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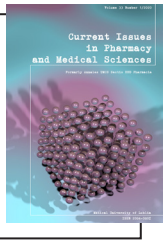

Cerebrospinal fluid inflammatory markers in children with aseptic meningitis

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ABSTRACT

Aseptic meningitis is one of the most common inflammatory disorders of the meninges of the central nervous system (CNS). The aim of our study was to investigate the cytokine profiles in the CSF and in the serum of children with aseptic meningitis to determine their role in CNS inflammation. Sixty-eight (68) children were kept under observation. Cytokine profile of CSF and blood (based on the results of determining of IL-1β, IL-4, IL-10, TNF-α levels) and procalcitonin in children were revealed, meningitis severity were estimated by AMSS score. It was found that in the majority of patients with aseptic meningitis, the levels of IL-1β, TNF-α, IL-10 in CSF were increased and exceeded the serum cytokines levels. The severe course of meningitis was characterized by significantly higher concentrations of IL-1β and TNF-α in CSF, which was confirmed by positive correlation between AMSS score and IL-1β concentration ($r=0.46$, $p<0.01$), IL-10 ($r=0.32$, $p<0.01$), TNF-α ($r=0.62$, $p<0.05$). The IL-10/TNF-α ratio was – 17.8. PCT level in CSF was within normal limits in the majority of patients with meningitis. Increasing of anti-inflammatory cytokine levels in aseptic meningitis contributes to preventing of excessive inflammatory/immune responses in the brain. This can cause a longer diseases course and a longer recovery period. This can an indicate active production of cytokines in the central nervous system due to intrathecal inflammation and activation of immune responses caused by viral infection, but not due to penetration across the blood-brain barrier.

INTRODUCTION

Aseptic meningitis is one of the most common inflammatory disorders of the meninges of the central nervous system (CNS). It occurs in all ages, although more commonly in children. The vast majority of aseptic meningitis cases are viral infections, with 85% caused by the non-polio enteroviruses [1,2]. Since distinguishing between aseptic meningitis and bacterial meningitis is often difficult in pediatric patients, various tools have been proposed to help clinicians to treat bacterial meningitis as early as possible and at the same time limit unnecessary antibiotic use and hospital admission for patients with aseptic meningitis [3]. Cytokines play an important role in the inflammatory process in aseptic meningitis. Therefore, levels of proinflammatory cytokines in cerebrospinal fluid (CSF) have been used successfully for the early diagnosis of aseptic meningitis, as well as in differentiating between bacterial and aseptic/viral meningitis in children.

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MATERIALS AND METHODS

This is a retrospective study of 68 children, aged 1-18 y. (average 5.1±1.7 y.), who were admitted to the Department of Pediatrics, Lviv Infectious Diseases Clinical Hospital, with the preliminary diagnosis of "aseptic meningitis" or "viral meningitis". On admission, the patients had the clinical signs and symptoms of acute meningitis (headaches, stiff neck, stupor, seizures).

The patients were divided into two groups. Group A had 47 patients with aseptic meningitis and Group B included 21 patients with symptoms of meningitis but without CNS involvement. Criteria for the inclusion of children into the meningitis group (A) included symptoms of the disease and cytosis (lymphocytic or neutrophilic counts $<1000\times10^6$/L) in the CSF (during the first days after the onset of the disease), normal or slightly elevated protein level, normal glucose concentration and lack of bacteria in the CSF culture. The control group (B) consisted of children with meningitis-like symptoms on admission, but the CSF examination disclosed a normal leucocyte and lymphocyte count; viruses and microorganisms were not detected in the CSF

during subsequent laboratory studies. On this basis and clinical follow-up, the diagnosis of meningitis was ruled out.

Blood and CSF samples for immunological studies were taken on the first day of treatment at the hospital (1.23±0.42 days from the onset of symptoms). The collected CSF and blood (serum) specimens were stored at -70°C for the analysis of IL-1β, IL-4, IL-10, TNF-α, PCT, which were conducted at the end of the study. The monoclonal antibody enzyme-linked immunosorbent assay (ELISA) kit (Vector Best, Russia) was utilized and the testing performed in the certificated laboratory of the Department of Clinical Immunology and Allergology of the Danylo Halytskyi Lviv National Medical University. The minimal concentrations detectable by the Vector Best ELISA Kit were: 1 pg/mL (IL-1β); 0,5 pg/mL (IL-4); 1 pg/mL (IL-10); 1 pg/mL (TNF-α); and 0,01 ng/ml (PCT).

