia, meningitis, or septicemia [(3)].

Frequently, warning symptoms and signs are modest and readily confused with non-infectious reasons as hypothermia, acute exacerbation of chronic lung disease and apnea. Therefore, biochemical and hematological indicators as platelet count, immature/total neutrophil ratio, different cytokines and C reactive protein (CRP) have been recommended as effective indicators for the early detection of neonatal sepsis [(4)].

The unwarranted antibiotics utilization, along with the emergence of bacterial resistance, will likely have negative consequences for this vulnerable population of newborns. Attempts have been conducted to utilize cytokine profiles, hematologic indices, and physiologic indications at the beginning of a suspected sepsis episode in order to accurately detect the onset of sepsis [(5)].

No individual test fits the requirements that could consider it an appropriate marker for early identification of neonatal sepsis, despite substantial research. Generally, complete blood count with differential, that may be complemented by other tests as CRP are the screening components [(4)].

Neopterin, a pyrazino-pyrimidine derivative, synthesized from guanosine triphosphate along the biopterin metabolic pathway. It is generated by macrophages in response to interferon gama secreted by activated T lymphocytes. It is a valuable marker of immunological activation that can be utilized for evaluation of inflammatory disorders. In circumstances with cell-mediated immune response, an elevated the serum neopterin concentration is found [(6)].

The current study was to assess the diagnostic value of serum neopterin level and risk factors in neonatal sepsis.

PATIENTS AND METHODS

A case control study was conducted at Pediatrics Department of Fakous General Hospital. Case group included 24 neonates in the first 72 hours had neonatal sepsis and control group included 24 healthy neonates in the first 72 hours.

The exclusion criteria were intra-uterine growth retardation, preterm, perinatal asphyxia, infant of diabetic mother, severe congenital anomalies, chromosomal abnormalities, and any medications given to the neonates other than antibiotics (steroids, plasma and blood transfusion).

All neonates were underwent thorough history taking from their mothers, complete physical and clinical examination (age, weight, height and reflexes). The serum neopterin level was determined by ELISA technology at the time of diagnosis. Also, Complete blood count (RBCs, Hb, WBCs and platelets) blood cultures for sensitivity and quantitative CRP and were measured.

Ethical Consideration:

This study was ethically approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Zagazig University. Written informed consent was obtained from

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parents/guardians of all participants. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical Analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 24 for windows. Qualitative data were defined as numbers and percentages. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as mean and standard deviation (SD), and independent sample t-test was used for comparison between groups. Finally, the ROC curve and Pearson's correlation were used for data analysis. P value ≤0.05 was considered to be statistically significant.

RESULTS

Table 1 showed that the demographic data and mode of delivery were comparable between the cases and controls, whereas maternal age was significantly different between cases and controls.

Table (1): Comparison of demographic data between cases and controls.

Variable			Cases (No.= 24)	Controls (No.= 24)	t-test	P-value
Neonatal age (hrs)	**Mean ± SD**		46.21 ± 7.703	45.75 ± 8.456	0.088	0.93
GA (wks)	**Mean ± SD**		37.54 ± 1.474	37.21 ± 0.833	0.965	0.34
Birth wt (kg)	**Mean ± SD**		2.925 ± 0.4998	2.721 ± 0.3989	1.564	0.125
Sex	**Female**	**No.**	12	12	X^2 0.000	1.00
		%	50.0%	50.0%		
	Male	**No.**	12	12		
		%	50%	50%		
Maternal age	**Mean ± SD**		30.63 ± 5.102	25.83 ± 4.788	2.494	0.016*
Mode of Delivery	**CS**	**No.**	12	13	0.083	0.77
		%	50%	54.2%		
	NVD	**No.**	12	11		
		%	50%	45.8%		

Lethargy, HR beat/min, temperature and respiratory distress were significantly different between cases and controls (**Table 2**).

Table (2): Comparison of clinical manifestations and respiratory distress between cases and controls.

