ESTIMATION OF IL-6 IN CSF FOR THE DIAGNOSIS OF BACTERIAL MENINGITIS

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Abstract

IL-6 level was measured in cerebrospinal fluid (CSF) from 160 clinically suspected cases of meningitis of all age. Mean IL-6 concentrations in CSF samples from patients with bacterial meningitis (2988 ± 1740pg/ml) were significantly higher than those in patients with aseptic meningitis (283±317pg/ml) and non meningitis control group (4±2pg/ml). The sensitivity of IL-6 was 95.38% while the specificity and positive predictive values were 100% and negative predictive value was 96.93%.

Introduction

Bacterial meningitis, an infection of the membranes and cerebrospinal fluid surrounding the brain and spinal cord, is a major cause of death and disability worldwide. It involves all age groups but infant and older children are especially prone to develop this disease[1]. Also in Bangladesh, acute bacterial meningitis is one of the major cause of childhood morbidity and mortality[2]. Pathophysiologic effects in Bacterial meningitis are due to production of intrathecal cytokines. Among the cytokines IL-6 is a multipotent cytokine that acts in a network of factors directing the inflammatory reaction of bacterial meningitis[3]. Cytokines may be detectable in CSF even in the patients getting antibiotic for 24 hours or more[4] CSF culture, cytology, Gram's stain and biochemical test are traditionally being done to diagnose and to differentiate pyogenic from aseptic meningitis. Culture yield is affected by prior antibiotic therapy, delay in transportation and inoculation. Gram's stain lacks specificity and has interpretation error[5]. In this event of difficulties in CSF cytological, bacteriological and biochemical results in bacterial meningitis cases we sought to measure the level of CSF IL-6 in suspected cases of meningitis. Measurement of IL–6 may be valuable in diagnosing bacterial meningitis and in distinguishing patients with bacterial meningitis from viral meningitis in both untreated and treated cases6. So this study was done to see the level of IL-6 in BM as well as distinguishing patient with BM from viral meningitis along with culture, gram stain, cytology and biochemistry.

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Materials and methods

The study was carried out in the department of Microbiology, Chittagong Medical College, during the period of April 2004 to May 2005. A total of 160 clinically suspected cases of meningitis of all ages were included in the study. The patients were selected from Paediatric and Medicine department of Chittagong Medical College and Chittagong Maa-O-Shishu Hospital. The samples were collected preferably before starting antibiotic or within 24 hours of antibiotic therapy[5].

Categorizing of patients

Bacterial meningitis :

High WBC count usually >100/ mm^3 with gross neutrophilia, positive CSF culture, Gramís stain, elevated protein (> 45 mg/dl) and decrease sugar concentration (<40mg/dl)[7,8].

Aseptic meningitis :

WBC count >5 cells/mm3 with lymphocytic predominance, elevated protein (> 45 mg/dl), normal or slightly reduced sugar concentration with negative CSF bacterial culture and Gramís stain[7,8].

Non meningitis control group :

Normal CSF cytologic and biochemical finding with negative culture and Gramís stain[7,8].

Sample

About 2ml of CSF were collected in aseptic conditions in three sterile screw capped tubes.

Laboratory procedure

Bed side inoculation of CSF was done on blood agar and chocolate agar media, placed in candle jar and incubated at 370C. MacConkeyís agar media was inoculated in the laboratory. Isolation and identification of bacteria was done by performing different appropriate tests. Immediately after reaching in the laboratory, one screw capped tube was centrifuged at 10,000g for 05 minutes. Supernants were divided in to 02 aliquots (100 &

600μL) and collected in two separate sterile eppendorp tubes & numbered 01&02 respectively. Eppendorp tube number 1 was preserved at ñ320C for cytokine study until assay was performed. Detection of cytokine (IL-6) was done by chemiluminescence technique as per manufactureís instruction. Eppendorp tube no. 02 was used for CSF protein and sugar estimation. CSF of one screw capped tube was used for cytology which was done as per standard procedure[9].

Calculation

(I) Normal value of IL-6 was determined by mean results of IL-6 in CSF of non meningitis control group + 2 SD[5].
Here mean results of IL- 6 was 4.07 and SD 2.65

Normal value = (Mean +2 SD)
= 4.07+5.3
= 9.37 pg/ml

(II) Pyogenic level of IL-6 was determined by mean value of IL-6 in CSF of aseptic meningitis+ 2 SD.
Here mean value of Il-6 was 283.44 and SD 317.33

So Pyogenic level of IL-6
= Mean value of IL-6 for aseptic meningitis +2SD
= 283.44 + 634.66
= 918.1 pg/ml

Statistical analysis

Cytokine (IL-6) level between the three study groups were compared by unpaired 't' test.