The severity of the aseptic meningitis was evaluated using the Aseptic Meningitis Severity Score (AMSS) as proposed by Hikita [4]. Statistical analysis was performed with SPSS software version 19.0 (SPSS Inc., Chicago, IL). A *p* value of less than 0.05 was set as statistical significance. The Student's t-test was used for continuous variables with normal distributions between 2 groups, and the Mann-Whitney test was used for continuous variables without a normal distribution. Pearson's coefficient of correlation was applied to analyse the relationship between the AMSS and cytokines and PCT concentration in the CSF. The study protocol was approved by the Lviv National Medical University Ethics Committee.

RESULTS

The severity of the disease was identified by the intensity and duration of the hyperthermia, headache, vomiting, hyperesthesia, photophobia, disturbance of consciousness, loss of appetite, restlessness and sleepiness. Based on the AMSS score, severe disease was found in 12 (25.5%), moderate – in 25 (53.2%), and mild – in 10 (21.3%) patients. The age distribution for children with severe disease was 6.2±1.3 y. and for mild disease was 3.5±0.7 y.

Children with aseptic meningitis, compared to the controls had a longer duration of fever (2.75±0.21 d. *vs* 2.27±0.17 d., p<0.05) and a longer duration of nuchal rigidity (2.95±0.20 d. *vs* 2.08±0.16 d.) (Table 1). There was no significant difference in persistence of vomiting, nor Kernig's sign.

Blood and CSF analysis in patients with meningitis demonstrated significantly elevated WBC and a higher ESR. The patients in the control group had no abnormalities in the CSF, while the meningitis group had a markedly elevated white blood cell count and an increase in the percentage of neutrophils and lymphocytes compared to the control group. The concentration of protein and glucose in the CSF did not differ between groups (Table 1).

The upper and lower limits of cytokines and PCT reference range in CSF were determined on the basis of the 95% confidence interval of these parameters in the children of the control group. Thus, the level of IL-1β in CSF within the range of 1,44-2,94 pg/ml was recognized as normal, IL-4 – 1.74-2.42 pg/ml, TNF-α – 0.87-1.70 pg/ml, IL-10 – 4.62-8.39 pg/ml, and PCT – 0.02-0.03 ng/ml.

In patients with aseptic meningitis, the concentration of proinflammatory and anti-inflammatory cytokines in CSF exceeded those of children in the control group. Levels of IL-1β and TNF-α were more than 3 times higher and the concentration of IL-10 was more than 10 times higher, while the average was 65.83±5.91 pg/ml (Table 2).

Table 1. Symptoms of the disease and results of blood and CSF analyses

Variable	Meningitis group	Control group	p
Age (month)	108.21±49.90	105.2±57.01	n.s.
Fever duration (days)	2.75±0.21	2.27±0.17	<0,05
Vomiting duration (days)	1.15±0.11	1.18±0.07	n.s.
Nuchal rigidity duration (if this symptom was present (days)	2.95±0.20	2.08±0.16	<0.05
CBC results			
WBC (x10^9/l)	11.21±0.62	9.02±0.42	<0.05
Hemoglobin (g/l)	131.64±10.33	130.35±11.27	n.s.
Granulocytes (%)	69.62±8.11	65.33±4.41	n.s.
Agranulocytes (%)	28.38±4.23	31.37±3.18	n.s.
ESR (mm/h)	14.69±1.45	10.5±1.43	<0.05
CSF results			
WBC (x10^6/l)	164.22±22.89	3.45±0.27	<0.05
Neutrophils (%)	38.81±4.56	9.13±3.58	<0,05
Lymphocytes (%)	61.19±5.78	90.87±10.41	<0.05
Protein (g/l)	0.36±0.05	0.28±0.02	n.s.
Glucose (mmol/l)	3.63±0.04	3.82±0.27	n.s.

