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Serum Neopterin Level in Early Onset Neonatal Sepsis

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ABSTRACT

Backgound: Neonatal sepsis is defined as a clinical syndrome of bacteremia with signs and symptoms of infection in the first four weeks of life. A better understanding of the neonatal inflammatory response to sepsis and identification of sensitive and specific markers of inflammation or rapid microbe-specific diagnostic tests would assist in the early detection of neonatal sepsis.

Objective: Evaluate of serum neopterin level as an early diagnostic marker in neonatal sepsis for early detection of neonatal sepsis and early implementation of the appropriate therapeutic strategies.

Patients and Methods: The current study included 90 newborns admitted into NICU with 30 of them septic, 30 suspected neonatal sepsis and 30 control at Aswan University Hospital during the study period after obtaining consent from the parents.

Results: Neopterin level was significantly higher in cases than control ($p < 0.001$). A highly significant positive correlations was found between serum neopterin with TLC, T. neutrophils, immature/total neutrophil (I/T) ratio, serum C-reactive protein (CRP) level, ESR 1st hour, ESR 2nd hour and sepsis score. On the other hands, negative correlation was found between serum neopterin level and gestational age, with poor Moro reflex & apnoea. Moreover, no significant relation was found between serums level of neopterin and socio-demographic data.

Conclusion: Combined use of one or more laboratory marker as Haematological scoring system (HSS) and CRP with neopterin will enhance the diagnostic accuracy, early detection and consequently prevention of complications of infected cases.

Keywords: Neopterin, Neonatal Sepsis, HSS, CRP.

INTRODUCTION

Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first month of life. Sepsis in newborn is a major health-care burden worldwide accounting for approximately 1.4 million neonatal deaths annually [(1)]. Premature infants are particularly susceptible to sepsis and have a higher risk of long-term complications and mortality. There is an inverse correlation between gestational age, birth weight and sepsis [(2)].

There are two clinical types of sepsis: Early onset sepsis (EOS) presents within the first 72 hours of life. In severe cases, the neonate may be symptomatic at birth. Infants with EOS usually presents with respiratory distress and pneumonia. Late onset sepsis (LOS) usually presents after 72 hours of age. The source of infection in LOS is either nosocomial (hospital-acquired) or community-acquired and neonates usually present with septicemia, pneumonia or meningitis [(2)].

Neonatal sepsis is clinically diagnosed by a combination of clinical signs, nonspecific laboratory tests and microbiologically confirmed by detection of bacteria in blood by culture [(3)]. Warning signs and symptoms are often subtle and can easily be confused with non-infective causes such as apnea, hypothermia, and acute exacerbation of chronic lung disease. So that hematological and biochemical markers such as immature/total neutrophil ratio, platelet count, CRP, various cytokines have been proposed as being useful indicators for identification of septic infants [(4)].

The unnecessary exposure to antibiotics, with emergence of bacterial resistance will lead to potential poor outcomes in this vulnerable population of neonates. Resistance to antibiotics is a global problem.

Reports of multiresistant bacteria causing neonatal sepsis in developing countries are increasing and this may be explained by wide availability of over the counter antibiotics and the inappropriate use of broad-spectrum antibiotics in the community [(5)]. Moreover, despite extensive investigations, no single test meets the criteria that would make it an ideal marker for early diagnosis of sepsis in the newborn. Generally, complete blood count with differential count that may be accompanied by other adjuvant tests such as C-reactive protein (CRP) may be useful[(6)].

Neopterin is a catabolic product of guanosine triphosphate (GTP). It serves as a marker of cellular immune system activation. Measurement of neopterin concentrations in body fluids like blood serum, cerebrospinal fluid or urine provides information about activation of cellular immune system [(7)]. There is a close relationship between high neopterin levels and septicemia. High neopterin

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concentrations in serum have been shown to be a reliable indicator for the severity of bacteria-induced infections [8]. Elevated levels of neopterin have been shown to be an early specific and sensitive marker responsible for activation of the cellular immune system in several clinical settings including acute bacterial infection, inflammatory and malignant diseases [9].

The purpose of this study was to evaluate the serum neopterin level as an early diagnostic marker in neonatal sepsis for early implementation of appropriate therapeutic strategies aiming to decrease morbidity and mortality in Neonatal Intensive Care Unit of Aswan University Hospital.

PATIENTS AND METHODS

This is a cross sectional comparative study that carried out on 90 newborns devided according to the presence or absence of clinical manifestations of sepsis into three groups:

Group I (Infected Group): It included 30 neonate cases with: (1) A proven clinical picture of sepsis such as respiratory signs (tachypnea, irregular respiration, apnea, cyanosis), cardiac signs (decreased cardiac output, poor perfusion, bradycardia), neurological signs (meningitis, ventriculitis), general signs (temperature instability) [10]. (2) Laboratory evidence of sepsis such as positive blood culture, elevated C-reactive protein > 6 mg/dl and Rodwell's hematological sepsis score above 3 [11].