Variable			Cases (No.= 24)	Controls (No.= 24)	X^2	P-value
Lethargy	**No**	**No.**	10	22	13.50	0.000*
		%	41.7%	91.7%		
	yes	**No.**	14	2		
		%	58.3%	8.3%		
HR beat/min	**Mean ± SD**		128.63 ± 22.23	111.88 ± 12.026	2.385	0.021*
Temp.	**Mean ± SD**		37.44 ± 0.5089	36.88 ± 0.20	4.926	0.000*
Respiratory distress	**No**	**No.**	0	24	48	0.000*
		%	.0%	100.0%		
	yes	**No.**	24	0		
		%	100%	.0%		

RBCs and Hb were comparable between cases and controls. Cases had a significantly increased TLC, total neutrophils, CRP and neopterin and had significantly decreased platelets than controls (**Table 3**).

Table (3): Comparison of CBC, CRP and Neopterin between cases and controls

Variable		Cases (No.= 24)	Controls (No.= 24)	t-test	P-value
RBCs (10^6/uL)	**Mean ± SD**	3.86 ± 0.21	3.37 ± 0.51	1.650	0.106
Hb (g/dL)	**Mean ± SD**	11.36 ± 2.72	11.096 ± 1.86	.390	0.698
TLC (10^3/uL)	**Mean ± SD**	31.59 ± 6.39	8.94 ± 1.04	9.409	0.000*
Total Neutrophils (10^3/uL)	**Mean ± SD**	15.97 ± 2.57	4.95 ± 0.95	9.154	0.000*
Platelets (10^3/uL)	**Mean ± SD**	133.17 ± 20.31	171.25 ± 22.20	-1.728-	0.041*
Variable		**Cases (No.= 24)**	**Controls (No.= 24)**	**t-test**	**P-value**
CRP (mg/L)	**Mean ± SD**	64.79 ± 12.7958	0.683 ± 0.04	7.337	0.000*
Neoptrin	**Mean ± SD**	987.11 ± 1.27	689.22 ± 1.01	.892	0.037*

Table 4 summarizes frequency of Gram negative and positive organisms.

Table (4): Gram +ve or –ve organisms among the studied cases.

Variable		No.	%
Gram +ve or –ve	**(-ve)**	21	87.5
	(+ve)	3	12.5

Table 5 showed that Klebsiella was the most frequent isolated microorganism.

Table (5): Causative microorganisms among the studied cases.

Variable		No.	%
Causative Organism	**Klebsiella**	8	33.3
	Pseudomonas	6	25
	Enterobacter	4	16.7
	Staph aureus	2	8.3
	Staph coagulase	2	8.3
	Streptococcus	2	8.3

Table 6 summarizes the fate of the studied neonates.

Table (6): Outcome among the studied cases.

Variable		No.	%
Outcome	**Survived**	17	70.8
	Died	7	29.2

Survived cases had significantly increased Neopterin level (nmoL/L) than died cases (**Table 7**).

Table (7): Comparison of Neopterin between survived and died cases.

Neoptrin conc. (nmoL/L)	Survived	Died	t-test	P-value
Mean ± SD	927.22 ± 157.06	1132.54 ± 105.38	-.350	.029*

*Significant.

There were significant positive correlations between Neopterin and maternal age, neonatal age, HR and CRP. There were insignificant correlations between Neopterin and other data (**Table 8**).

Table (8): Correlation between Neopterin and other data in sepsis group.

Demographic and laboratory data	Neopterin	
	R	P-value
Neonatal age (hrs)	0.453	0.026
GA (wks)	0.075-	0.727
Birth wt (kg)	0.166	0.438
Maternal age	0.003	0.017*
APGAR 1min	0.094-	0.664
HR beat/min	0.694	0.000*
Temp.	0.002	0.993
RBCs (103/uL)	0.115-	0.594
Hb (g/dL)	0.032-	0.881
TLC (103/uL)	0.217-	0.308
Total Neutophils (103/uL)	0.110-	0.608
Plate (103/uL)	0.331-	0.114
CRP (mg/L)	0.168	.033*

*Significant.

