

Serum Neopterin Level in Early Onset Neonatal Sepsis

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Abstract: Background: Sepsis is the commonest cause of neonatal mortality and is probably responsible for 30-50% of the total neonatal deaths each year in developing countries. Diagnosis of neonatal sepsis remains a major challenge, as early signs of sepsis are often non-specific and the laboratory criteria are also not fully reliable. This leads to unnecessary exposure to antibiotics before the presence of sepsis has been proven with potentially poor outcomes. Several attempts have been made to use physiologic parameters, hematologic indices, and cytokine profiles, at the time of onset of the suspected sepsis episode to identify accurately neonates with sepsis. Elevated serum level of neopterin has been shown to be an early specific and sensitive marker responsible for activation of the cellular immune system and has also been proposed to aid in the diagnosis of bacterial infection. Objective: To evaluate the usefulness of serum neopterin level as an accurate diagnostic tool for neonatal sepsis and compare it with Rodwell's hematological sepsis score and C-reactive protein for predicting infection and outcome in neonates with sepsis. Methods: The study comprised 20 neonates with a clinical proven sepsis, 20 neonates with a clinical suspicion of sepsis and 20 healthy neonates of matched gestational age who were considered as the normal control group. All groups were subjected to full history taking and clinical examination. Laboratory investigations done were complete blood count, total and differential leucocytic count, blood culture, serum levels of CRP and neopterin. Results: Serum neopterin levels were significantly higher in the infected and suspected groups compared with the control group ($p=0.0001$) and correlated positively with both CRP levels ($r=0.8$, $p=0.0001$) and the Hematological Sepsis Score ($r=0.5$, $p=0.04$). Significant positive correlations were detected between serum neopterin level, maternal age ($r=0.5$, $p=0.02$), gravidity ($r=0.5$, $p=0.01$), respiratory distress ($r=-0.5$, $p=0.03$), and lethargy ($r=0.2$, $p=0.05$) in septic neonates. Conclusion: Serum neopterin may be used as an early diagnostic tool with high sensitivity (78.09%), specificity (85%), positive predictive value (93.8%), negative predictive value (82.6%) in neonates with suspicion of sepsis especially when combined with routine hematological sepsis score and C-reactive protein.

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1. Introduction:

Each year, an estimated four million neonatal deaths occur globally. Infections account for about 36% of these deaths. Forty percent of these four million neonatal deaths occur in developing countries¹. Neonatal sepsis has been used to describe the systemic response to infection in the newborn infant younger than one month of age and is categorized as early or late neonatal sepsis². Early signs of sepsis in the newborn are often non-specific leading to the initiation of treatment before the presence of sepsis has been proven. Blood culture is currently the gold standard for the diagnosis of sepsis, however, in addition to the fact that cultures reports are available only after 48-72 hours, they frequently give false negative results due to the small amount of blood that can be drawn from neonates³.

So the unnecessary exposure to antibiotics, with emergence of bacterial resistance will lead to potential poor outcomes in this vulnerable population of neonates. To identify accurately neonates with sepsis, attempts have been made to use physiologic parameters, hematologic indices, and cytokine profiles, at the time of onset of the suspected sepsis episode⁴. C-reactive protein (CRP) has been extensively investigated but there has been more interest in chemokines, cytokines, and other markers to diagnose the neonatal sepsis as procalcitonin, fibronectin, haptoglobin, lactoferrin, and neopterin⁵.

Neopterin a pyrazino – pyrimidine derivative is formed from guanosine triphosphate within the biosynthetic pathway of biopterin. It is produced by the human macrophages when stimulated by interferon gamma released from activated

T lymphocyte⁶. 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 allograft rejection, acute bacterial infection, inflammatory and malignant diseases⁷. Therefore, neopterin production appears to be closely associated with activation of the cellular immune system⁸ and increased concentrations are related to endothelial damage and risk for septic complications⁹. In this study, we aimed to evaluate the value of serum neopterin as an early diagnostic marker for early onset sepsis during the first 48 hours and to compare the three variables, serum neopterin level, Rodwell's hematological sepsis score and C-reactive protein for predicting infection and outcome in neonates with sepsis.

