

ESTIMATION OF INTERLEUKIN-6 FOR THE DIAGNOSIS OF SEPTICEMIA IN CHILDREN

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Summary

Septicemia is a common cause of morbidity and mortality among children in developing world. Clinicians are in need of better diagnostic markers to diagnose septicemia. This study was carried out to evaluate the usefulness of interleukin-6 (IL-6) as a marker for the diagnosis of septicemia in children. Blood samples from 202 clinically suspected cases of septicemia and 20 age and sex matched healthy children were studied. On the basis of clinical features, blood culture and C-reactive protein (CRP) analysis results, study populations were categorized as culture proven septicemia 35(15.77%), probable septicemia 125 (56.31%), non-septicemic febrile patient 42(18.92%), and healthy control 20(9.0%). Out of 35 culture positive cases, IL-6 estimation was done in 32 samples. In culture proven septicemia group, 28 (87.5%) had IL-6 levels above cut-off value (>137 pg/ml). In 20 probable septicemia and 10 non-septicemic febrile group, IL-6 level above cut-off value were found in 06 (30%) and 01(10%) cases respectively. None of the healthy control group showed the concentration of IL-6 above cut-off value. The sensitivity and specificity of IL-6 were 65.38% and 95% respectively. Measurement of IL-6 may be a good marker to diagnose septicemia in children but this needs further evaluation on larger population.

Key words

Interleukin-6; septicemia; CRP

Introduction

Septicemia is the systemic illness caused by spread of microorganisms or their toxins via the blood stream¹. The incidence rate of septicemia among industrialized nations was 50 to 100 cases per 100,000 individuals and in most developing countries it was 1 in 10 intensive care unit (ICU) patients².

The reported incidence of neonatal sepsis varies from 7.1 to 38 per 1000 live births in Asia, from 6.5 to 35 per 1000 live births in Africa and from 3.5 to 8.9 per 1000 live births in South America and the Caribbean³. A study in Khulna medical college hospital on neonatal infection had shown that septicemia (34.6%) was the commonest major infection⁴. Despite all the advances in medical practices in recent years, septicemia continues to be a serious problem that needs immediate attention and treatment⁵. Blood culture, the gold standard for the diagnosis of septicemia requires up to 48 to 72 hours before the results are known and almost half of the positive samples do not show growth⁶. IL-6 is an important cytokine of the early host response to infection. Its concentration increases sharply after exposure to bacterial products and precedes the increase in CRP. Blood IL-6 has been consistently shown to be a sensitive marker for diagnosing septicemia within 72 hours of infection, the sensitivities and negative predictive values being 87-100% and 93-100% respectively⁷. So, this study has been designed to evaluate cytokine (IL-6) as biomarker for the diagnosis of septicemia in children.

Materials and methods

It was a cross sectional study, carried out in the department of Microbiology, Chittagong Medical college, during the period June 2006 to May 2007. Approval from Ethical review committee of Chittagong Medical College was duly taken. 202 clinically suspected cases of septicemia in the age group 0 day to 15 years and 20 age and sex matched healthy control were included in the study. The patients were selected randomly from the department of paediatrics of Chittagong Medical College and healthy controls were selected from the healthy children attending the patients in hospital with their parents.

Inclusion criteria

Patients were selected according to the following clinical features and laboratory findings⁸

Temperature : >38°C or <36°C

Tachypnoea :

Respiratory rate > 40/min. in children.

Tachycardia :

Heart rate > normal limit for the age of child.

Leukocyte count : Leukocytosis > 15,000/cmm. or

Leukopenia < 5,000/cmm. and/or Band form >10%

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Original

At least two of the above four conditions with one or more of the followings, with or without localized site of infection were considered as suspected septicemia case.

- Fever with chills and rigor.
- Convulsion.
- Vomiting.
- Abdominal distension.
- Poor feeding.
- Lethargy/Listlessness.
- Skin septic spots.
- Jaundice.
- Hypotension.
- Hepato-splenomegaly.
- Obtundation of mental faculty – confusion, irritability or coma.