Table 9 and Figure 1 show that sensitivity of Neopterin was 91%, specificity 684.7%, PPV 91.9% and NPV 88%.

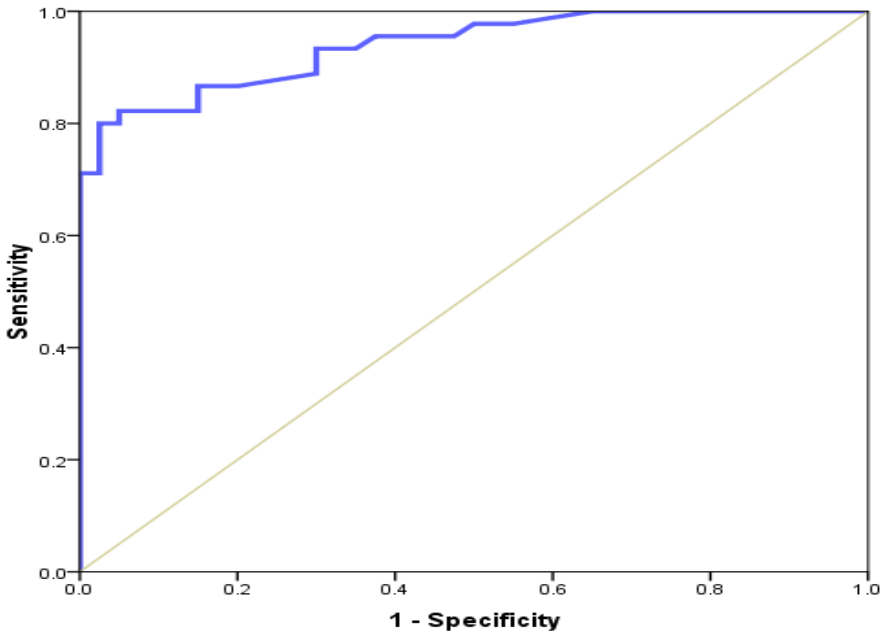

Figure (1): ROC curve for Neopterin in diagnosis of cases.

Table (9): Neopterin for diagnosis of neonatal sepsis.

Neopterin (AUC=0.91) (Cut off value=499)	Sensitivity	Specificity	PPV	NPV
	91%	84.7%	91.9%	88%

DISCUSSION

In the present research, age and sex were comparable between the studied groups. **El-Gendy *et al.*** [7] intended to assess the apolipoprotein A1 (Apo A1) value in neonatal sepsis diagnosis and prognosis in NICU of Menoufia University Hospitals. Their research involved 60 neonates admitted to NICU and were separated into 2 groups: group I (cases) contained 40 neonates with a sepsis and group II (controls) included 20 healthy neonates without sepsis and concluded an insignificant difference in sex and age between cases and controls.

Up to 50 % of cases in this research were males. Numerous investigations had demonstrated a male predominance among cases with neonatal sepsis [8,9]**.** In addition, **Higazi *et al.*** [10] reported a male preponderance (60 %) in their research of assessment of the diagnostic and prognostic performance of serum amyloid A and urinary interleukin-18 in neonatal sepsis in comparison to CRP.

In the current research, gestational age and weight were comparable between the studied groups. This was in line with **El-Gendy *et al.*** [7] who reported an insignificant difference in gestational age and weight. Additionally, **El-Mazary *et al.*** [11] reported an insignificant difference in body weight between sepsis and healthy groups.

The present work found a significant increase in maternal age among newborns with sepsis. This is consistent with **Ocviyanti *et al.*** [12] who revealed that the risk of neonatal sepsis elevated with maternal age in pregnant women.

