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ORIGINAL PAPER

Cerebrospinal fluid levels of interleukin-6 and interleukin-12 in children with meningitis

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Abstract
Purpose Certain cytokines play important roles in the pathophysiology of meningitis. The main purpose of this study was to investigate if the levels of interleukin-6 (IL-6) and interleukin-12 (IL-12) in cerebrospinal fluid (CSF) could be diagnostic predictors of bacterial meningitis in children.
Methods CSF was obtained from 95 patients suspected with meningitis. These cases were classified to the bacterial meningitis (n=12), aseptic meningitis (n=41), and non-meningitis (n=42) groups. The levels of IL-6 and IL-12 in CSF were measured using the enzyme-linked immmuno-sorbent assays test.
Results The CSF IL-6 levels in the bacterial meningitis group (45.2±50.0 pg/ml) were significantly higher than those in the aseptic meningitis group (12.9±10.2 pg/ml) and the nonmeningitis group (6.5±7.8 pg/ml; $p<0.05$). The CSF IL-12 levels in the bacterial meningitis group (69.8±67.1 pg/ml) were significantly higher than those in the aseptic meningitis group (22.9±10.8 pg/ml) and the non-meningitis group (15.3±11.2 pg/ml; $p<0.05$). With regard to diagnosis, the measurement of CSF IL-6 and IL-12 levels showed sensitivities of 96% and 96%, respectively, and specificities of 51% and 75%, respectively.
Conclusion It is suggested that the CSF IL-6 and IL-12 levels are useful markers for distinguishing bacterial meningitis from aseptic meningitis.

Keywords Aseptic meningitis · Bacterial meningitis · Interleukin-6 · Interleukin-12

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Introduction

Meningitis is the most common serious infection of the central nervous system (CNS). Bacterial meningitis is characterized by a high mortality rate despite today's advanced intensive medical care and more effective antibiotics treatment. The rapid diagnosis of childhood bacterial meningitis is generally believed to be essential to avoid a poor outcome [1, 2]. Recent attention has been directed towards the study of the role of cytokines in the regulation of inflammation and host responses to infection [3–7]. Studies in bacterial meningitis, both in animal models and clinical studies, suggest that the release of certain cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ), may be responsible for the severe inflammatory responses in meningitis and may correlate with morbidity and mortality [8–13].

IL-6 is produced mainly by macrophages and monocytes in infectious disease such as septicemia, endotoxemia, and necrotizing enterocolitis and plays an important role in the processes of tissue damage and the modulation of immune responses [14–17]. It is also the most important regulator of C-reactive protein (CRP) synthesis [18–21]. It has been shown that high levels of IL-6 can occur in the cerebrospinal fluid (CSF) of patients with acute CNS infection as a sensitive marker in the early diagnosis of bacterial meningitis [22, 23]. The combination of CRP and CSF IL-6 has been

proved to be useful to differentiate bacterial meningitis [23]. Interleukin-12 (IL-12) may contribute to the natural immunity to microorganisms in the CSF compartment during the acute phase of bacterial meningitis [24]. The aim of the present study was to assess the CSF IL-6 and IL-12 levels in children with meningitis and the roles as predictors of bacterial meningitis.

Materials and methods

From January 1999 to January 2003, 95 patients admitted to the pediatric ward of Taipei Veterans General Hospital with suspected meningitis were enrolled into this study. Sixty-three patients were boys and 32 were girls. Their ages ranged from 1 month to 15 years old. These patients were admitted for acute febrile illness with CNS symptoms requiring a lumbar puncture to rule out CNS infection. CSF samples were taken, and CSF routine and CSF cultures for bacteria, virus, and fungus were done on the first day of admission. Those patients with other severe infection, e.g., acute otitis media and pneumonia or medicated with antibiotics in the past 4 weeks were excluded.

According to the clinical picture and CSF findings, the patients were divided into three groups: (1) the first group was bacterial meningitis, defined as patients with a positive bacterial culture from the CSF samples (n=12); (2) the second group was aseptic meningitis, defined as patients with a positive CSF culture for the virus or pleocytosis ($\geq$5 mm^{-3}) in CSF with negative CSF bacteriologic studies (including CSF culture, CSF gram-stained smears, and bacterial antigen test) [13] or pleocytosis in CSF with a viral isolation from other sources such as a throat and rectal swab (n=41); (3) acute febrile patients without meningitis were served as controls. These patients were admitted for acute febrile illness with CNS symptoms requiring a lumbar puncture to rule out CNS infection (n=42).

According to the total duration of fever (including before admission and after admission), the bacterial and aseptic meningitis patients were further divided into two groups: one group of those with fever longer than 3 days and one group of those with fever three or less days. According to the symptoms of seizure attack, one group was classified with seizure attack and the other group was classified without seizure attack. Blood and CSF samples were taken on the first day of admission, and CSF and centrifuged serum were stored at −70°C until assay. CRP in serum was measured by immunonephelometry (Behring, San Jose, CA, USA).