Group II (Suspected Group): It included 30 cases of neonates with: (1) High risk maternal factors of sepsis such as intrapartum fever > 37.8 °C, chorioamnionitis, premature rupture of membrane >18 hours, meconium stained amniotic fluid, antepartum hemorrhage, pregnancy induced hypertension and diabetes mellitus [2]. (2) High risk fetal factors of sepsis such as low birth weight infant and meconium aspiration syndrome [12]. (3) Non-specific laboratory markers such as white blood cells < 5000 or > 30.000 cells/mm^3, immature/total leucocyte count ratio < 0.2 and C-reactive protein < 6mg/dl.

Group III (Non-infected group): It included 30 healthy neonates with: (1) Absence of clinical picture of sepsis. (2) Normal laboratory markers such as white blood cells between 5000-30.000 cells/mm^3, immature/ total leucocyte count ratio < 0.2 and C-reactive protein < 1mg/dl.

All cases and control groups conducted from Aswan University Hospital (NICU and Obstetric Departments). The study started on December 2017 to May 2018.

Inclusion Criteria:

- Preterm and full-term newborns (29-42 weeks).
- Neonates with signs and symptoms of sepsis.
- Neonates of 72 hours of age or less.
- New borns with high risk of sepsis but without clinical manifestations of sepsis:
  - ❖ High risk fetal factors:
  - Low birth weight.
  - Meconium aspiration.
  - ❖ High risk maternal factors:
  - Intrapartum fever >37.5C.
  - Chorioamnionitis.
  - Premature rupture of membrane >18 hours.
  - Meconium stained amniotic fluid.
  - Antepartum hemorrhage.
  - Pregnancy induced hypertension (PIH).
  - Diabetes mellitus.
  - ❖ Nonspecific laboratory markers such as (WBCs < 5000 or > 30.000 cells/mm^3, immature/total leucocytes count > 0.2 and C-reactive protein > 6mg/dl).

Exclusion Criteria:

- Neonates of more than 72 hours of age.
- Neonates suffering from:
  - Cardiac problems as congenital heart disease.
  - Gastrointestinal problems as duodenal atresia.
  - Neurological problems as hypoxic ischemic encephalopathy.
  - Renal failure, chromosomal abnormalities or metabolic diseases.

All included neonates in the present study were subjected to the following:

A- History: Prenatal history, natal history and postnatal history.

B- Full clinical examination: Examination was done to all newborns with special emphasis on clinical manifestation of sepsis, which included vital signs, central nervous system, respiratory, cardiovascular system, gastrointestinal tract and hematological.

C- Assessment of gestational age using New Ballard Score [13].

D- Growth assessment, which included mean body weight, lenghth, and head circumference. This was done by standardized procedures.

E- Laboratory Investigations: Blood samples were collected from all cases to confine infected group, suspected group and the control group.

Sampling: Five to seven ml (5-7 ml) venous blood samples were withdrawn under complete aseptic condition and devided as follow: Two ml of blood on EDTA tubes for CBC, I/T ratio and ESR. 3 ml of blood had been collected in

neonatal bottles for blood culture. The remaining part of sample was put on plan tubes for routine investigations. samples in plan tubes were left in water bath for 20 minutes at 37 ºC for clotting then centrifuged at 3000 rpm for serum separation, which was used for assessment of CRP (done on the same day). The remaining serum was stored at -20°C for assessment of neopterin later on.

Ethical consideration:

The Institutional Review Board and Ethic Committee of of Medical Sciences, Aswan University, Egypt approved the study protocol. Written consent had been obtained from all patients' parents before getting them involved in the study. The steps of the study, the aim of the study, the potential benefit and hazards, all had been discussed with the patient's parents. Confidentiality of all data had been ensured.

Statistical Methods and Analysis

Statistical Package for social science (SPSS) program version 20 was used for analysis of data. Data were summarized as mean and SD. Non parametric test (Mann Whitney U) was used for analysis of two qualitative data. One-way ANOVA test was used to compare the mean difference between groups more than two variables followed by post Hocc test with Bonferroni corrections for detection of significance.

Chi-square test was used to compare proportions between groups. Kruskal-Wallis test was used to compare the median difference between groups. Simple linear correlation (Pearson's correlation for quantitative data) was done to detect the relation between neopterin, CRP, sepsis score, gestational age and birth weight with all other demographic and laboratory data. "r "value was considered weak if < 0.25, mild if ≥ 0.25-< 0.5, moderate if ≥ 0.5 -< 0.75 and strong if ≥ 0.75. ROC curve was used to compare neopterin and CRP in diagnosing neonatal sepsis and prediction of its outcome.

Receiver operating characteristic (ROC) curve was used to assess the best cut off point with its sensitivity, specificity, positive predictive value and negative predictive value. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: P > 0.05: Non-significant, P ≤ 0.05: Significant & P < 0.01: Highly significant.