Our research demonstrated an insignificant difference in the mode of delivery between cases and controls. This was in line with **Rass *et al.*** [13] who found that mode of delivery was insignificantly different between the infected and the uninfected neonates.

Some environmental factors, as a filthy atmosphere and incompetent personnel, are responsible for neonatal sepsis in neonates born via SVD.

This was in disagreement with a previous case control study [14] which included 35 newborns hospitalized to NICUs with sepsis and 35 healthy neonates with no clinical or biological evidence of infection as controls. They revealed that rate of CS delivery was significantly increased in neonates with sepsis than controls.

This also was in disagreement with a research which found that vaginally delivered infants were more susceptible to exhibit EOS than those delivered via CS. This may be attributable to intrapartum chemoprophylaxis and effective sterilization, which significantly reduced the sepsis incidence in neonates delivered by CS [15]**.**

In our clinical evaluation of the septic group, RD was the most common clinical manifestations of sepsis (100%). According to **Payaslı *et al.*** [16], RD was the most prevalent clinical manifestation (80%).

Regarding CBC, our findings demonstrated a substantial increase in WBCs in cases compared to controls. Our findings concur with those of **Awad *et al.*** [17] and **Ahmed *et al.*** [18] who observed a significant elevation in WBCs in the septic group compared to the controls. This is in accordance with the findings of **El-Mazary *et al.*** [11] who discovered that WBCs were significantly elevated in patients than in controls.

This result was comparable to that of **Srinivasan *et al.*** [19]**.** They demonstrated that TLC is valuable for evaluation of sepsis probability. Although, **Laurent *et al.*** [20] reported that the TLC is of limited use in identifying the neonatal infection.

In the current research, platelet count was significantly decreased in cases compared to controls. In line with this finding, **Shalaby *et al.*** [21] revealed a significant reduction in the platelet count in sepsis group compared to controls. Also, **El-Mazary *et al.*** [11] demonstrated that patients had considerably lower platelet count than controls.

We found that cases had significantly higher CRP level than controls. This was in line with **Awad *et al.*** [17]**,** **Ahmed *et al.*** [18] and **Shalaby *et al.*** [21] who reported increased CRP level among confirmed septic cases.

However various new infection markers have been studied in recent years, CRP is still a substantial, sensitive, and specific acute-phase protein for the prediction of sepsis, particularly in impoverished nations as indicated by some research [22]**.**

These findings were consistent with those of **Naglaa *et al.*** [23] and **Nora *et al.*** [24]. They revealed that CRP was the most prevalent test, that it can accurately diagnose neonatal sepsis, easily evaluated, and is more cost-effective. CRP is a simple measure for neonatal sepsis identification, especially when resources are limited.

In the present research, Neopterin was significantly different between cases and controls. In line with our findings, **El Nemer *et al.*** [6 reported that sepsis cases had considerably higher Neopterin levels compared to controls group in a research involved 88 neonates recruited from NICU.

Similarly, **Boseila *et al.*** [25] showed that serum neopterin was considerably higher in the infected and suspected groups than controls.

In our investigation, Gram negative bacteria were the most prevalent (87.5%). This was in accordance with **Elmashad *et al.*** [26] who revealed that Gram-negative organisms were the most prevalent in the affected group.

Our findings revealed that Klebsiella demonstrated the highest proportion (33.3%) then pseudomonas (25%), Enterobacter (16.7%), S. aureus (8.3%), S. coagulase (8.3%) and streptococcus (8.3%). These results were in

line with **Hashim *et al.*** [27] who found that Klebsiella is the most prevalent isolated bacteria in septic infants, with a range of 35-56 % of all isolates.

Boseila *et al.* [25] reported that 35% of the blood culture organisms were Klebsiella, Pseudomonas (20%), S. coagulase (10%), Group B Streptococci (10%), S. aureus (10%) and Enterobacter (15%).