2. Patients and Methods

The study was conducted on 60 neonates of different gestational age, who were admitted to the NICU in El Kasr El-Aini and Abou El-Reish Pediatric Hospitals, Cairo, Egypt between June 2007 and August 2008 after collecting signed approval consents from their parents. According to their clinical picture they were divided into 3 groups. Group (1) had a proven clinical picture of sepsis and group (2) with a suspected clinical picture of sepsis. 20 neonates of matched gestational age with no evidence of sepsis served as control group (3), they were admitted to NICU for causes other than sepsis. Careful prenatal, natal and postnatal history was taken also full clinical examination was done.

Diagnosis of sepsis was based on the presence of one or more of the following clinical signs: tachypnea, respiratory distress, apnea, cyanosis, lethargy, tachycardia, bradycardia, hypotonia, seizures and irritability. Laboratory criteria of sepsis were; positive blood culture, elevated C-reactive protein level >6 mg/dl and Rodwell's hematological sepsis score above 3¹⁰.

Clinically suspected infection was defined when there were (1)high risk maternal factors of sepsis such as intrapartum fever $>37.5^{\circ}\text{C}$, chorioamnionitis, premature rupture of membrane, meconium stained amniotic fluid, antepartum hemorrhage, pregnancy induced hypertension(PIH), and diabetes mellitus or (2)high risk fetal factors of sepsis such as low birth weight and meconium aspiration syndrome ,(3) non specific laboratory markers such as white blood cells count below 5000 or above 30.000cell/m³, immature/total leucocytes count >0.2 and C-reactive protein >6 mg/dl are indicative.

Blood sample collection and storage:

Two ml of blood were withdrawn from a peripheral vein after taking an informed consent from parents of patients and controls. The sera were separated by centrifugation at 3500 rpm for 10 minutes. Sera were stored at -20°C till the time of assay. A follow up sample of blood of suspected neonates was collected for estimation of the serum neopterin level after 48 hours to confirm sepsis.

Laboratory investigations included complete blood count (CBC) with differential measured by automated cell counter system Coulter T680. The differential leucocytic counts were performed manually on leishman stained blood films. Neutrophils were classified as immature (band) forms when width of the nucleus at any constriction was not less than one third of its widest portion.¹¹

Blood cultures:

Aerobic and anaerobic cultures were done on blood agar plates at 10%CO₂ and on MacConkey agar plates. Isolated colonies were further identified by examination of their colony morphology, gram stained smears and biochemical and enzymatic reactions. True bacteremia was considered when the blood culture was positive within 72 hours. If no growth was detected, the bottles were incubated up to 10 days with further subcultures every other day on solid media. If no growth appeared after 10 days of incubation, blood culture was considered negative. Antibiotic sensitivity test was done by Kirby Baur Technique¹².

Hematological Sepsis Scoring system(HSS) :

The previously validated hematologic criteria were used as indicators for hematological sepsis scoring system : (1)Abnormal total leucocyte (TLC)count <5000 or >30.000 , (2)Abnormal total neutrophil count, (3)Elevated immature neutrophil count, (4)Elevated immature to total neutrophil ratio >0.2 (I/T), (5)Immature to mature neutrophil (I/M) ratio >0.3 , (6)Platelet count less than or equal to 150.000/mm³ (7)Pronounced degenerative changes in total neutrophil count. The higher the score the greater was the likelihood of sepsis. With score ≤ 2 the likelihood that sepsis was absent was 99%¹⁰.

CRP assay:

C-reactive protein serum level was assessed by slide latex agglutination test (Rapidex CRP kit). It was considered positive when the titer was >6 mg/L.

Serum Neopterin assay:

Neopterin serum level was determined using Human Neopterin ELISA kit. These tests are species-specific >32.2nmol/l during the first 48 hours.

