ORIGINAL ARTICLES

Interleukin-6: A Sensitive Parameter for the Early Detection of Neonatal Sepsis

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Abstract:

Background: Neonatal sepsis is a major cause of neonatal mortality and morbidity throughout the world. Though blood culture is the gold standard and has higher sensitivity and specificity over the hematological value and cytokine, it is not available in our community health situation and also in most of health care facilities. It is also time consuming. Therefore hematological value and interleukin-6 can be evaluated for the early diagnosis of neonatal bacterial infection. **Objective:** This study was conducted to see the usefulness of IL-6 as an early marker of neonatal sepsis and also to compare the sensitivity in comparison with CRP, hematological value and blood culture. **Study Design:** It was a quasy experimental study. **Setting:** This study was carried out in the neonatal unit of pediatric department, BSMMU during the period of September, 2005 to February, 2006. **Method:** Forty five suspected septic cases were enrolled in the study and thirty healthy newborn were taken for comparison. Venous blood sample from peripheral vein was collected on the 1st day of symptoms and/or 1st day of admission and was sent for IL-6 estimation within half an hour and estimation of IL-6 was done by using immunolyte DPC USA which employed automated chemiluminescent immunoassays. **Results:** Out of forty five cases of suspected-neonatal sepsis, IL-6 were positive in twenty five cases. In culture proven sepsis 100% cases had raised IL-6. In control group only five babies had raised IL-6. Three cases were culture positive, of which all were also positive for IL-6 (100%). Among the cases twenty six were CRP positive, of which twenty were also positive for IL-6 (76.92%). **Conclusion:** In the present study IL-6 was found to be an early marker of neonatal infection. Sensitivity was more than CRP and other hematological parameter in the first twenty four hours.

Key word: Interleukin-6, Neonatal Sepsis.

Introduction:

Neonatal infections are a frequent and important cause of neonatal morbidity and mortality. Neonatal septicemia is a clinical syndrome of systemic illness accompanied by bacteraemia occurring in the 1st day of life[1]. Neonatal sepsis may be categorized as early onset and late onset sepsis. Eighty five percent of newborns with early onset infection present with in 24 hours, five percent present at 24-48 hours and smaller percentage of patients between 48 hours and 6 days of life.

In the global perspective, the microorganisms most commonly associated with early onset infection include group B streptococcus (GBS), Escherichia Coli, Heamophilus influenzae and Listeria monocytogens[2]. In case of late onset infection causative organisms are coagulase negative staphylococci, staphylococcus arueus, E. coli, Klebsiella, Pseudomonas, Enterobacter, Candida, GBS, Serratia, Acinobacter and anaerobes[3].

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The reported incidence of neonatal sepsis varies from 7.1[4] to 38[5] per 1000 live births in Asia, from 6.5[6] to 8.9[7] per 1000 live births in Africa, and from 3.5[8] to 8.9[9] per 1000 live births in South America and the Caribbean. By comparison, rates reported in the United States and Australasia range from 1.5 to 3.5 per 1000 for early onset sepsis and up to 6 per 1000 live births for let onset sepsis, a total of 6-9 per 1000 for neonatal sepsis.[10-13].

CRP (C reactive protein), hematological parameter and blood culture have good predictive value in the detection of neonatal sepsis [14,15]. CRP is an acute phase reactant, which has been used in the diagnosis of bacterial infection in the neonates[16]. IL-6 is a proinflammatory cytokine produced by monocytes and macrophages activated by bacterial infection.[17] Interleukin-6 is one of the markers of infection which appears earlier and remains elevated in first 24 hours after which it declines. This initiates the formation of CRP which is elevated 24-48 hours after the onset of infection and persist upto the time until the infection is not resolved. As interleukine-6 plays a critical role in the induction of CRP synthesis in the liver it was

hypothesized that this cytokine could be detected earlier in the blood than CRP during the course of bacterial infection. IL-6 decreased in 24 hours to undetectable level in the majority of the infected infants [18]. Sensitivity of IL-6 in CRP negative newborns on admission was 100% in newborns with blood culture positive and clinical sepsis [18]. IL-6 was more sensitive than CRP in infected newborn on admission 73% vs 58%[18].

Though blood culture is the gold standard and has higher sensitivity and specificity over the hematological value and cytokine but this highly sensitive microbiological parameter is not available in our community health situation and it is time consuming also. Therefore hematological value and interleukin-6 can be evaluated for the early diagnosis of neonatal bacterial infection. This study was carried out to measure the level of interleukin-6 for the diagnosis of neonatal sepsis and compare the level of interleukin-6 with CRP, hematological value and blood culture.

Methodology
Study design:
It was a quasy experimental study. This study was carried out in the neonatal unit, Department of Pediatric, BSMMU during the period of September 2005 to February 2006. Forty five cases of suspected septicemia were enrolled in the study and thirty healthy newborn were taken for comparison. The samples were randomly collected from neonatology unit, Department of Pediatrics BSMMU. In this hospital, high risk neonates of suspected sepsis are referred from obstetric department of the same hospital (in born) as well as from different hospitals and clinics of Dhaka and outside Dhaka (out born).