Exclusion criteria

- Neonatal asphyxia
 - Haemorrhagic disease
 - Congenital malformation
 - Chromosomal anomalies
- Study populations were grouped on clinical features, blood culture result and CRP concentration in blood⁹ as follows-

1. Culture proven septicemia group
2. Probable septicemia group
3. Non-septicemic febrile group
4. Healthy control group

Laboratory procedure

Blood cultures were performed by conventional method using Trypticase soy broth with 1-3 ml of blood from each patient. Subcultures were done in Blood agar, Chocolate agar and Mac-Conkey's agar plates after 24 hours, 48 hours and once before discarding the culture i. e. after 14 days. All plates were examined after 18-24 hours and discarded after 48 hours if no visible growth occurs. The organisms were identified by their colony morphology, staining characters, pigment production, motility and relevant biochemical tests as per standard methods¹⁰.

2 ml of blood from each patient were collected in a clean test tube and centrifuged at 10,000 g for 5 minutes. Serum were taken by using micropipette in two eppendorp tube, 50 µl in tube no 1 and 500 µl in tube no 2. Tube no 1, was used for CRP estimation and tube no 2, was preserved at - 20°C for estimation of IL-6. Qualitative CRP estimation was done by latex agglutination method. Cytokine (IL-6) estimation was carried out by an automated method using chemiluminescence (Immulate DPC).

Results

On the basis of blood culture yield, CRP estimation results and clinical signs of septicemia, the study cases and controls were categorized into 4 groups. 35(15.77%) were blood culture proven septicemia (both blood culture and CRP positive), 125(56.31%) were probable septicemia (blood culture negative, CRP positive), 42(18.92%) were non-septicemic febrile patient (both culture and CRP negative) and 20(9%) were healthy control. Healthy control (20) subjects were included in the study population for baseline cytokine (IL-6) study (Table-I).

Among 35 culture positive cases, *S. typhi* 17(48.57%) was the most commonly isolated bacteria followed by *Klebsiella* spp. 6(17.14%) *Acinetobacter* spp. 5(14.29%), *S. aureus* 2(5.71) and *Pseudomonas* spp. 2(5.71), *E. coli* 01(2.86%), *Serratia* spp. 01 (2.86%) and *N. meningitidis* 01 (2.86%) (Fig-1)

Table II shows the results of CRP among study cases. Out of 202 cases of clinically diagnosed septicemia in children 160(79.21%) were CRP positive which includes culture positive cases and 42(20.79%) were negative.

It appears from table-III that mean IL-6 levels (pg/ml) of blood culture proven septicemia, probable septicemia, non-septicemic febrile patient and healthy control group were 527.31, 233.58, 48.04 and 3.90 respectively. Significant differences were found when blood culture positive group were compared with blood culture negative groups ($p < 0.01$).

Cut off value of IL-6 for septicemia was 137pg/ml. IL-6 values of 32 septicemia cases were in the range of 35.6 – 1000 pg/ml of which 28 (87.5%) were above the cut off value and 04(12.5) were below. The concentration of IL-6 in probable septicemia were in the range of 2-1000 pg/ml, in non-septicemic febrile patients were 2-253 pg/ml and in healthy control group were 2-12.6 pg/ml (Table-IV).

The sensitivity of IL-6 was 65.38%, specificity 95% and positive and negative predictive values were 97.14% and 51.35% respectively (Table-V).

Table I : Categorization of cases and controls in different groups. (n=222).

Study groups	No. of patients	Percentage
Culture proven septicemia (Both blood culture & CRP positive)	35	15.77
Probable septicemia (Only CRP positive)	125	56.31
Non-septicemic febrile patient (Both blood culture & CRP negative)	42	18.92
Healthy control	20	9.0
Total	222	100

Table II : Results of CRP in blood among study cases. n =202

CRP	No. of patients	Percentage
Positive (> 6 mg / l)	160	9.21
Negative (< 6mg / l)	42	20.79
Total	202	100

Table III : Mean cytokine (IL-6) level in blood among different groups of study population.