Statistical analysis:

Statistical Package for Social Science (SPSS) program version 9.0 was used for analysis of data. Values were summarized as mean± SD, range or as number of subjects and proportions. Non parametric test (Mann Whitney U) was used for analysis of two qualitative data. One way ANOVA was done for analysis of more than two variables followed by post Hoc test for detection of significance. Simple linear correlation (Pearson's correlation for quantitative data and Spearman correlation for qualitative data) were done to detect any relationship between the variables. P-value is considered significant if < 0.05*.

The sensitivity and specificity for the measured variables were calculated according to construct the receiver operator characteristic curves.

3. Results:

The demographic characteristics of the study and control groups are summarized in table (1). There were no significant differences in gestational age means (35.3 versus 35.6 v 35.7 weeks), birth weight means (2.2 v 2.6 v 2.7 kg), Apgar scores at one and five minutes, and male to female ratio between the infected, suspected, and control groups respectively.

Neonatal sepsis was predisposed by maternal and neonatal risk factors. Premature rupture of membrane (PROM) occurred in 8 neonates (40%) of the infected group while in the suspected group it occurred in 9 neonates (45%). 12 neonates (60%) of the infected group were delivered preterm and in the suspected group 11 neonates (55%) were delivered preterm. Maternal temperature more than 38°C was detected in mothers of 2 neonates (10%) of the infected group and in 4 mothers (20%) of the suspected group. There were no significant changes between the 3 groups, table (2).

The most frequent clinical presentations of septic and suspected cases were poor Moro (100% and 80% respectively), poor suckling reflexes (80% and 60% respectively), fever (35% and 35 % respectively), respiratory distress (100 % and 65% respectively), tachycardia (55% and 25% respectively), lethargy (60% and 45% respectively) and hypotonia (65% and 50% respectively), (Fig.1) .

Pathogenic organisms were isolated from blood cultures of all neonates of the infected group (group I), gram negative organisms were the most common pathogens identified (80%). The most common species identified were Klebsiella (7cases, 35%),

Pseudomonas (4cases,20%), Enterobacter (3cases,15%), Staph Coagulase Negative (2cases,10%), followed by gram positive organisms which were predominant (20%) of the neonates, they were Staphylococcus aureus (2 neonates, 10%), and Streptococcus (2 cases,10%). In group II, gram negative organisms were also predominant, they were isolated from 4 (70%) neonates, they were Klebsiella (6 cases, 30%), Pseudomonas (3 cases, 15%), Enterobacter (2 cases, 10%), Staph Coagulase Negative (3cases, 15%), followed by gram positive organisms in 6 cases (30%) of the neonates, they were Staphylococcus Aureus (3 neonates, 15%), and Streptococcus (3 cases, 15%). The causative bacterial agents are listed in table (3).

Different laboratory parameters of the three studied groups are shown in table (4). The I/T ratio and I/M ratios were significantly higher in the infected and the suspected groups compared to the control group, while platelet and RBC counts were significantly lower ($p=0.001$) in both the infected and the suspected groups compared to the control group.

Serum levels of neopterin of newborns in the sepsis group had a mean of 51.6 ± 22.2 nmol/l ranging between 28.0 - 124.0 nmol/l, while in the suspected group, it ranged between 26.0- 91.0 nmol/l with a mean of 41.4 ± 17.6 nmol/l in the first day of life and ranged between 40.0-147.0 nmol/l with a mean of 66.5 ± 24.0 nmol/l in the third day of life. In the controls, it ranged between 2.4-38.0 nmol/l with mean of 12.8 ± 9.7 nmol/l. The level of serum neopterin was significantly higher in cases than controls ($P=0.0001$) being higher in infected group, as shown in table (4).

Mean serum CRP levels, in the neonates with proven sepsis were found to be significantly higher than in the suspected and control groups. ($P=0.0001$). These levels are shown in table (4).

In the group with confirmed sepsis, a highly significant positive correlation was found between serum neopterin level and the serum CRP level ($r=0.8$, $p=0.0001$) it correlated as well significantly with the sepsis score ($r=0.5$, $p=0.04$). A significant positive correlation was also detected between sepsis score and serum CRP level ($r=0.5$, $p=0.01$), Table (5).