RESULTS

Table (1): Socio-demographic and maternal risk factor differences between groups

Parameter	Sepsis G (1) (No.=30)	High Risk G (2) (No.=30)	Control G (3) (No.=30)	P-value
Age in days	1.87 ± 0.7	1.83 ± 0.6	1.77 ± 0.7	= 0.841*
P-value**	1 vs 2 = 0.848	2 vs 3 = 0.701	1 vs 3 = 0.564	
Gender				= 0.721***
Male	20 (66.7%)	18 (60%)	17 (56.7%)	
Female	10 (33.3%)	12 (40%)	13 (43.3%)	
Maturity				= 0.725***
Full-term	18 (60%)	16 (53.3%)	19 (63.3%)	
Preterm	12 (40%)	14 (46.7%)	11 (36.7%)	
Gestational Age (weeks)	35.70 ± 3.3	36.13 ± 2.6	36.97 ± 1.8	= 0.174*
P-value**	1 vs 2 = 0.527	2 vs 3 = 0.225	1 vs 3 = 0.067	
Maternal Age (years)	26.13 ± 3.9	27.03 ± 4.9	26.70 ± 4.1	= 0.720*
P-value**	1 vs 2 = 0.424	2 vs 3 = 0.767	1 vs 3 = 0.614	
Mode of Delivery				= 0.207***
CS	19 (63.3%)	24 (80%)	18 (60%)	
VD	11 (36.7%)	6 (20%)	12 (40%)	
Maternal Disease				
Negative	20 (66.7%)	13 (43.3%)	30 (100%)	< 0.001***
Positive	10 (33.3%)	17 (56.7%)	0 (0%)	

*****ANOVA test was used to compare the mean difference between groups

**Post-hoc test with Bonferroni corrections

***Chi-square test was used to compare proportions between groups

90 neonates were enrolled in the study. There were 30 neonates with proven sepsis, 30 neonates suspected to be septic and the last 30 neonates were controls. Out of these 55(61.1%) were males and 35(38.9%) were females.

There were 53 (58.9%) full term and 37 (41.1%) were preterm neonates among all groups. There were no significant differences in age means (1.87 versus1.83 vs 1.77 days), gestational age means (35.7 vs 36.13 vs 36.97 wks.), maternal age means (26.13 vs 27.03 vs 26.70 years), male to female ratio, maturity and mode of delivery between the sepsis, suspected and control groups respectively. As regard maternal risk factors, there was significant difference between groups in comparing maternal disease ($p < 0.001$). Among neonates with sepsis, there were 10 cases (33.3%) and of suspected sepsis, 17 cases (56.7%) had positive maternal disease compared to the control, which had no maternal disease (Table 1).

Table (2): Other risk factor differences between groups

Parameter	Sepsis G (1) (No.=30)	High Risk G (2) (No.=30)	Control G (3) (No.=30)	P-value
Risk Factors				
No	0 (0%)	0 (0%)	30 (100%)	
AP_Hge	2 (6.7%)	6 (20%)	0 (0%)	
DM	3 (10%)	9 (30%)	0 (0%)	
PROM	10 (33.3%)	6 (20%)	0 (0%)	< 0.001*
Fever	5 (16.7%)	3 (10%)	0 (0%)	
Meconium	5 (16.7%)	3 (10%)	0 (0%)	
PIH	5 (16.7%)	9 (30%)	0 (0%)	
Consanguinity				
Negative	23 (76.7%)	23 (76.7%)	22 (73.3%)	= 0.207***
Positive	7 (23.3%)	7 (23.3%)	8 (26.7%)	
Socioeconomic Status				
High	16 (53.3%)	14 (46.7%)	14 (46.7%)	= 0.942*
Low	14 (46.7%)	16 (53.3%)	16 (53.3%)	

*Chi-square test was used to compare proportions between groups

Antepartum hemorrhage (AP-He) occurred in 2 mothers of neonates from sepsis group (6.7%) while in the suspected group it occurred in 6 mothers (20%). 3 mothers (10%) of septic neonates were diabetic and in the suspected group 9 (30%) mothers were diabetic. Premature rupture of membrane (PROM) occurred in 10 neonates (33.3%) of the sepsis group and in 6 neonates (20%) of the suspected group. Maternal fever more than 38 °C was detected in mothers of 5 neonates (16.7%) of the sepsis group and in 3 mothers (10%) of the suspected group. Meconium-stained fluid was found in mothers of 5 neonates (16.7%) of the sepsis group and in 3 mothers (10%) of the suspected group. Pregnancy-induced hypertension (PIH) occurred to 5 mothers (16.7%) of septic neonates, while occurred in 9 mothers (30%) of suspected sepsis neonates. There were high significant differences between the studied groups as regard maternal risk factors ($p < 0.001$). No significant difference between the groups regarding consanguinity or socioeconomic status (Table 2).