Cytokines assays

The IL-6 and IL-12 levels were determined using a commercially available enzyme-linked immmunosorbent assays test (R&D systems, Minneapolis, MN, USA). The minimum detectable concentration for IL-6 was 0.7 pg/ml and for IL-12 was 5.0 pg/ml.

Statistical analysis

All of the data were expressed as mean ± standard deviation. One-way analysis of variance followed by Scheffe's multiple range test was used to compare CSF IL-6 and IL-12 levels and serum CRP levels among the bacterial meningitis, aseptic meningitis, and nonmeningitis groups. Mann–Whitney U test was used to compare CSF IL-6 and IL-12 levels and serum CRP levels between the patients with different clinical manifestations in the bacterial and aseptic meningitis groups. For all tests, a p value below 0.05 was considered significant. The sensitivity (percent of positives detected correctly identified), specificity (percent of negatives detected correctly identified), and predictive value (percent of class matching) of each subject tested were determined.

Results

In these 95 patients, the mean of fever duration before admission were 1.4±0.6 days. The mean of fever duration before admission was 1.2±0.2 days in the bacterial meningitis group, 1.8±0.7 days in the aseptic meningitis group, and 1.7±0.8 days in the nonmeningitis group. There were no significant differences in the fever duration before admission among these three groups. In the bacterial meningitis group, the etiology agents were: *Streptococcus pneumoniae* (n=4), Group B streptococcus (n=2), *Hemophilus influenza* (n=2), *Enterobacter* spp. (n=1), *Pseudomonas aeruginosa* (n=1), *Streptococcus morbillorum* (n=1), and *Streptococcus viridans* (n=1).

The comparison of CSF IL-6 and IL-12 levels and serum CRP levels in children with meningitis is shown in Table 1. The CSF IL-6 levels in the bacterial meningitis group (45.2±50.0 pg/ml) were significantly higher than those in the aseptic meningitis group (12.9±10.2 pg/ml) and nonmeningitis group (6.5±7.8 pg/ml; p<0.05). The CSF IL-12 levels in the bacterial meningitis group (69.8±67.1 pg/ml) were significantly higher than those in the aseptic meningitis group (22.9±10.8 pg/ml) and nonmeningitis group (15.3±11.2 pg/ml; p<0.05). The serum CRP levels in the bacterial meningitis group (8.8±9.3 mg/dl) were significantly higher than those in the aseptic meningitis group (1.9±4.8 mg/dl) and nonmeningitis group (0.8±1.8 mg/dl; p<0.05). There were no significant differences in CSF IL-6 and IL-12 levels and serum CRP levels between the aseptic meningitis and nonmeningitis groups.

The CSF IL-6 and IL-12 levels and serum CRP levels in different clinical manifestations of the bacterial meningitis

Table 1 Comparison of cerebrospinal fluid interleukin-6 (IL-6) and interleukin-12 (IL-12) levels and serum C-reactive protein (CRP) levels in children with meningitis

	IL-6 (pg/ml)	IL-12 (pg/ml)	CRP (mg/dl)
Bacterial meningitis (n=12)			
Mean ± SD	45.2±50.0[a,b]	69.8±67.1[a,b]	8.8±9.3[a,b]
Median (range)	48.5 (3.9–115.2)	73.2 (8.5–157.4)	10.7 (0.9–20.4)
Aseptic meningitis (n=41)			
Mean ± SD	12.9±10.2	22.9±10.8	1.9±4.8
Median (range)	10.6 (1.1–29.7)	19.8 (7.9–32.5)	0.8 (0.4–7.4)
Nonmeningitis(n=42)			
Mean ± SD	6.5±7.8	15.3±11.2	0.8±1.8
Median (range)	4.4 (0.7–17)	13.5 (7.2–33.4)	0.4 (0.3–3.2)

Data were expressed as mean ± SD
[a] Significant differences between bacterial meningitis and aseptic meningitis (p<0.05)
[b] Significant differences between bacterial meningitis and nonmeningitis (p<0.05)

and aseptic meningitis groups are listed in Table 2. In the bacterial meningitis group, the CSF IL-6 levels in these patients with fever >3 days (52.8±61.2 pg/ml) are significantly higher than those in patients with fever ≤3 days (30.0±37.5 pg/ml; p<0.05). In the aseptic meningitis group, the CSF IL-6 levels in these patients with fever >3 days (21.5±16.6 pg/ml) are significantly higher than those in patients with fever ≤3 days (8.9±6.7 pg/ml; p<0.05), and the CSF IL-12 levels in these patients with seizure attack (34.4±22.9 pg/ml) were significantly higher than those patients without seizure attack (21.3±8.9 pg/ml; p<0.05).

The sensitivity, specificity, and predictive values of positive and negative of CSF IL-6 and IL-12 levels to distinguish bacterial meningitis from aseptic meningitis are shown in Table 3. The sensitivities of CSF IL-6 and IL-12 levels to distinguish bacterial meningitis from aseptic meningitis are 96% and 96%, respectively; the specificities of CSF IL-6 and IL-12 levels are 51% and 75%, respectively.