Study group	Cytokine(IL-6) pg/ml Mean ± SD	Range
Culture proven septicemia n=32	527.31± 359.30	168.01 – 886.61
Probable septicemia. n=20	233.58 ± 344.98	111.40 – 578.56
Non-septicemic febrile patient. n=10	48.04 ± 76.27	28.23 – 124.31
Healthy control n = 10	3.90 ± 3.29	0.61- 7.19

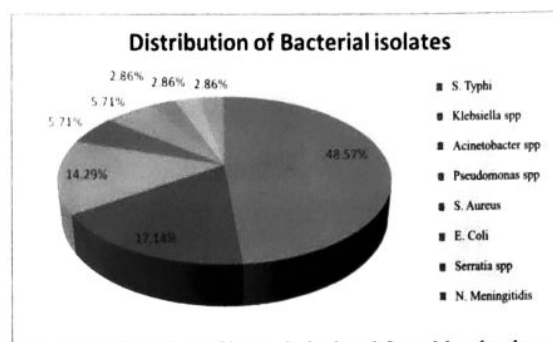
Table IV : Result of cytokine (IL-6) estimation in blood among study population.

Study group	No. of patients with IL-6 levels in pg/ml		
	>137	<137	Range
Culture proven septicemia n=32	28 (87.5%)	04(12.5%)	35.6 - 1000
Probable septicemia n=20	06 (30%)	14(70%)	2 - 1000
Non-septicemic febrile patient n=10	01(10%)	09 (90%)	2 - 253
Healthy control n=10	0	10 (100%)	2 – 12.6

Values >1000 pg/ml were considered as 1000 pg/ml and <2pg/ml were considered as 2 pg/ml.

Table V : Sensitivity, Specificity and predictive values of cytokine (IL-6) results in study population

	Cytokine (IL-6)
Sensitivity	65.38%
Specificity	95%
Positive predictive value	97.14%
Negative predictive value	51.35%

**Fig 1:** Distribution of bacteria isolated from blood culture

Discussion

In this study suspected septicemia cases were categorized on the basis of blood culture yield, CRP estimation results and clinical signs. Culture proven septicemia (both blood culture and CRP were positive) were 35(15.77%) cases, probable septicemia (only CRP positive) were 125(56.31%) and non-septicemic febrile patient (both culture and CRP were negative) were 42(18.92%) cases. Healthy control 20(9%) children were sampled for baseline cytokine levels. Similar categorization was done by Bhartiya et al. (2000)⁶ in India, Panero et al. (1997)¹¹ in Italy and Messer et al. (1996)¹² in France. Messer et al. (1996)¹² in France found 15.49% cases of proven septicemia, 49.29% probable septicemia and 35.21% cases was non-septicemic febrile patient. This is consistent with the present study.

In our study, total 35 strains of bacteria were isolated and *S. typhi* (48.57%) was the most frequently isolated bacteria, followed by *Klebsiella* spp. (17.14%), *Acinetobacter* spp. (14.29%), *Pseudomonas* spp. (5.71%), *s.aureus* (5.71%), *E.coli* (2.86%), *Serratia* spp.(2.86%) and *N. meningitidis* (2.86%). Our results are similar with those of Phetsouvanh et al. (2006)¹³ in Laos who found 50.9% *S.typhi* isolates from blood culture. Brooks et al. (2005)¹⁴ in Bangladesh also found *S.typhi* as the common pathogen (75.4%). Septicemia due to *Salmonella* was predominant in children of Bangladesh because of living in unhygienic conditions, lack of sanitation facilities and taking of unhygienic foods and unsafe water. In contrast to our findings, Ahmed et al. (2002)¹⁵ in Bangladesh, from Dhaka Shisu hospital revealed that the principal organisms were *E.coli* (30%) followed by *Klebsiella* spp (23.3%).