In the septic neonates, serum neopterin level correlated positively with the maternal age ($r = 0.5$, $p=0.02$) and gravidity ($r = 0.5$, $p= 0.01$). On the other hand it correlated negatively with the gestational age with ($r=-0.4$, $p=0.07$). No significant correlations between serum neopterin concentration, sepsis score and serum CRP with the other demographic data of the infected neonates were detected. As regards the clinical manifestations, 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 ($r=0.2, p=0.05$) table (5).

In this study 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). This was also the case regarding the serum CRP level, which was significantly higher in dead septic neonates compared with those who survived with a significant p value of 0.002 table (6).

The sensitivity, specificity, positive and negative predictive values of neopterin, CRP and

HSS for determining neonatal sepsis are summarized in table (7). In case of neopterin, it was found to be 78.9%; sensitive in identifying sepsis, the specificity was 95%; the predictive value of a positive test was 93.8%, while that of a negative test was 82.6%. The sensitivity of CRP was found to be 65.1%, specificity 95.5%, positive predictive value 97%, negative predictive value 60.5%, while the HSS sensitivity was 63.4%, specificity 100%, positive predictive value 100% and negative predictive value 57.1%.

Table (1): Demographic and clinical characteristics of the studied neonates

Variables	Infected (n=20)	Suspected (n=20)	Controls (n=20)	P-value
Maternal age (yrs) mean±SD range	27.2± 6.7 18-40	27.5 ± 5.8 18-39	26.7 ± 6.1 17-40	0.7
Gestational age (wks) mean±SD range	35.3 ± 3.1 29-39	35.6 ± 3.6 29-40	35.7 ± 4.1 28-40	0.5
Birth weight(kg) mean±SD range	2.2 ± 0.8 1-3.5	2.6 ± 1.1 1.1-4.1	2.7 ± 1.1 1.1-4.2	0.2
Apgar1min mean±SD range	4.4 ± 0.6 3-5	4.5 ± 1.5 2-7	6.3 ± 1.8 5-8	0.09
Apgar 5 min mean±SD range	8.9 ± 0.4 8-9	9.0 ± 0.6 8-10	9.2 ± 0.5 8-10	0.09

Table (2): Risk factors for neonatal sepsis in the different studied groups

Variables	Infected No. (%)	Suspected No. (%)	Controls No. (%)
Sex			
Males	12 (60)	13(65)	12(60)
Females	8 (40)	7 (35)	8(40)
Maternal risk factors			
PROM>18h	8 (40)	9 (45)	0
Fever>37.8	2 (10)	4 (20)	0
Diabetes	2 (10)	1 (5)	0
Pregnancy ind. hypertension	1 (5)	1 (5)	1 (25)
Polyhydramonos	2 (10)	1 (5)	1 (25)
Meconal amniotic fluid	5 (25)	2 (10)	0
Neonatal age			
Preterm	12(60)	11(55)	9 (45)
Full term	8(40)	9(45)	11 (55)
Low birth weight < 2499 g	9(45)	8(40)	6(30)
Mode of Delivery			
Vaginal delivery	11(55)	12(60)	11 (55)
Cesarean section	9(45)	8(40)	9 (45)
Sepsis score			
< 3	2(10)	1 (5)	20 (100)
> 3	18(90)	19 (95)	0
Outcome			
Died	7(35)	9 (45)	2(10)
Survived	13(65)	11(55)	18(90)