Table 2 Cerebrospinal fluid interleukin-6 (IL-6) and interleukin-12 (IL-12) levels and serum C-reactive protein (CRP) levels in different clinical manifestations of bacterial and aseptic meningitis

	IL-6 (pg/ml)	IL-12 (pg/ml)	CRP (mg/dl)
Bacterial meningitis (n=12)			
Duration of fever (days)			
>3 days (n=7)	52.8±61.2[a]	75.9±73.5	9.1±10.8
≦3 days (n=5)	30.0±37.5	57.6±56.2	8.1±5.0
Seizure attack			
Yes (n=4)	48.5±52.1	77.5±74.3	9.7±10.5
No (n=8)	40.6±46.8	59.0±56.8	7.5±7.9
Aseptic meningitis (n=41)			
Duration of fever (days)			
>3 days (n=18)	21.5±16.6[a]	25.1±11.8	2.1±5.9
≦3 days (n=23)	8.9±6.7	21.9±10.3	1.7±4.3
Seizure attack			
Yes (n=4)	17.2±14.4	34.4±22.9[b]	3.6±5.9
No (n=37)	12.3±9.8	21.3±8.9	1.6±4.8

Data were expressed as mean ± SD
[a] Significant differences between patients with fever >3 days and ≦3 days (p<0.05)
[b] Significant differences between patients with and without seizure (p<0.05)

Discussion

The principal finding of our study is that CSF IL-6 and IL-12 levels and serum CRP levels were higher in patients with bacterial meningitis. With regard to diagnosis, measurement of CSF IL-6 and IL-12 levels showed sensitivities of 96% and 96%, respectively, and specificities of 51% and 75%, respectively. Therefore, our study suggests that CSF IL-6 and IL-12 levels may contribute to the early differential diagnosis of infection in the CNS. Our results demonstrated that higher CSF IL-6 levels were found in bacterial and aseptic meningitis patients, who suffered from fever for more than 3 days. Higher CSF IL-12 levels were also found in aseptic meningitis patients with seizure attack. These findings suggest that the CSF IL-6 and IL-12 levels seem to reflect the severity of bacterial and aseptic meningitis.

Recent studies suggested that certain cytokines may participate in the acute inflammatory responses of the CNS

Table 3 Predictive value of cerebrospinal fluid (CSF) of interleukin-6 (IL-6) and interleukin-12 (IL-12) levels and serum C-reactive protein (CRP) levels to distinguish bacterial meningitis from aseptic meningitis

	CSF IL-6 (cutoff 10 pg/ml)	CSF IL-12 (cutoff 50 pg/ml)	Serum CRP (cutoff 1 mg/dl)
Sensitivity (%)	96	96	71
Specificity (%)	51	75	79
Predictive value of positive test	0.19	0.24	0.25
Predictive value of negative test	0.98	0.98	0.96

[8–11, 14, 25, 26]. Studies on the relationship between cytokines and inflammation revealed the potential roles of TNF-α, IL-1β, IL-6, IL-8, and INF-γ in the pathogenesis of bacterial meningitis [6, 10–12]. IL-6 is a multiple potential cytokine that acts within a network of factors directing the inflammatory reaction of bacterial and aseptic meningitis [18, 27]. IL-6 is an endogenous pyrogen which acts on cells in hypothalamic regions of the brain to induce fever [28]. Moreover, CSF IL-6 measurement was markedly elevated from most patients with bacterial meningitis compared to patients with viral meningitis [22, 29, 30]. Elevations in cerebrospinal fluid concentrations of IL-6 in postneurosurgical bacterial meningitis were also described [31]. CRP is an acute phase protein, and serum CRP levels is often correlated with the severity of the bacterial meningitis [28, 29]. Serum CRP and CSF IL-6 levels are considered to be useful to differentiate bacterial meningitis and viral meningitis [23]. It is concordance in our study that serum CRP and CSF IL-6 shown high sensitivity and high negative predictive values.

IL-12 is an important inducer of T helper type 1 cells [32]. CSF IL-12 levels were significantly elevated in patients after trauma [33]. Kornelisse et al. suggested that IL-12 may contribute to the immunity against microorganisms in bacteria meningitis [24]. However, Mastroianni et al. showed that CSF TNF-α and INF-γ were elevated in patients with tuberculosis meningitis (TBM), while CSF IL-12 was undetectable in all TBM patients [34]. An increase in interleukin-12 mRNA was detected in the brain of mice having undertaken pilocarpine-induced seizures, but the roles of IL-12 in human seizure have not been documented yet [35].

The circulating levels of IL-6 and IL-12 were elevated in sera from patients with various infectious diseases [36–38] and in CSF from patients with bacterial meningitis [24]. These findings were also confirmed by the present study. In conclusion, this study suggests that the CSF IL-6 and IL-12 levels are useful markers for distinguishing bacterial meningitis from aseptic meningitis.

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