Table 2. Cytokines and PCT in the CSF

Variable	M	SE	95% CI	
IL-1β (pg/ml)				
Meningitis group	10.97*	1.15	8.66	13.29
Control group	2.19	0.36	1.44	2.94
TNF-α (pg/ml)				
Meningitis group	3.68*	0.36	2.96	4.39
Control group	1.29	0.20	0.87	1.07
IL-4 (pg/ml)				
Meningitis group	3.31*	0.23	1.68	8.92
Control group	2.08	0.16	0.87	8.39
IL-10 (pg/ml)				
Meningitis group	65.83*	5.91	53.93	77.73
Control group	6.50	0.90	4.62	8.39
PCT (ng/ml)				
Meningitis group	0.04*	0.001	0.03	0.04
Control group	0.02	0.001	0.02	0.03

* – p<0.05, differences between the meningitis group and the control group

Simultaneously with higher mean values of the CSF cytokine levels in children with meningitis, we found the level of the pro-inflammatory cytokine IL-1β to be higher than normal in 39 of 47 children, the TNF-α to be higher than normal in 34 of 47 children and the concentration of IL-10 exceeded the reference range in all 47 patients (Table 3). The level of anti-inflammatory cytokine – IL-4 was elevated in only 13 of 47 children of the meningitis group. In 37 patients with aseptic meningitis, the concentration of procalcitonin in CSF did not differ from the normal, and only in 10 patients was it higher than the normal reference range.

Table 3. Number of children with meningitis and with low, normal, high levels of cytokines and PCT in CSF

Variable	Lower than normal	Normal	Higher than normal
IL-1β	1 (2.1%)	7 (14.9%)	39 (83.0%)
TNF-α	-	13 (27.6%)	34 (72.4%)
IL-4	6 (12.8%)	28 (59.6%)	13 (27.6%)
IL-10	-	-	47 (100%)
PCT	4 (8.5%)	33 (70.2%)	10 (21.3%)

Comparing the concentration of cytokines in CSF with that in blood in children with meningitis and the control group, the cytokines levels in CSF were significantly higher than in serum in patients with aseptic meningitis. The levels of TNF-α and IL-1β in the CSF were also significantly lower than in serum in the control group (Fig. 1, 2).

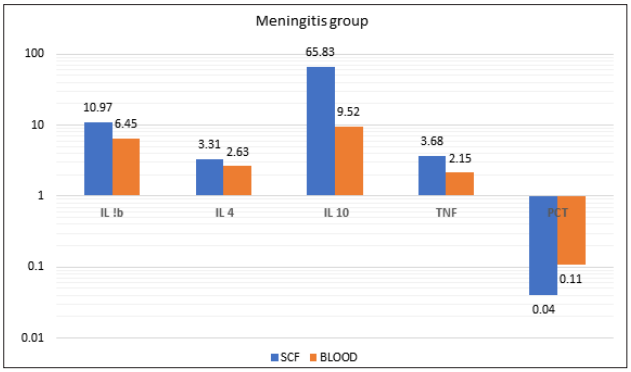

Figure 1. Cytokines and PCT levels in CSF and blood in children with aseptic meningitis (vertical axis: cytokines concentration in ng/ml; logarithmic scale)

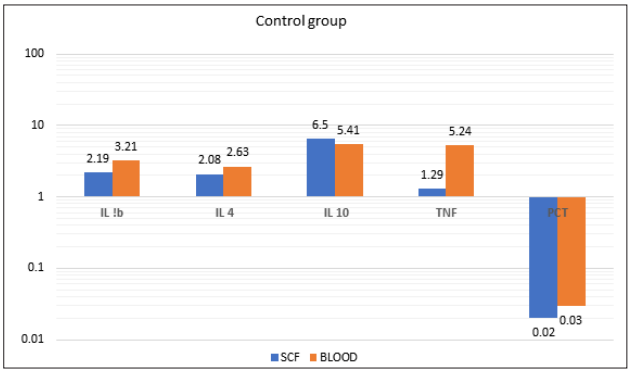

Figure 2. Cytokines and PCT levels in CSF and blood in the children of the control group (vertical axis: cytokines concentration in ng/ml; logarithmic scale)

The CSF cytokine profile also depended on the severity of the disease. A severe course of meningitis was characterized by significantly higher concentrations of IL-1β, IL-10 and TNF-α in the CSF (Table 4). There was also a positive correlation between the AMSS score and the concentrations of IL-1β ($r=0.46$, $p<0.01$), IL-10 ($r=0.32$, $p<0.01$), and TNF-α ($r=0.62$, $p<0.5$).