These results may imply that each neonatal unit has its unique pattern of microorganisms, that fluctuates over time, and that antimicrobial combinations should be modified based on culture results. This disparity may be attributable to differences in bacterial profile, antibiotic resistance, and intrapartum antibiotic utilization between countries.

Gram-negative organisms may have been existed in our research due to lack of hygienic measures at birth site, the uncontrolled and inappropriate antibiotic utilization, an improper cord care, and unhygienic newborn care practices.

This study revealed that 29.2% of the examined cases had fatal outcomes. This was in line with **Kim *et al.*** [28] who reported that the total mortality rate was 37.8% (17/45). **Jumah *et al.*** [29] revealed that the death rate was higher in EOS cases (62.9%) than in LOS cases (36.5%).

In our investigation, the survived group had much lower levels of neopterin than those who died. This is in accordance with **El Nemer *et al.*** [6], who demonstrated a substantial rise in serum neopterin in died cases compared to survived cases. This is also consistent with the findings of **Tasdelen Fisgin *et al.*** [30] who reported a significant correlation in sepsis cases between serum Neopterin concentration and fatality rate suggested that Neopterin may contribute to tissue damage resulting from elevated cellular apoptosis.

In our study regarding Neopterin in diagnosis of cases, sensitivity of neopterin was 91%, specificity 684.7%, PPV 91.9% and NPV 88%. This was in consistent with **El Nemer *et al.*** [6] who reported 94.7% sensitivity and 88.6% specificity of Neopterin for sepsis detection. Also, **Boseila *et al.*** [25] concluded that serum Neopterin level is a valid test for the early diagnosis of bacterial infection and may be useful for commencing antibiotic treatment in neonates.

CONCLUSION

In infants with EOS, the serum level of neopterin rises significantly.

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REFERENCES

1. **Simonsen A, Anderson-Berry L, Delair F *et al.* (2014):** Early-onset neonatal sepsis. Clinical Microbiology Reviews, 27(1):21-47.
2. **Zou Q, Wen W, Zhang C (2014):** Presepsin as a novel sepsis biomarker. World J Emerg Med., 5:16-9.
3. **Stoll J (2012):** Infections of the Neonatal Infant.In: Kliegman RM, Behrman RE, Jenson HB and Stanton BF (eds). Nelson Textbook of Pediatrics, 19 editions. Elsevier, 109:794-811.
4. **Camacho-Gonzalez A, Spearman W, Stoll J (2013):** Neonatal infectious diseases: evaluation of neonatal sepsis. Pediatr Clin N Am., 60:367-89.
5. **Benitz E (2010):** Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. Clin Perinatol., 37(2):421-38.
6. **El Nemer S, Midan R, Mohamed F (2015):** Serum neopterin level in early onset neonatal sepsis. Am J Biosci., 3(3):80-6.
7. **El-Gendy M, El-Lahony R, Midan A *et al.* (2018):** Diagnostic value of apolipoprotein A1 in neonatal sepsis. Menoufia Med J., 31:1011-7.
8. **Belling L (2004):** Neonatal sepsis (review). Eur J Pediatr., 155:120-6.
9. **Gabri F, Eltabakh I, Osman O *et al.* (2021):** Acute kidney injury in neonatal sepsis: prevalence, and outcome. SVU-International Journal of Medical Sciences, 4(2):76-89.
10. **Higazi M, Mahrous M, Sayed Z *et al.* (2016):** Assessment of Urinary Interleukin-18 and Serum Amyloid A Efficacies against C-Reactive Protein in Diagnosis and Follow-up of Neonatal Sepsis. J Clin Cell Immunol., 7:446-50.
11. **El-Mazary M, Afifi F, Maher E *et al.* (2010):** Neutrophil CD64 in early-onset neonatal sepsis. Egyptian Journal of Pediatric Allergy and Immunology, 8(1):19-25.
12. **Ocviyanti D, Wahono T (2018):** Risk Factors for Neonatal Sepsis in Pregnant Women with Premature Rupture of the Membrane. Journal of Pregnancy, 3:1-6.
13. **Rass A, Talat A, Arafa A *et al.* (2016):** The Role of Pancreatic Stone Protein in Diagnosis of Early Onset Neonatal Sepsis. BioMed Research International, 15(3):1-8.
14. **Mohsen A, Kamel A (2015):** Predictive values for procalcitonin in the diagnosis of neonatal sepsis. Electronic Physician, 7(4):1190-8.
15. **Stoll J, Gordon T, Korones B *et al.* (1996):** Late-onset sepsis in very low birth weight neonates. A report from the national institute of child health and human development neonatal research network. Journal of Pediatrics, 129(1):63-71.
16. **Payaslı M, Özkul A, Ayaz S *et al*. (2013):** A New Marker for Early Diagnosis in Neonatal Sepsis: Polymorphonuclear Leucocyte Elastase Levels. Erciyes Med J., 35(2):46-51
17. **Awad M, Hawary A, Kholef M *et al.* (2020):** Serum Neopterin Level in Early Onset Neonatal Sepsis. The Egyptian Journal of Hospital Medicine, 81(1):1193-203.
18. **Ahmed S, Mahmoud M (2015):** Evaluation of cord blood and serum Hepcidin levels as biomarkers for neonatal sepsis. Al-Azhar Assiut Medical Journal, 13(3):155-9.
19. **Srinivasan L, Harris C (2012):** New technologies for the rapid diagnosis of neonatal sepsis. Curr Opin Pediatr., 24(2):165-71.
20. **Laurent R, Caroline S, Eric K *et al.* (2013):** A composite score combining procalcitonin, C-reactive protein and temperature has a high positive predictive value for the diagnosis of intensive care- acquired infections. BMC Infectious Diseases, 13:159-66.
21. **Shalaby M, Sobeih A, Abdulghany E *et al.* (2017):** Mean