Inclusion Criteria:
i.   Newborn babies who had more than one clinical features of suspected sepsis.

ii. In absence of clinical features the babies having risk factors (one major or two minor) were also taken as suspected septic cases.[19]

Risk factors:

Major risk factors:
Rupture of membrane > 24hrs., Chorioamniontis Maternal intrapartum fever >100.4^0/38^{0}c, sustained fetal heart rate > 160.

Minor risk factors:
Rupture of membrane > 12hrs., Maternal WBC count > 15000/cmm, Low APGAR score at 5 min. Preterm labour, Low birth weight. Foul Lochia, maternal colonization

Exclusion Criteria: Neonates with major congenital abnormality

Measurements of outcome variables:
1.   Clinical features: Lethargy, Reluctant to feed etc.
2.   Hematological variables: Total count of WBC & Platelet count.
3.   Immunological variables: CRP, IL- 6

Procedure and Technique:
After taking a careful history thorough physical examination was carried out. A specified questionnaire was designed and attendants were interviewed. The answer were compiled by the investigator.

The following investigations had been done:
In the 1st day of symptom and 1st day of admission, CBC (total leukocyte count, platelet count), blood for culture and sensitivity (C/S), C-reactive protein (CRP) and blood for interleukin-6 was done and after 48-72 hours of symptom blood for C-reactive protein (CRP) was done. On evaluation of various Haematological parameters, TLC < 5 x10^9 / L (leukopenia) or 25 x 10^9/L (leukocytosis), Thrombocytopenia if platelet count <100 x 10^9 / L was taken as standard. For blood culture in thirty cases blood was taken in conventional bottle and fifteen cases blood was taken in FAN bottle.Cytokines (IL-6) were measured using IMMULITE, DPC, USA, which employed automated chemiluminescent

immuno- assays. As per instruction in the manual provided by the company normal value of IL-6 is <10 pg/ml.CRP value was estimated by latex agglutination method with CRP kit manufactured by CHRONOLAB Ag, Switzerland. As per instruction in the manual provided by the company CRP value < 10mg/L was taken as negative. Data was compiled and statistically analyzed by using SPSS version 10.

Results
Table-I Shows that Lethargy (37.8%), reluctant to feed (33.3%), abdominal distension (33.3%), respiratory distress (31.1%), apnoea (22.2%) and hyperthermia (22.2%) were the common features of neonatal sepsis in this series.

In this study, suspected septic babies had high leukocyte count (>25000). This count was compared with IL-6 level. IL-6 had a high sensitivity (85.71%), negative predictive value (95%) with an accuracy of 56% (Table –II)

Table-III shows the comparison of decreased platelet count (<100000) with IL-6 level in suspected sepsis cases. IL-6 had high sensitivity (88%), specificity (51.35%) with an accuracy of 56%.

IL-6 level was compared with CRP positive suspected sepsis cases (Table –IV). In twenty six CRP positive cases, IL-6 was positive in twenty and among nineteen CRP negative cases IL-6 was positive in five. Here sensitivity (77%), specificity (74%), positive predictive value (80%) and negative predictive value (70%) were high with an accuracy of 78%.

In comparison of IL-6 with blood culture (Table -V) three babies had culture proven sepsis and forty two culture negative sepsis. Culture proven three babies were also IL-6 positive and among culture negative forty two babies, twenty two were IL-6 positive. Sensitivity of IL-6 was (100%), and negative predictive value was also found (100%) with an accuracy of 51.11%.

When control and case groups were compared for CRP and IL-6, the level of significance was much higher for IL-6 (<.001) than CRP (<.05) (Table-VI).

Table-I

Presenting features of the cases (n=45)

Presenting features	Number of patients	Percentage
Lethargy	17	37.8
Reluctant to feed/vomiting	15	33.3
Abdominal distension	15	33.3
Respiratory distress	14	31.1
Apnoea	10	22.2
Hyperthermia	10	22.2
Bleeding	6	13.3
Hypothermia	4	8.8
Sclerema	2	4.4

Table II

Validity of IL 6 test in comparison to total leukocyte count in cases (n=45)

Total leukocyte count		IL 6 test			
		Positive		Negative	
	No.	No.	(%)	No.	(%)
Raised (>25000)	7	6	(85.7)	1	(14.3)
Normal	38	19	(50.0)	19	(50.0)
Total	45	25	(55.6)	20	(44.4)

Sensitivity	:	85.71%
Specificity	:	50%
Positive predictive value	:	24%
Negative predictive value	:	95%
Accuracy	:	55.56%

Table III

Validity of IL 6 test in comparison to platelet count in cases (n=45)

Platelet count		IL 6 test			
		Positive		Negative	
	No.	No.	(%)	No.	(%)
Decreased (<100000)	8	7	(87.5)	1	(12.5)
Normal	37	18	(48.6)	19	(51.4)
Total	45	25	(55.6)	20	(44.4)

Sensitivity	:	87.5%
Specificity	:	51.35%
Positive predictive value	:	28%
Negative predictive value	:	95%
Accuracy	:	57.78%