We also found high levels of the anti-inflammatory IL-10 cytokine in the CSF in patients with aseptic meningitis. The CSF IL-10/TNF-α ratio in children of this group was high and was equal to 17.8, and in the control group – 4.6. Increasing anti-inflammatory cytokine levels in aseptic meningitis contributes to preventing excessive inflammatory/immune responses in the brain, however, high initial levels of IL-10 (a marker of the inhibition of both innate and T-cell dependent immune responses) are gradually reduced over a long period of time. This can cause a prolong disease course and a recovery period.

Table 4. CSF cytokine levels depending on meningitis severity

Cytokine	Meningitis severity (AMSS score)		
	Mild (n=10)	Moderate (n=25)	Severe (n=12)
IL-1β (pg/ml)	3.57 (2.55-4.58)	6.99 (5.81-8.10)	21.71 (17.02-26.41) *
TNF-α (pg/ml)	1.62 (1.26-1.97)	3.02 (2.55-3.41)	6.37 (4.76-7.85) *
IL-4 (pg/ml)	2.88 (1.72-4.04)	3.15 (2.63-3.87)	4.10 (2.05-5.77)
IL-10 (pg/ml)	27.05 (22.66-32.02)	59.74 (55.15-64.80)	112.79 (93.04-144.62) *

* – $p<0.05$; the data were presented as median (95% CI), groups were compared using Kruskal-Wallis test

DISCUSSION

Inflammatory reactions of the CNS are associated with penetration through the blood-brain barrier of infectious pathogens or other molecular substances. This initiates the immune response, which leads to the activation of a cascade of inflammatory and anti-inflammatory cytokines. The nature of the inflammation is the result of the interaction between the pathogen and the mechanisms of anti-infective protection, and mostly depends on the spectrum and concentration of produced cytokines. Classically, the diagnosis of acute meningitis is made using a lumbar puncture with cerebrospinal fluid (CSF) analysis. The traditional markers of meningitis include CSF cell count, presence of proteins and glucose in the CSF, Gram stain, cultures and polymerase chain reaction detection of viruses in the cerebrospinal fluid. Blood cytokines in children with meningitis have been studied for more than ten years. Levels of the proinflammatory cytokines IL-1β and TNF-α are considered to be highly effective markers for the differential diagnosis of meningitis of viral and bacterial etiology. As an example, the concentration of IL-1β exceeding 20 pg/ml was observed in 80% of all patients with bacterial meningitis and only in 4% of all patients with viral meningitis [5]. Researchers have recently focused on the role and significance of the cytokine status in the cerebrospinal fluid in patients with meningitis. Cytokines appear in the cerebrospinal fluid due to the secretion of these substances by perivascular macrophages and resident cells of the central nervous system, such as astrocytes, glial cells and nerve cells. Some cytokines are essential components of active protection, particularly in bacterial meningitis [6]. Increased levels of TNF, IL-1β, IL-6 in CSF can be considered as biomarkers of neonatal meningitis [7]. The concentration of IL-6 may in particular be an excellent marker for meningeal inflammation; IL-6 is produced by fibroblasts, monocytes (macrophages), and lymphocytes in response to a variety of stimuli, and their presence has been associated with a variety of septic insults [8].

Significantly higher levels of cytokines in CSF in bacterial meningitis may be related to the presence of more potent inducers of cytokines production in this pathology, primarily the components of the bacterial cell wall and bacterial toxins. If immune cells of the brain (astrocytes, microglia)

identify the components of the bacteria, they will react by releasing a large number of cytokine-hormone-like mediators that attract other immune cells and stimulate other tissues to participate in the immune response. In aseptic meningitis, in comparison with bacterial meningitis, some proinflammatory cytokines were detected in CSF at relatively low concentrations, but anti-inflammatory cytokines were identified in the CSF at early stages of the illness [9].

While planning the future study of CSF cytokines levels in patients with meningitis, it must be taken into account that the concentration of pro-inflammatory and anti-inflammatory cytokines in the CSF in meningitis decreases relatively slowly in the dynamics of the disease and only during the latter few weeks does it return to normal range. Studies have shown that the time for cytokines to decrease in the cerebrospinal fluid takes much longer than in blood. It has been found in experimental studies that blood cytokines, for example, IL-2, have a half-life of only few minutes during intravenous infusion [10]. This can be the reason why in diseases with a high levels of cytokines in the CNS, e.g. in patients with bacterial meningitis, cytokines may not be detected in the blood.