Weinstein et al. (1997)¹⁶ from U.S.A. reported that the most common blood stream isolate was *S.aureus* (50.6%). These different findings in USA may be due to geographical difference, increased use of invasive procedures, extensive surgery, intravascular devices and increase in the number of immunocompromized persons (Kurruvillas 1988)¹⁷. In the present study out of 202 suspected cases of septicemia in children, 160 (78.21%) patients were CRP positive (>6mg/l) and 42 (20.79%) patients were CRP negative (<6mg/l). Similarly Makhija et al. (2005)¹⁸ in India found CRP positive in 84.3% cases of suspected septicemia.

Mean level of IL-6 in culture proven septicemia, probable septicemia, non-septicemic febrile patient and healthy control group were 527 pg/ml, 233 pg/ml, 48 pg/ml and 3.90 pg/ml respectively. Significant differences were found when blood culture proven septicemia group were compared with blood culture negative groups ($p < 0.001$). Similarly Makhija et al. (2005)¹⁸ in India found that mean IL-6 level was 480 pg/ml in proven septicemia, 264 pg/ml in clinical septicemia, 160 pg/ml in non-septicemic control group and 2 pg/ml in healthy control group. Similar findings were also observed by panero et al. (1997)¹¹ in Italy and Messer et al. (1996)¹² in France.

In our study the cut off value of Il-6 was 137pg/ml for septicemia. Similarly Kallman et al. (1999)¹⁹ in Sweden used a cut-off value of 135pg/ml. Messer et al. (1996)¹² in France found a cut-off value of 100pg/ml. Some authors have proposed 50-500 pg/ml as a cut-off value (Bont et al. 1994)⁹. Variations in cut-off values are mainly due to different methods of measuring IL-6. In this study IL-6 values of 32 proven septicemia were in the range of 35.6 - > 1000 pg/ml. Out of 32 proven septicemia 28 (87.5%) showed IL-6 levels above and 4 (12.5%) cases below cut-off value for septicemia. IL-6 values of 20 probable septicemia cases were in the range of 2-1000 pg/ml. Only 6 (30%) cases had IL-6 levels above cut-off value and 14 (70%) cases showed concentration of IL-6 below cut-off value. These large false negative results might be due to sampling later in the course of infection, delay in transportation, processing and preservation of samples for cytokine study. Only one patient in non-septicemic febrile group had IL-6 value above cut-off level. None of healthy control group showed the concentration of IL-6 above the cut-off value. Similar findings were also observed by Bhartiya et al. (2000)⁶ in India who found 81.25% of proven septicemia had IL-6 levels above the cut-off value and 18.75% had levels below the cut-off point.

In the present study sensitivity, specificity, positive predictive value and negative predictive value of IL-6 were 65.38%, 95%, 97.14% and 51.70% respectively. Similarly Gaini et al. (2006)²⁰ in Denmark found sensitivity 70.3%, specificity 89.5%, positive predictive value 96.3% and negative predictive value 43.6% in diagnosing sepsis. Huang et al. (2003)²¹ in Taiwan showed that as a diagnostic test in children with sepsis serum IL-6 yielded a sensitivity of 68%, specificity of 88%, a positive predictive value of 71% and a negative predictive value of 58% which is similar to our findings. Lacour et al. (2001)²² in Switzerland found sensitivity 79% and specificity 66% and Messer et al. (1996)¹² in France showed that presence of detectable level of IL-6 in blood was 83.3% sensitive and 90.3% specific for septicemia. In the present study slightly lower sensitivity rate than others could be due to smaller size of study population.

Conclusion and recommendation

Measurement of IL-6 may be a good marker in diagnosing septicemia. It needs a long term broad based study. All the conventional tests done to diagnose septicemia have limitations. So newer methods of diagnosis should be explored.

Disclosure

All the authors declared no competing interestes

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