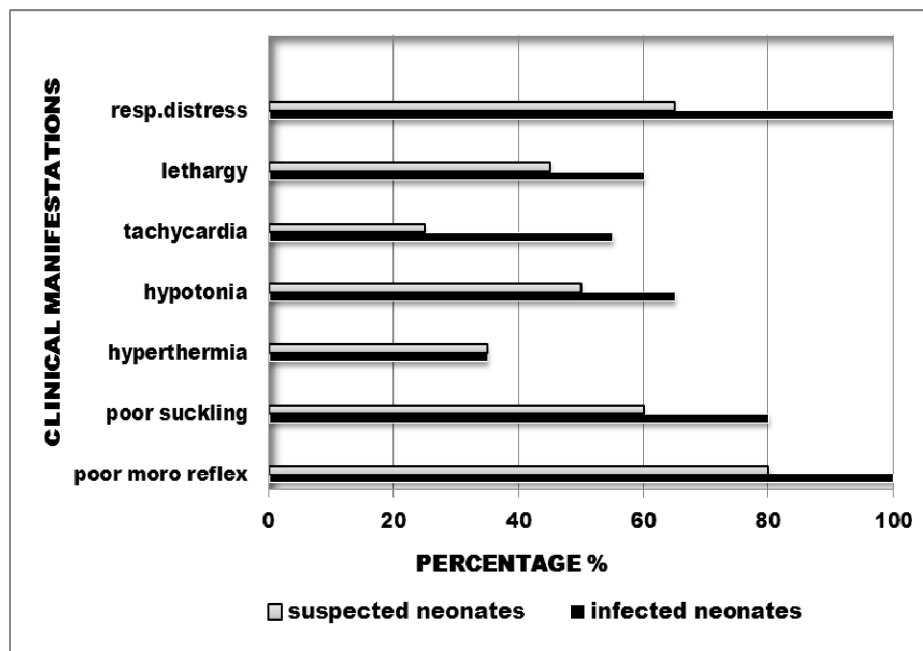


Fig. (1) Clinical manifestations of infected and suspected neonates

Table (3) Organisms isolated from blood culture of infected and suspected neonates

Organisms isolated	Infected No (%)	Suspected No (%)	P value
Gram negative bacteria	16(80%)	12(60%)	NS
Klebsiella	7 (35)	6 (30)	
Enterobacter	3 (15)	2 (10)	
Pseudomonas	4 (20)	2 (10)	
Staph coagulase -ve	2 (10)	2 (10)	
Gram positive bacteria	4(20%)	8(40%)	NS
Staph aureus	2 (10)	4 (20)	
Streptococcus	2 (10)	4 (20)	

Table (4): Laboratory data of the studied neonates

Variables		Infected	Suspected	Control	P-value
TLC (10 ³ /ul)	Range	2.9-53	2.8-40.6	5.4-18.2	0.8
	Mean+SD	13.4± 12.6	17.8± 14.9	11.5± 43.6	
Total Neutrophil (10 ³ /ul)	Range	1.2-22.9	11.3-27.0	2.1-7.0	0.9
	Mean+SD	7.74± 6.88	9.21± 9.81	5.02±1.99	
Immature Neutrophil (10 ³ /ul)	Range	1.7-52	2.5-72.9	0.2-1.1	0.3
	Mean+SD	16.89± 17.28	21.96± 23.97	6.59± 2.97	
I/T ratio	Range	0.2 -0.3	0.2 - 0.3	0.1- 0.2	0.0001*
	Mean+SD	0.2± 0.04 ^a	0.2± 0.03 ^a	0.1± 0.02 ^b	
I/M ratio	Range	0.2 - 0.4	0.2 - 0.4	0.1 - 0.2	0.0001*
	Mean+SD	0.2± 0.04 ^a	0.3± 0.05 ^a	0.1± 0.04 ^b	
Hb (g/dl)	Range	7.6-16.6	6.5-13.4	9.7 -17.3	0.0001*
	Mean+SD	10.4± 2.7 ^a	9.3± 2.0 ^a	14.1± 2.0 ^b	
RBCs (10 ¹⁰ /ul)	Range	2.2 – 5.5	2.1-6.2	3.4-6.2	0.0001*
	Mean+SD	3.9 ± 0.8 ^{ab}	3.6 ± 1.0 ^a	4.5 ± 0.7 ^b	
Platelet (10 ³ /ul)	Range	56.7-382.0	43.0-253.0	101.0-370.0	0.0001*
	Mean+SD	88.50± 99.07 ^a	130.5± 55.3 ^b	247.3± 82.5 ^c	
CRP (mg/l)	Range	12-48	0-6	0-3	0.0001*
	Mean+SD	28.8±16.7	2.9±1.1	1.3±0.7	
Neopterin (nmol/l)	Range	28-124	(Day 1) 26-91 41.4± 17.6a	2.4 - 38	0.0001*
	Mean+SD	51.6± 22.2 ^a	(Day 3) 40-147 66.5±24a	12.8± 9.7 ^b	
HSS	Range	3-7	3-7	0-1	0.0001*
	Mean+SD	5.6±1.3	5.1±0.9	0.2±0.4	

P value is significant if < 0.05*

Different symbols indicate significance

Table(5): Correlation between serum neopterin, CRP levels and sepsis score with demographic and clinical data of the septic neonates.