platelet volume and serum uric acid in neonatal sepsis: A case-control study. Annals of Medicine and Surgery, 20:97-102.

22. **Ucar B, Yildiz B, Aksit A *et al.* (2008):** Serum amyloid A, procalcitonin, tumor necrosis factor-alpha, and interleukin-1beta levels in neonatal late-onset sepsis. Mediators Inflamm., 15(2):910-9.
23. **Naglaa B, Abeer S, Mohammad A *et al.* (2012):** Procalcitonin and C- Reactive Protein as Diagnostic Markers of Neonatal Sepsis. Australian Journal of Basic and Applied Sciences, 6(4):108-14.
24. **Nora H, Wilhelm M, Bernhard R (2013):** The Role of C-Reactive Protein in the Diagnosis of Neonatal Sepsis. Licensee InTech., 10(5):752-5.
25. **Boseila S, Seoud I, Samy G *et al.* (2011):** Serum Neopterin Level in Early Onset Neonatal Sepsis. Journal of American Science, 7(7):343-52.
26. **Elmashad M, Elsayed M, Omar A *et al.* (2019):** Evaluation of serum amyloid A protein as a marker in neonatal sepsis. Menoufia Med J., 32:1094-8.
27. **Hashim M, AboulGhar H, Hamam A (2004):** Evaluation of serum cortisol and ACTH level in neonatal sepsis. Egypt J Neonatol., 3:135-43.
28. **Kim J, Kim E, Park H *et al.* (2019):** Clinical features and prognostic factors of early-onset sepsis: a 7.5-year experience in one neonatal intensive care unit. Korean Journal of Pediatrics, 62(1):36-41.
29. **Jumah D, Hassan K (2007):** Predictors of mortality outcome in neonatal sepsis. The Medical Journal of Basrah University, 25(1):11-8.
30. **Tasdelen Fisgin N, Aliyazicioglu Y, Tanyel E *et al.* (2010):** The value of neopterin and procalcitonin in patients with sepsis. South Med J., 103(3):216-9.