Table IV

Validity of IL 6 test in comparison to CRP in cases.(n=45)

CRP test		IL 6 test			
		Positive		Negative	
	No.	No.	(%)	No.	(%)
Positive	26	20	(76.9)	6	(23.1)
Negative	19	5	(26.3)	14	(73.7)
Total	45	25	(55.6)	20	(44.4)

Sensitivity	:	76.92%
Specificity	:	73.68%
Positive predictive value	:	80%
Negative predictive value	:	70%
Accuracy	:	75.56%

Table V

Validity of IL 6 test in comparison to blood culture in cases.(n=45)

Blood culture		IL 6 test			
		Positive		Negative	
	No.	No.	(%)	No.	(%)
Positive	3	3	(100.0)	0	
Negative	42	22	(52.4)	20	(47.6)
Total	45	25	(55.6)	20	(44.4)

Sensitivity	:	100%
Specificity	:	47.61%
Positive predictive value	:	12%
Negative predictive value	:	100%
Accuracy	:	51.11%

Table VI

Comparison of CRP and IL 6 levels between cases
(n=45) and control (n=30)

Neonatal sepsis	Control (n=30)		Case (n 45)		P value
	No.	(%)	No.	(%)	
CRP					
Positive	9	(30.0)	26	(57.8)	
					<0.05[*]
Negative	21	(70.0)	19	(42.2)	
IL 6					
Positive	5	(16.7)	25	(55.6)	
					<0.001[***]
Negative	25	(83.3)	20	(44.4)	

Chi square test

*/*** = Significant

Sensitivity of IL-6 in neonatal sepsis : 55.6%

Specificity of IL-6 in neonatal sepsis : 83.3%

Discussion

In neonatal sepsis commonly observed clinical features were lethargy (37.8%), reluctant to feed (33.3%), abdominal distension (33.3%), respiratory distress (31.1%), apnoea (22.2%) and hyperthermia (22.2%). A good number of babies had more than one clinical features. Theses observations are similar to the findings of other workers[20-22]. Out of 45 cases of suspected neonatal sepsis blood culture was found positive in only 3 cases and negative in 42 cases. The validity of IL-6 test in comparison to blood culture cases sensitivity was 100%, specificity 47.61%, positive predictive value 12%, negative predictive value 100% and test of accuracy 51.11%. In many studies the incidence of culture positive sepsis was not more than 10%[23]. In our study less number of culture positive reports may be due to late arrival and sample collection after giving antibiotic. This conforms with the study of others[24]. Marked abnormality of leukocyte count was observed in only 15.5% cases, but in 84.5% patient's leukocyte count was within the normal limit. The possibility of septicemia cannot be ruled out by normal leukocyte count. The leukocyte was not increased in all cases probably due to early collection, lab errors in counting and previous low levels. The validity of IL-6 in comparison to the total leukocyte count for cases was identified i.e. sensitivity 95.71%, specificity 50%, positive predictive value 24%, negative predictive value 95%, accuracy 55.56%.

Out of 45 cases of suspected sepsis thrombocytopenia was observed in 17.8% and normal count 82.2%. The validity IL-6 test in comparison to platelet count for cases was identified i.e. sensitivity 87.5%, specificity 51.4%, positive predictive value 28%, negative predictive value 95%, accuracy of 57.8%. Out of 45 cases of suspected neonatal sepsis, CRP was raised in 57.8% and negative in 42.2%. In culture proven sepsis, 100% cases had raised CRP which is an important marker of sepsis. Similar findings were reported by other investigators [20, 24, 25]. In control group 9 babies (30%) had raised CRP. One baby had history of PROM, 5 babies had history of mild perinatal asphyxia and 3 babies had meconium aspiration syndrome (MAS), which may be associated with raised CRP [26,27]. Value of CRP compared between cases and controls was found statistically significant. Validity of IL-6 test in comparison to CPR in cases was identified i.e. sensitivity 76.92%, specificity 74.68%, positive predictive value 80%, negative predictive value 70%, accuracy 75.92%. This is consistent with the finding of Magudumana 2000[24]. In this study in cases of suspected neonatal sepsis the value of IL-6 was raised in 55.6% cases and was normal in 44.4%. This relatively high figure of normal IL-6 in cases was due to delay in bringing the patient in hospital. It is known that IL-6 is an early marker and the raised level start declining usually after 24 hours. In control group IL-6 was found positive in 5 babies (16.7%) and negative in 25 babies (83.3%). The validity of IL-6 for cases was identified i.e. sensitivity 55.6% and specificity 83.3%. The levels of IL-6 in comparison to control and cases is statistically significant (p value is <.001).

Conclusion

Interleukin-6 is a very early marker in the diagnosis of neonatal infection. In the present study IL-6 level was raised in suspected septic cases. IL-6 was positive in more cases within twenty four hours of the onset of sepsis in comparison to other tests. It may be concluded that estimation of IL-6 done at the time of onset of signs and symptoms suggestive of infection is useful in the early diagnosis of neonatal sepsis.

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