The dynamics of cytokine levels in aseptic/viral meningitis is known from only a few studies with a limited number of patients, as lumbar punctures are not always repeated in patients with viral meningitis [1,4].

The Hikita study [4] showed that in viral meningitis, the concentration of cytokines in CSF depends on the causative agent of the disease. In his study, the level of IL-10 in CSF in enterovirus meningitis was 14.3 pg/ml, and in mumps, virus meningitis was 264.2 pg/ml. Determination of TNF-α, IL-6, and IL-8 levels is important for the differential diagnosis of purulent versus aseptic meningitis. This is particularly relevant for meningococcal meningitis, as these pathogens are susceptible to antibiotics and following one or more days of antibiotic treatment, the CSF is rapidly dampened and changes are detected that usually are observed in aseptic meningitis – low lymphocytic pleocytosis and slightly elevated protein content. At the same time, in patients with bacterial meningitis, the levels of the cytokines TNF-α, IL-6, and IL-8 in CSF remain high and are significantly higher than in patients with aseptic/viral meningitis [9].

CONCLUSIONS

The results of our studies show that in most patients with aseptic meningitis, the levels of the anti-inflammatory cytokines IL-1β, TNF-α and IL-10 in the CSF significantly exceed that in the control group. Moreover, PCT level in CSF was within normal limits in the majority of patients with meningitis. Also, in the patients with meningitis, in contrast to the control group, the levels of most CSF cytokines are much higher than that in the blood. This indicates active production of cytokines in the central nervous system due to intrathecal inflammation and activation of immune responses caused by viral infection, and not their penetration to the CNS via the blood-brain barrier.

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REFERENCES

1. Voloha AP. Aseptic meningitis in children. *Sovremennaya pediatriya*. 2014;1:39-43.
2. Dalwai A, Ahmad S, Al-Nakib W. Echoviruses are a major cause of aseptic meningitis in infants and young children in Kuwait. *Virol J.* 2010;7:236-7.
3. Dubos F, Korczowski B, Aygun DA, Martinot A, Prat C, Galetto-Lacour A, et al. Distinguishing between bacterial and aseptic meningitis in children: European comparison of two clinical decision rules. *Arch Dis Child.* 2010;95(12):963-7.
4. Hikita N, Seto T, Yamashita K, Iritani N, Aata M, Ogura H, et al. Relationship between severity of aseptic meningitis and cerebrospinal fluid cytokine levels. *Osaka City Med J.* 2015;61(2): 63-71.
5. Tang RB, Lee, BH, Chung, RL, Chen, SJ, Wong, TT. Interleukin-I-beta and tumor necrosis factor-alpha in cerebrospinal fluid of children with bacterial meningitis. *Childs Nerv Syst.* 2001;17:453-6.
6. Srinivasan L, Kilpatrick L, Shah S, Abbasi S, Harris M. Elevations of novel cytokines in bacterial meningitis in infants. *PLoS One.* 2018;13(2):e0181449.
7. Krebs VL, Okay TS, Okay Y, Vaz FA. Tumor necrosis factor-alpha, interleukin-I-beta and inter-leukin-6 in the cerebrospinal fluid of the newborn with meningitis. *Ar. Neuropsijuiatr.* 2005:63;7-13.
8. Vázquez JA, Adducci Mdel C, Coll C, Godoy Monzón D, Iserson KV. Acute meningitis prognosis using cerebrospinal fluid interleukin-6 levels. *J Emerg Med.* 2012;43(2):322-7.
9. Prasad R, Kapoor R, Srivastava R, Mishra OP, Singh TB. Cerebrospinal fluid TNF-α, IL-6, and IL-8 in children with bacterial meningitis. *Pediatr Neurol.* 2014;50(1):60-5.
10. Lahrtz F, Piali L, Spanaus KS, Seebach J, Fontana A. Chemokines and chemotaxis of leukocytes in infectious meningitis. *J Neuroimmunol.* 1998;85(1):33-43.