Result

A total of 160 clinically suspected meningitis patients were studied. On the basis of gram's stain, culture, cytological and biochemical findings of CSF, the study patients were categorized into 3 groups. 65 (40.62%) patients were diagnosed as bacterial meningitis, 42 (26.25%) as aseptic meningitis and 53 (33.13%) as non-meningitis control group (Table-I).

Table I : Distribution of study population in different categories (n = 160)

Study groups	No of Patient	Percentage
1. Bacterial M	65	40.62
2. Aseptic M	42	26.25
3. Non meningitis control group	53	33.13
Total	160	100.00

Cut off values of IL - 6 for bacterial meningitis were found 918 pg/ml. IL-6 values of 65 bacterial meningitis cases were found in the range of (800 - >4000) pg/ml of which 62 (95.38%) were above the cut off value (918 pg/ml) and 03(4.61%) were below. (Fig 1)

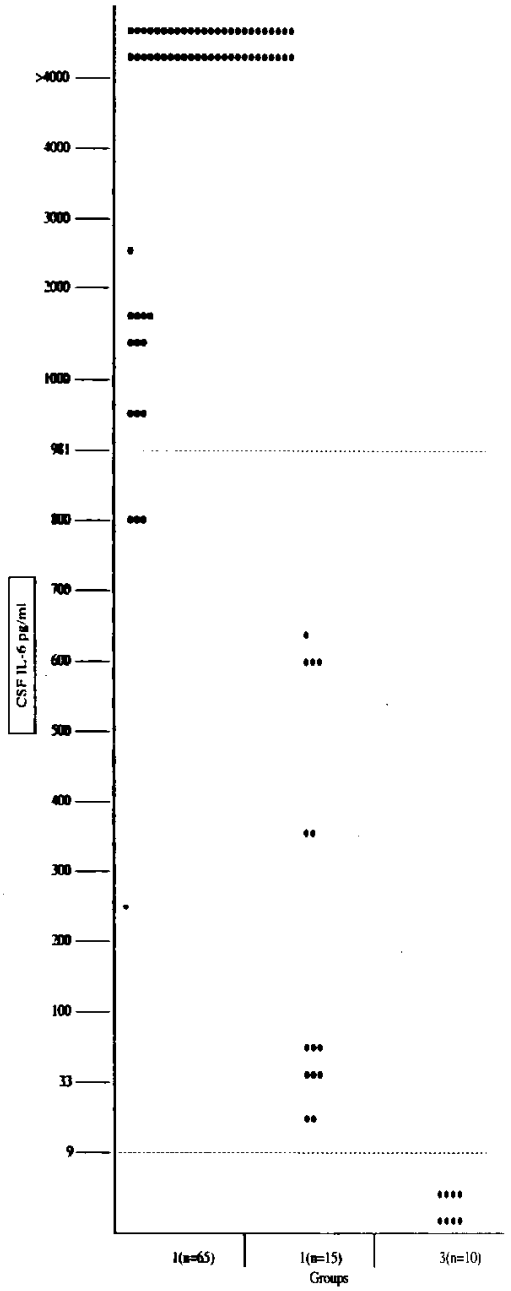

Fig 1 : CSF levels of IL-6 in patients among : bacterial meningitis, aseptic meningitis & non-meningitis control group.

The concentration of IL-6 in aseptic meningitis were in the range of (28-600) pg/ml and non meningitis ontrol groups were (1-9)pg/ml. Statistically significant difference were found when bacterial meningitis was compared with other two groups (P<. 001) (Table- II).

Table II : Result of cytokine (IL-6) estimation in CSF among study cases.

Study groups	No. of patients with IL-6 levels in pg/ml		
	9-917	>918	range
Bacterial meningitis n=65	3(4.61)	62(95.38)	800- >4000
Aseptic meningitis n=15	15(100)	0	28-600
Non meningitis control groups n=10	0(0)	0	1-9

Figure within parenthesis indicate percentages. Values > 4000 pg/ml were considered as 4000 pg/ml.

It appears from Table -III that mean 1L-6 levels (pg/ml) of bacterial meningitis, aseptic meningitis and non-meningitis control group were 2988.78, 283.44 and 4.07 respectively. Significant differences were found when bacterial meningitis were compared with aseptic meningitis and non-meningitis control groups (P<0.001).

Table III : Mean cytokine level (IL-6) in CSF among the different categories of patient.