Inflam. markers	MA	GA	BW	Gravidity	AS(1)	AS(5)	Resp. Distress	Lethargy	Neopterin	CRP	HSS
Neopterin											
r	0.5	-0.4	-0.3	0.5	0.3	-0.01	0.5	0.2	—	0.8	0.5
p	0.02*	0.07	0.2	0.01	0.2	0.9	0.03*	0.05*		0.0001*	0.04*
CRP											
r	0.5	-0.3	-0.3	0.5	0.3	-0.2	0.5	0.1	0.8	—	0.5
p	0.02*	0.2	0.36	0.02*	0.2	0.4	0.03*	0.6	0.0001*		0.01*
HSS											
r	0.2	-0.1	-0.01	0.2	-0.1	-0.1	0.3	-0.2	0.5	0.5	—
p	0.5	0.7	0.9	0.5	0.7	0.6	0.2	0.3	0.04*	0.01*	

Maternal age (MA) Gestational age (GA), Agar score (AS), C reactive protein (CRP)

P-value is significant if < 0.05*

Table (6): The relation of serum neopterin level, serum CRP level and sepsis score with the outcome of the infected group.

Variables	Survival Mean ± SD N= 13	Death Mean ± SD N= 7	p-value
Neopterin (nmol/L)	41.3 ± 11.4	70.8 ± 25.4	0.001*
CRP (mg/L)	20.3 ± 13.3	44.6 ± 9.1	0.002*
Sepsis score (HSS)	5.2 ± 1.4	6.3 ± 0.8	0.1

value is significant if < 0.05

P-

Table (7): Sensitivity, Specificity and Predictive values of neopterin, CRP serum levels and HSS as a marker in early onset neonatal sepsis

Variable (Cut-off)	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Neopterin 32.2(nmol/L)	78.9%	95 %	93.8 %	82.6 %
CRP 6 (mg/dl)	65.1%	95.5%	97.5%	60.5%
HSS	63.4%	100%	100%	57.1%

The multiple regression analysis of neopterin was done and revealed that the most important factor affecting neopterin level was serum CRP ($r^2=0.63$, p value= 0.0001).

4. Discussion

Sepsis and septic shock in newborn infants have a high risk of morbidity and mortality. Despite advances in medicine, diagnosis of neonatal sepsis

remains as a major challenge. Early clinical signs are nonspecific and the laboratory criteria are also not fully reliable. 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 haematological and biochemical markers such as immature/total neutrophil ratio, platelet count, C-reactive protein (CRP), various cytokines have been proposed as being useful indicators for early identification of septic infants¹³.

Incidence of sepsis increases with low birth weight and preterm infants, due to the relatively immunodeficiency condition and the possibility of undergoing some invasive monitoring procedures¹⁴. In our study, 60% and 55% of the infected and suspected group respectively were born preterm while 40% and 45% respectively had low birth weight. This goes concomitantly with other previous reports where prematurity and low birth weight were found to have higher incidence of sepsis and high case fatality rates^{15,16}. Prolonged leaking and premature rupture of membranes is considered as a major risk factor for sepsis because of the danger of ascending infection. In the present study, 40% of the neonates with sepsis and 45% of suspected had PROM. Veskari et al., (2000)¹⁷ reported that premature rupture of membranes occurred in 19% of his cases and reached up to 75% of cases in the study done by Khalada et al, (2010)¹⁸.

Most previous studies have shown a predominance of males among neonates with neonatal sepsis¹⁹, this is confirmed in our study, were males formed around 60% of the patients. A gene located on x-chromosome has been postulated to be involved in the function of thymus or with synthesis of immunoglobulins²⁰.