Table (3): APGAR scores at 1 min. and 5 min. between groups

Parameter	Sepsis G (1) (No.=30)	High Risk G (2) (No.=30)	Control G (3) (No.=30)	P-value
APGAR Score at 1 min.	4.03 ± 0.8	5.00 ± 1.6	6.50 ± 1.2	< 0.001*
P-value**	1 vs 2 = 0.003	2 vs 3 < 0.001	1 vs 3 < 0.001	
APGAR Score after 5 min.	8.23 ± 0.7	8.83 ± 0.8	9.43 ± 1.1	= 0.001*
P-value**	1 vs 2 =0.061	2 vs 3 =0.061	**1 vs 3 <0.001**	

*ANOVA test was used to compare the mean difference between groups **Post-hoc test with Bonferroni corrections

In table **(3),** there were high significant differences between the studied groups regarding APGAR (1 min.) and APGAR (5 min.) with mean (4.03 ± 0.8, 8.23 ± 0.7 vs 5.00 ± 1.6, 8.83 ± 0.8 vs 6.50 ± 1.2, 9.43 ± 1.1 respectively) in sepsis , suspected and control groups with APGAR (1min) ($p < 0.001$) and APGAR (5min) ($p = 0.001$).

Table (4): Haematological scoring system (HSS) among the studied groups

Parameter	Sepsis G (1) (No.=30)	High Risk G (2) (No.=30)	Control G (3) (No.=30)	P-value
HSS score				
Mean ± SD	4.90 ± 0.2	3.50 ± 0.2	0.47 ± 0.1	< 0.001*
Median (Range)	5 (4 - 7)	3 (2 - 7)	0 (0 - 2)	
P-value**	1 vs 2 <0.001	2 vs 3 <0.001	1 vs 3 <0.001	
HSS Classification (Sepsis)				
Unlikely	0 (0%)	8 (26.7%)	30 (100%)	<0.001***
Possible	13 (43.3%)	16 (53.3%)	0 (0%)	
Very Likely	17 (56.7%)	6 (20%)	0 (0%)	

*ANOVA test was used to compare the mean difference between groups **Post-hoc test with Bonferroni corrections
***Chi-square test was used to compare proportions between groups

As regards, HSS among studied groups, there were significant differences between the groups ($p < 0.001$) being higher in infected cases compared to suspect and control groups with mean of (4.90 ± 0.2 versus 3.50 ± 0.2 and 0.47 ± 0.1 respectively). According to the classification, there were 13 neonates (43.3%) possible and 17 neonates (56.7%) very likely among infected neonates, while there were 16 neonates (53.3%) possible and 6 neonates (20%) very likely of suspected group as shown in table (4).

Table (5): Correlation between serum neopterin level and the studied parameters

Variable	Neopterin (nmol/l)	
	r*	P-value**
Age in days	**-0.058**	= 0.292
Gestational Age/weeks	-0.197	= 0.032
Maternal Age/years	-0.033	= 0.377
HR	0.532	< 0.001
RR	0.690	< 0.001
Temperature	0.352	< 0.001
Weight	-0.158	= 0.068
Length	-0.157	= 0.069
TLC	0.659	< 0.001
T. Neutrophil	0.664	< 0.001
I/T Ratio	0.787	< 0.001
Platelet Count	-0.128	= 0.115
HG	0.012	= 0.455
RBCs	0.068	= 0.389
CRP	0.667	< 0.001
ESR 1st hour	0.369	< 0.001
ESR 2nd hour	0.474	< 0.001
HSS score	0.771	< 0.001

* Pearson Correlation Coefficient **P≤0.05 is considered significant

A highly significant positive correlations were found between serum neopterin level and heart rate with ($r = 0.532$, $p < 0.001$) and respiratory rate with ($r = 0.690$, $p < 0.001$) and temperature with ($r = 0.352$, $p < 0.001$). It correlated as well significantly highly positive with TLC ($r = 0.659$, $p < 0.001$), T.neutrophils ($r = 0.664$, $p < 0.001$) and I/T ratio ($r = 0.787$, $p < 0.001$). Additionally, a highly significant positive correlations were found between serum neopterin level and the serum CRP level ($r = 0.667$, $p < 0.001$), ESR 1st hour ($r = 0.369$, $p < 0.001$), ESR 2nd hour ($r = 0.474$, $p < 0.001$) and sepsis score ($r = 0.771$, $p < 0.001$). Negative correlation was found between serum neopterin level and gestational age with ($r=-0.197$, $p=0.032$) as shown in table (5).

Table (6): Serum level of Neopterin in relation to Socio-demographics among the studied Cases (n=60)

Variable	Category	Serum level of Neopterin (nmol/l) Mean ± SD	P-value*
Sex	• **Male**	178.83 ± 44.2	= 0.283
	• **Female**	117.54 ± 14.9	
Maturity	• **FT**	131.46 ± 16.7	= 0.312
	• **PT**	188.71 ± 63.1	
Delivery Mode	• **CS**	170.99 ± 40.2	= 0.405
	• **VD**	121.35 ± 14.6	
Maternal Dis.	• **Negative**	164.41 ± 39.1	= 0.606
	• **Positive**	133.02 ± 14.1	
Consanguinity	• **Negative**	164.74 ± 35.9	= 0.539
	• **Positive**	124.87 ± 23.1	
Social Status	• **High**	173.70 ± 52.9	= 0.512
	• **Low**	137.10 ± 19.7	

* T-test was used to compare the mean difference between groups

Among suspected and sepsis groups, there was no significant relation between serum level of neopterin and socio-demographic data as shown in table (6).