Study groups	Cytokine (IL-6) (pg/ml)Mean ±SD	Range
Bacterial meningitis n=65	2, 988.78 ± 1740.32	1248.46 -4729.1
Aseptic meningitis n=15	283.44 ± 317.33	33.89 – 600.77
Non meningitis control group n=10	4.07 ± 2.65	1.42 – 6.72

The sensitivity of 1L-6 was 95.38%, while the specificity and positive predictive values were 100% and negative predictive value was 96.93%. (Table –IV).

Table IV : Sensitivity, specificity and predictive values of cytokine (IL-6) results in bacterial meningitis (n=65).

Study groups	Cytokine (1L-6)
Sensitivity	95.38 %
Specificity	100 %
Positive predictive value	100 %
Negative predictive value	96.93 %

Discussion

Bacterial meningitis is a life threatening illness resulting from bacterial infection of meninges. Bacterial meningitis has an unacceptable mortality rate and frequency of neurological sequellae. Mortality rates vary with age. Despite effective antimicrobial and supportive therapy mortality rates among neonates remain high. The poor outcome is partly attributed to the induction of an excessive host immune response by bacterial cell wall products that resulted in an intrathecal overproduction of cytokines and other inflammatory mediators which exert a detrimental effect on the central nervous system,[10,11]. We have studied 160 CSF samples of clinically suspected meningitis patients. These patients were categorized into 3 groups. 65(40.62%) patients were diagnosed as bacterial meningitis, 42 (26.25%) were aseptic meningitis and 53 (33.13%) were non meningitis control group. Dulkerian et al. in USA[8] detected 32.25% BM cases while Deivanayagain et al. in India[12] found 48.24% cases. Chowdhury et al. in Bangladesh[13] detected 26.92% bacterial meningitis cases. Our result was nearer to Dulkarian et al. and Deivanayagain et al. but higher than Chowdhury et al. This higher rate of detection in the present study may be due to seasonal and geographical variation in the epidemiology of BM and difference in selection criteria of study samples. In the present study the cut off value of IL-6 was ≥ 918 pg/ml for BM. 1L-6 values of 65 BM cases were in the range of (800->4000) pg/ml. Out of 65 BM cases 62(95.38%) showed IL-6 level above the cut off value (>918pg/ml) for BM and 03(4.61%) cases had IL-6 level below 918 pg/ml. None of the patients of aseptic meningitis and non-meningitis control groups showed the concentration of IL-6 above the cut off value for BM. All 15 (100%) aseptic meningitis patients had IL-6 level between 38-600pg/ml, which was not in the level of BM. Our result was closely related with Rusconi et al. in Italy[14] who found that 36(85.71%) out of 42 bacterial meningitis patients had IL-6 level above the cut off value for pyogenic meningitis and 06 (14.28%) had levels below the cut off value. None of the aseptic meningitis and non meningitis control groups had level above the cut off values for BM. Similar findings were also reported by Torre et al. in Italy[15]. In the present study 03(4.61%) cases of BM had IL-6 concentration <918 pg/ml which may be

due to intravenous administration of antimicrobial therapy prior to collection of sample, delay in transportation, processing and preservation of sample for cytokine study. In the present study mean level of IL-6 in BM, aseptic meningitis and non-meningitis control groups were 2988 pg/ml, 283 pg/ml and 4 pg/ml respectively. Similar findings were also observed by Azuma et al. in Japan[16] and Chavnet et al. (1992) in France[4]. Alhan et al. in USA[17] found that 82.4% of BM had mean IL-6 level 661 pg/ml, 50% of aseptic meningitis had 75 pg/ml. In contrast to Alhans findings the present study had higher level of IL-6. This may be due to difference in selection criteria and higher sensitivity of chemiluminescence technique used to measure cytokine. Higher level of CSF IL-6 in BM cases may suggest that these cytokines are produced in the subarachnoid space in patients with BM and probably take part in the early local inflammation. In the present study sensitivity and specificity of IL-6 were 95.38% and 100% respectively. Dulkerian et al. (1995) in USA[8] found that the presence of detectable level of IL-6 in CSF was 100% sensitive and 79% specific for BM. In the present study specificity is higher than other study which may be due to chemiluminescence technique which is at least 10 times more sensitive than ELISA. Goldsby et al[18].

Conclusion

Measurement of IL-6 may be valuable in diagnosing as well as distinguishing patients with bacterial meningitis from viral meningitis. But it can not detect the bacterial pathogen causing the disease. Antimicrobial drug sensitivity pattern is not attainable by IL-6. So it act as useful diagnostic procedure in those patients who have nonpurulent CSF and negative gram stains and cultures and in patients who have received previous antimicrobial treatment.

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