Klebsiella dominated the organisms isolated from the blood culture (35%), followed by Pseudomonas (20%), Coagulase Negative Staphylococci (10%), Group B Streptococci (10%), Staph. Aureus (10%) and Enterobacter (15%). These pathogens are commonly responsible for early onset disease as in other studies done in Egypt^{21,22,23,24}. In most of the developing countries, gram-negative bacteria form the majority of the isolates in neonatal sepsis where Klebsiella was the commonest isolate recovered in Tanzania²⁵ and in Nigeria it was E. coli followed by Staphylococcus aureus²⁶. The predominance of an organism causing septicemia in the unit can be due to selective pressure of antibiotics, this has been found to be true with neonatal septicemia due to Klebsiella pneumoniae²⁷.

Rodwell et al. 1988¹⁰ evaluated the role of hematologic findings as a screening test for neonatal sepsis, one must keep in mind, however, the wide variability in the diagnostic accuracy of leukocyte indices in neonatal sepsis, especially the band count and its derived immature/total neutrophil ratio⁴. The HSS has practical advantages; it is applicable to all

infants, including those who have received antibiotic therapy prior to evaluation and simplifies the interpretation of hematologic profile¹⁸. In the present study, total leucocytic count (TLC) was not much informative for the diagnosis of neonatal sepsis, this may be because septic infants, in contrast to adults in whom haematopoiesis is developmentally mature, may deplete their neutrophil reserve and develop neutropenia during overwhelming infection²⁸. Thurlbeck and Meintoch (1987)²⁹ also stated that the TLC is often unhelpful in the diagnosis of sepsis because the normal range is wide and varies with gestational age and postnatal age. 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 controls, these results are concordant with the results of Varsha et al, (2003)³⁰ and Abou El-Ela et al., (2005)³¹.

Most of our studied patients in the infected group were thrombocytopenic which is similar to previous studies^{32,33}. This could be due to direct toxic injury of platelets, megakaryocytic suppression, increased peripheral consumption as in DIC or presence of immune component due to increased level of platelet associated immunoglobulins³⁴.

Although, in recent years, several new markers of infection have been investigated, some studies suggested that CRP remains to be the best sensitive and specific acute phase reactant for diagnosis of neonatal sepsis with a higher likelihood ratio for the prediction of sepsis¹³. CRP level in the septic group of our patients was found to be elevated when compared with both suspected and control groups. A highly significant positive correlation between CRP and HSS was found ($P=0.01$), this is in concordance with Black et al, 2004³⁵.

Neopterin has been proposed to aid in the diagnosis of bacterial infection³⁶. Human monocytes/macrophages produce neopterin when stimulated by interferon- γ released from activated T cells. Therefore, neopterin production appears to be closely associated with activation of the cellular immune system. Increased concentrations are related to endothelial damage and risk for septic complications³⁷. The current study revealed highly significant increase in serum neopterin concentrations in the infected and suspected groups compared with the control group

($P=0.0001$), indicating that the serum neopterin level is a good marker for diagnosis of early onset neonatal sepsis, this is in agreement with Czynewska et al, 2005³⁸. The optimal cutoff points have not been 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 this study,

serum neopterin level correlated positively with both CRP ($p=0.0001$) levels and the Hematological Sepsis Score ($p=0.04$) which are laboratory markers of neonatal sepsis pointing to their usefulness as additional markers of sepsis, this is in agreement with the study of Czyewska et al, 2005³⁸. 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 newborn³⁹. In our study, 35% of the infected group and 55% of the suspected group, died, a highly positive significant correlation was detected between the outcome of the infected neonates and serum neopterin level. This positive significant predictor of mortality in the studied patients ($p=0.02$), is similar to the results of Murr et al, 2001³⁹ and Ruokonen et al, 2002⁴⁰, who reported an increase of serum neopterin level with the severity of infection and a higher level in non-survivors.

In conclusion, neopterin may be used as a diagnostic marker for early onset neonatal 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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