Table (7): Diagnostic performance of serum level of neopterin and CRP for diagnosis of neonatal sepsis, analysed as area under the curve (95% CI)

	AUC*	95% CI+	SE**	P-value***
Serum level of Neopterin	0.992	0.978 - 1.000	0.007	< 0.001
CRP	0.933	0.880 - 0.986	0.027	< 0.001

*AUC = Area under the Curve **SE = Standard Error +CI = Confidence Interval ***Null hypothesis: true area = 0.5

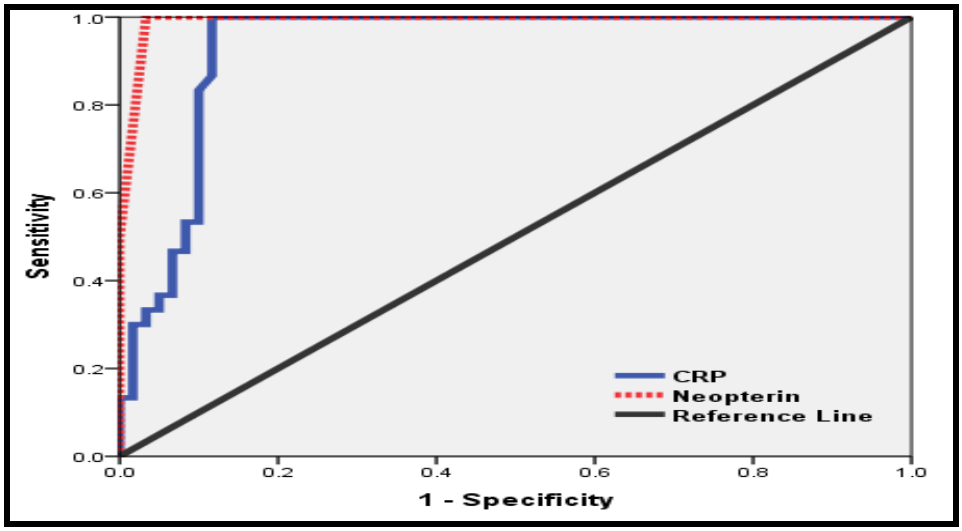

Figure (1): ROC curve for Serum level of Neopterin and CRP for the studied Cohort

The diagnostic value of serum neopterin levels to diagnose sepsis (AUC = 0.992, p < 0.001; 95% CI 0.978-1.000) was comparable to that of serum CRP (AUC = 0.933, p < 0.001; 95% CI 0.880-0.986) as shown in table (7) and fig (1).

Table (8): Goodness criteria of the serum level of neopterin and CRP for diagnosis of neonatal sepsis

	Goodness criteria	
	Serum level of Neopterin	CRP
AUC	0.992	0.933
Cut-off	100.3	36
Accuracy	94.2%	89.5%
Sensitivity, %	100%	88%
Specificity, %	88.3%	91%
PPV, %	89.5%	90.7%
NPV, %	100%	88.3%

*Sensitivity (true positives/all diseased); specificity (true negatives/all non-diseased); PPV (true positives/all test positives); NPV (true negatives/all test negatives).

As regards neopterin, it was found to be 100%; sensitive in identifying sepsis, with specificity 88.3%, positive predictive value 89.5% and negative predictive value 100% with the best cut of point between cases and controls was 100.3 nmol/l. The sensitivity of CRP was found to be 88%, specificity 91%; the predictive value of the positive test was 90.7%, while that of the negative test was 88.3% as shown in table (8).

Table (9): Serum level of Neopterin in relation to Outcome among the studied Groups

Parameter	Survived (No.=37)	Died (No.=23)	P-value
Neopterin (nmol/l)			
Mean ± SD	156.52 ± 109.8	267.77 ± 184.9	= 0.610*
Median (Range)	107 (79 - 515)	105 (79 - 2377)	

*Mann-Whitney U was used to compare the median difference between groups

In table (9), the relation between serum neopterin and outcome was illustrated and showed that there was no statistically significant difference between the studied groups.

Table (10): Diagnostic performance of serum level of neopterin for outcome prediction of neonatal sepsis, analysed as area under the curve (95% CI).

	AUC*	95% CI+	SE**	P-value***
Serum level of Neopterin	0.625	0.537 - 0.776	0.107	= 0.047

*AUC = Area under the Curve
**SE = Standard Error +CI = Confidence Interval
***Null hypothesis: true area = 0.5

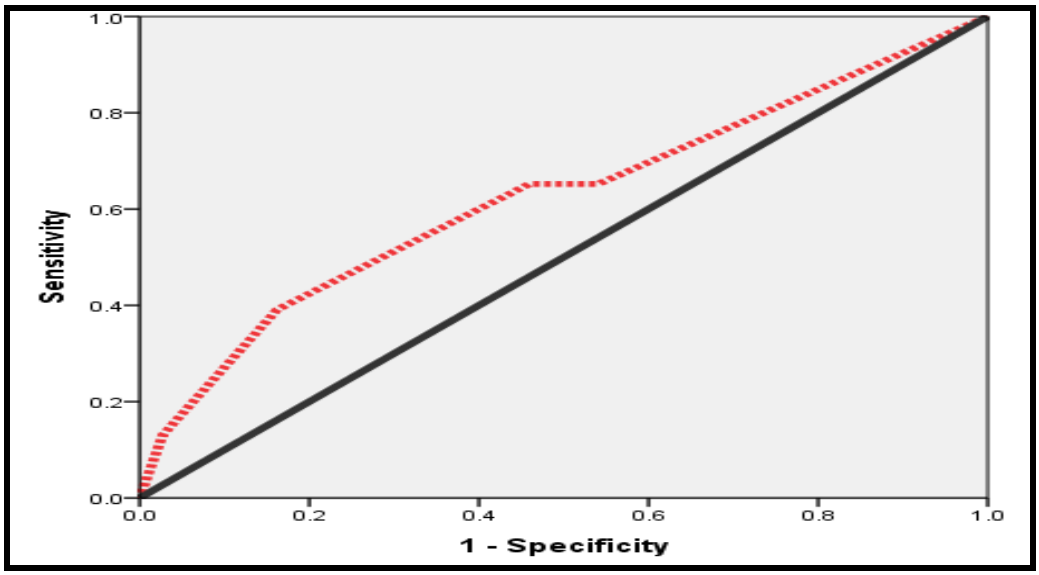

Figure (2): ROC curve for Serum level of Neopterin for Outcome Prediction

The prognostic value of serum neopterin to predict outcome of sepsis (AUC = 0.625, p = 0.047; 95% and CI 0.537-0.775) as shown in table (10) and fig (2).

Table (11): Goodness criteria of the serum level of neopterin for outcome prediction of neonatal sepsis

	Goodness criteria
	Serum level of Neopterin
AUC	0.625
Cut-off	100.9
Accuracy	56.1%
Sensitivity, %	65.2%
Specificity, %	46.9%
PPV, %	55.1%
NPV, %	57.4%

*Sensitivity (true positives/all diseased); specificity (true negatives/all non-diseased); PPV (true positives/all test positives); NPV (true negatives/all test negatives).

Neopterin as a prognostic marker for outcome (between died and survived) in patient group was found to be 65.2% sensitive, the specificity was 46.9%, positive predictive value 55.1% and negative predictive value was 57.4% as shown in table (11).

DISCUSSION

In our study, 90 neonates were enrolled in the study. There were 30 neonates with proven sepsis, 30 neonates suspected to be septic and the last 30 neonates were controls. Out of them, 66.7% of the infected group were males and in the suspected group, 60% were males. This comes in agreement with, **El Nemer *et al.* [9].** They studied 88 neonates dividing them into group I (35 cases suspected sepsis), group II (38 neonates proven sepsis) and group III (15 healthy neonates as controls). They found that males were 55.3% in infected group and 45.7% in suspected group. This also is in line with **Jyoti and Mahadevi [14]** who found that male babies were more affected by sepsis than female babies with distribution of 56.4% and 43.6% respectively. In addition, **Gupta *et al.* [15]** found predominance of males by 64.7% in his study. In agreement with us, **Boseila *et al.* [16]** found that males formed around 60% of the patients. This is due to A gene located on x-chromosome that has been postulated to be involved in the function of thymus or with synthesis of immunoglobulins.

As regards maturity, we found predominance of sepsis among term infants (60%) than preterm one (40%). This is in accordance with **Caughey *et al.* [17]** who found that the rates of immediate neonatal morbidity increased with increasing gestational age. **Ellahony *et al.* [18]** and **Adatara *et al.* [19]** reported that 90% and 71.8% of their patients were full-term in clinically positive blood culture sepsis respectively. In contrast to our results, **Dhumal *et al.* [20]** concluded that 58% and 52% of their patients were preterm in proved and probable septicaemia respectively.

In our study there was predominance of cesarean section than normal delivery in sepsis and suspected groups (60% and 80%). This is in agreement with **Agrawal *et al.* [21]** study who compared IgG antibody content in the blood of cesarean newborns and neonates by natural labor. They discovered that the blood IgG antibody levels in newborns delivered by cesarean section was significantly lower than that of newborns delivered by natural labor.

In our study, among neonates with sepsis, there were (33.3%) and of suspected sepsis (56.7%) had positive maternal disease compared to the control, which had no maternal disease. The incidence of premature rupture of membranes (PROM) in septic group was (33,3%) and in suspected one was (20%). PROM is considered as a major risk factor for sepsis because of the danger of ascending infection. In agreement with current study, **Boseila *et al.* [16]** found that, PROM occurred in 8 neonates (40%) of the infected group while in the suspected group it occurred in 9 neonates (45%). In addition, **Anggara *et al.* [22]** showed that PROM occurred in eight neonates (34.8%), meconeal amniotic fluid occurred in seven infants (30.4%), and maternal fever during delivery in two infants (8.7%). In contrast, **Utomo [23]** found no significant difference among sepsis that associated with PROM.

As regard other maternal risk factor, we found that maternal fever more than 38 °C was detected in mothers of 5 neonates (16.7%) of the sepsis group and in 3 mothers (10%) of the suspected group. This is in agreement with **Boseila *et al.* [16]** who found that fever in mothers of 2 neonates (10%) of the infected group and in 4 mothers (20%) of the suspected group.

The current study showed that there were high significant differences between the studied group regarding APGAR (1 min) and APGAR (5min) in sepsis, suspected and control groups with APGAR (1min) ($p < 0.001$) and APGAR (5min) ($p = 0.001$). This is in agreement with **El Nemer *et al.* [9]** who found that there were high significant differences between the studied groups regarding APGAR (1 min) and APGAR (5 min) with $p < 0.001$**.** In addition, in accordance with the study of **Yousef *et al.* [24]** who observed that a 5-minute Apgar score < 7 carries a significantly higher risk of sepsis than infants with higher scores and that Apgar score less than 5 at one minute may be due to sepsis, especially with the presence of risk factors for infection**.** Furthermore, low Apgar scores usually necessitate more prolonged and aggressive resuscitation, which is a known risk factor for sepsis as reported by **Gomella *et al.* [25].** However in contrast, **Boseila *et***

al. [16] found no significant differences in Apgar scores at one and five minutes, between the infected, suspected, and control groups respectively.

In our study, we found that the I/T ratio was significantly higher in the septic and high-risk neonates (p=0.001) (p=0.001) respectively in comparison with the control. These results are concordant with the results of **Rusia *et al.*** [26] and **Abou El-Ela *et al.*** [27]**.** Thus the ratio of immature to total neutrophil (I/T) and immature to mature neutrophil (I/M) were much informative, as they were significantly higher in the septic and suspected neonates in comparison with the control [9]**.**

As regards HSS among studied groups, there were significant differences between the groups ($p < 0.001$) being higher in infected cases compared to suspect and control groups. This comes in agreement with **El Nemer *et al.*** [9] who found high significant differences between the case groups regarding hematological sepsis score**.** This agrees with **Das *et al.*** [28] who reported that HSS was significantly higher in patients with infection than in patients with no infection and that HSS of the septic group was ≥ 3. In addition, in harmony with current observation, **Badrawi *et al.*** [29] reported that, HSS score ≥ 3 should detect septic infants with a sensitivity of 98%. They also suggested that HSS score ≥ 5 are highly predictive of sepsis until a reliable diagnostic test is available.

In current study, among the infected group there were 20 neonates (66.7%) culture positive, while in suspected group only 5 neonates (16.7%) were culture positive. Therefore, there was significant difference between the two groups ($p < 0.001$). Staph aureus showed the highest percent of incidence being 40% in blood cultures of sepsis group followed by Klebseilla (35%), Enterobacter (20%) and lastly Coagulase negative staph (5%). In suspected group, Staph aureus showed the highest percent of incidence being (40%) and Klebseilla (40%) followed by Pseudomonas (20%). These pathogens were commonly responsible for early onset disease as in other studies done in Egypt [30]**.**

In current study, Neopterin level was significantly higher in cases than controls ($p < 0.001$). This is in agreement with **Mitaka** [31] who observed that neopterin level have been increased in patients progressing from gram-negative sepsis to septic shock. In addition, they reported that neopterin level are higher in patients with septic shock than in patients with non-infectious SIRS. This finding can be easily explained because neopterin closely reflects the activation of both monocytes macrophages and endothelial cells, which have a central role in the pathogenesis of septic shock. In line with our study, **El Nemer *et al.*** [9] found high significant difference between the studied groups regarding neopterin level being higher in sepsis group (108.37 ± 22.38) than suspected group (44.46 ± 24.72) and control group (5.35 ± 2.34). The same results were observed in the studies done by **Boseila *et al.*** [16] and **Baydar *et al.*** [32]**,** which revealed that serum neopterin was significantly increased in the infected group than in the control group and explained this by that neopterin is a biomarker of cellular immunity and therefore increased level of neopterin may reflect septic complications.

As regards relation of serum neopterin to socio-demographic data, we found that it correlated negatively with the gestational age (r=-0.197, p=0.032). In agreement with us **Boseila *et al.*** [16] found that in septic neonates, serum neopterin level correlated negatively with the gestational age (r=-0.4, p=0.07). In contrast to our study they found neopterin correlated positively with the maternal age (r = 0.5, p=0.02) and gravidity (r =0.5, p= 0.01).

In our study, a highly significant positive correlations were found between serum neopterin level and TLC ($p < 0.001$), T. neutrophils ($p < 0.001$), I/T ratio ($p < 0.001$), serum CRP level ($p < 0.001$), ESR 1st and 2nd hour ($p < 0.001$) and sepsis score ($p < 0.001$), which are laboratory markers of neonatal sepsis. This is in agreement with the study of **Coetzee *et al.*** [33] who revealed that serum neopterin level correlated positively with both CRP (p =0.0001) levels and the HSS (p=0.04) which are laboratory markers of neonatal sepsis pointing to their usefulness as additional markers of sepsis. High levels of neopterin can be explained by the hygiene hypothesis. It is probable that the presence of infection by bacteria or viruses in a sterile condition such as in the intrauterine period will activate the immune system, which could increase the levels of regulatory T cells resulting in higher neopterin levels [34]**.**

As regard serum neopterin in relation to clinical manifestations, we found that the level significantly increased with poor moro reflex and with apnea among suspected and sepsis cases. On other hand, **Boseila *et al.*** [16] found that, serum neopterin and CRP concentrations correlated significantly positive with respiratory distress (r= 0.5, p= 0.03) while lethargy correlated significantly positive with serum neopterin level only (r=0.2, p=0.05). The optimal cutoff points was not established yet. This may be due to the wide variation between the studies in the methods or the relative small numbers of patients studied. In our study, the diagnostic value of serum neopterin levels to diagnose sepsis (AUC = 0.992, $p < 0.001$; 95% CI 0.978-1.000) was comparable to that of serum CRP (AUC = 0.933, $p < 0.001$; 95% CI 0.880-0.986). As regards neopterin, it was found to be 100% sensitive in identifying sepsis, with specificity 88.3%, positive

predictive value 89.5% and negative predictive value 100% with the best cut of point between cases and controls was 100.3 nmol/l. While, the sensitivity of CRP was found to be 88%, specificity 91% and the predictive value of the positive test was 90.7%, while that of the negative test was 88.3%. This comes in agreement with **El Nemer *et al.*** [(9)] who found that neopterin had a better sensitivity value (94.7%) than CRP, which had sensitivity value (65.8%). The specificity of neopterin was 88.6%, which was higher than that of CRP (60%). Their study also revealed that the best cutoff value of serum neopterin to detect sepsis is 70.56 nmol/L with sensitivity 94.7% and specificity 88.6%. On the other hand, **Boseila *et al.*** [(16)] agrees with us in that neopterin was superior to CRP in sensitivity (78.9% and 65.1%) and NPV (82.6% and 60.5%) respectively. But they found that CRP was superior to neopterin in specificity (95.5% and 95%) and PPV (97.5% and 93.8) respectivily. **Boseila *et al.*** [(16)] and **Murr *et al.*** [(35)] found that the combination of serum neopterin level and CRP is a reliable test for the diagnosis of early onset bacterial infection and may be helpful in establishing antibiotic therapy in newborn.

In our study, the relation between serum neopterin and outcome showed that no statistically significant difference between the studied groups. Neopterin as a prognostic marker for outcome (between died and survived) in patient group was found to be 65.2% sensitive, the specificity was 46.9%, positive predictive value was 55.1% and negative predictive value was 57.4%. In contrast to our study, **Murr *et al.*** [(35)] and **Ruokonen *et al.*** [(36)] reported an increase of serum neopterin level with the severity of infection and a higher level in non-survivors. In agreement with them **El Nemer *et al.*** [(9)] found significant increase in serum neopterin in non-survived (109.69 ± 34.97 nmol/l) than in survived patients (68.75±36.46 nmol/l). This agrees also with **Boseila *et al.*** [(16)] who found that 7 cases (35%) of the infected group and 9 cases (45%) of the suspected group died. Their serum neopterin level was significantly higher than that of the living neonates (p value = 0.001).

We can explain our result on basis that due to meticulous care in our unit with good antibiotics so that the survival rate in septic case was high, or may be due to the variation between the studies in their methods. Also, it may be due to the relative small numbers of patients studied, or the death may be due to another cause not sepsis.

CONCLUSION AND RECOMMENDATIONS

- Highly significant increase in serum neopterin level in newborn with early onset sepsis when compared to control. Therefor, may be used as a diagnostic marker for early onset neonatal sepsis.
- There was significant increase in mean serum neopterin level among septic neonates with high risk factors for sepsis.
- Combined use of one or more laboratory marker as HSS and CRP with neopterin will enhance the diagnostic accuracy, early detection and consequently prevention of complications of infected cases.
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RECOMMENDATIONS

- However, this study could be used as an initial or baseline research for further, larger research on neopterin levels in neonatal sepsis, especially in developing countries. The study population was small, so further study is needed for evaluation of the prognostic value of neopterin in neonatal sepsis.
- Evaluation of serum neopterin level among newborn is recommended as an early marker to diagnose early onset sepsis.
- Despite the advance in supportive care and availability of potent antimicrobial agents, mortality from sepsis is a leading cause of death in neonatal intensive care unit.
- Prevention and early management of neonatal sepsis is vital to improve outcome.
- Risk factors should be avoided to decrease incidence of early onset sepsis.

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