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CEREBROSPINAL FLUID INTERLEUKIN-6 CONCENTRATION IN PATIENTS WITH PURULENT, BACTERIAL MENINGITIS – OWN OBSERVATIONS

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ABSTRACT

AIM. This study aimed at evaluating the usefulness of determining cerebrospinal fluid (CSF) interleukin-6 (IL-6) concentration in adults with purulent, bacterial meningoencephalitis.
MATERIAL AND METHODS. A study group consisted of 16 patients hospitalized in the Department of Infectious Diseases of the Medical University of Silesia in Bytom in 2008 – 2012 due to purulent, bacterial meningoencephalitis. All of them were classified into two groups based on clinical severity, assessed on admission: group I – severe condition, group II – moderately severe or mild condition. CSF IL-6 concentration was measured in all patients on the first day of hospitalization.
RESULTS. Mean concentrations of IL-6 in CSF were assessed at 391.54 pg/mL and 110.51 pg/mL in patients in severe (group I) and moderately severe or mild condition (group II), respectively. Differences between CSF mean concentrations of this cytokine in both groups were statistically significant ($p<0.01$). No correlations between CSF IL-6 concentrations and other CSF inflammatory parameters were determined. Control testing performed in 5 patients of group I revealed only slight decrease of CSF IL-6 concentration in fatal cases. In case of patients who recovered from disease, IL-6 concentration in CSF was evidently decreased compared to its initial value.
CONCLUSIONS. Results suggest the usefulness of determining CSF interleukin-6 concentration to estimate inflammation intensity in the subarachnoid space, and indirectly, patient's clinical severity. IL-6 concentration may be also of prognostic importance in purulent, bacterial meningoencephalitis.

Key words: *interleukin-6, cerebrospinal fluid, purulent, bacterial meningoencephalitis*

INTRODUCTION

Bacterial infections of the central nervous system (CNS) are still an important clinical problem of modern medicine. Irrespective of advances in pharmacotherapy and intensive care, bacterial, purulent meningoencephalitis remains a condition of uncertain prognosis and relatively high mortality. Furthermore, permanent, neurological sequelae of infection are commonly reported (1). Results of routine laboratory testing of cerebrospinal fluid (CSF), i.e. pleocytosis and cytogram, concentration of protein, glucose, chloride, and to a lesser extent, lactic acid seem not to entirely reflect the actual inflammation of the brain in such diseases (1,2).

Cytokines play an important role in the pathogenesis of inflammation of the central nervous system, including i.a. tumour necrosis factor alpha, interleukin 1-beta and interleukin-6 (2,3,4).

Interleukin-6 (IL-6) is a multifunctional cytokine of various properties in both physiological and pathological processes. It is produced by different cells, including monocytes, pulmonary alveolar macrophages, fibroblasts, endothelial cells, mesangial cells, keratinocytes, B and T cells, eosinophiles and in the central nervous system (CNS) – astrocytes and glial cells. Production of IL-6 is induced by endotoxins, DNA and RNA viruses and several cytokines (interleukin-1, tumour necrosis factor, lymphotoxin, PDGF – platelet-derived growth factor, TGF-β – transforming growth factor beta). A list of the most important biological activities of IL-6 includes:

- stimulation of B cells for antibody production (humoral immune response),

- T cell activation mainly by inducing the synthesis of interleukin 2 receptor (IL-2) and stimulating the cytokine production, in the presence of IL-2 IL-6 it induces cytotoxic T cell proliferation and differentiation,
- participation in activating T cells by monocytes,
- regulation of platelet formation,
- participation in the initiation of acute-phase response,
- induction of fever by stimulating prostaglandin production (5-10).

It was demonstrated that IL-6 plays a role in the pathophysiology of several conditions such as injuries, burns, rheumatoid arthritis, septic shock, acute pancreatitis, ocular inflammatory disease and AIDS (8-10)

This paper aimed at evaluating the usefulness of determining cerebrospinal fluid (CSF) interleukin-6 (IL-6) concentration in diagnosing patients with purulent, bacterial meningoencephalitis.

MATERIAL AND METHODS

A total of 16 patients hospitalized in the Department of Infectious Diseases of the Medical University of Silesia in Bytom in 2008 – 2012 were enrolled in the study. This group consisted of 12 males (75%) and 4 females (25%) with the youngest and oldest participant aged 18 and 71 years; mean age was about 48 years. Patients were admitted to the department from a suspicion of meningoencephalitis. On a basis of CSF analysis, all patients were diagnosed with purulent, bacterial meningoencephalitis. *Streptococcus pneumoniae* and *Neisseria meningitidis* etiology were determined in 6 (37.5%) and 2 (12.5%) patients, respectively. In case of 8 patients (50%), etiological agents of meningoencephalitis were not determined.

Patient were classified based on clinical severity, assessed on admission. Group I consisted of 10 patients in severe condition (7 males and 3 females; mean age-about 55 years), presenting with disturbances of consciousness, symptoms of focal CNS injury, generalized seizures (in the period preceding hospitalization or at 1 day of hospitalization). In case of these patient, the number of points in Glasgow Coma Scale (GCS) did not exceed 9. The etiological agents of infections were: *Streptococcus pneumoniae* in 4 patients and *Neisseria meningitidis* in one patient. Etiology was not determined for 5 patients. Group II was composed of 6 patients in moderately severe or mild condition (5 males and 1 female; mean age-about 40 years). These patients did not present with severe disturbances of consciousness. No symptoms of focal CNS injury or generalized seizures were reported. The number of points in GCS exceeded 10. Infections were caused by *Streptococcus pneumoniae* (2 patients) and *Neisseria meningitidis* (1 patient). In case of 3 patients, etiology was not determined.

On admission, all patients were subject to lumbar puncture and CSF analysis, including pleocytosis and cytogram, concentration of protein, glucose, lactic acid and interleukin-6 (IL-6). Quantikine Human IL-6 Immunoassay, R&D Systems Inc. (USA) was used to measure the concentration of IL-6.

Furthermore, at day 10 of treatment, 5 patients from group I were subject to control CSF analysis. Of them, 2 patients recovered from disease and 3 patients died.

Student's t-test was used to compare mean pleocytosis, concentration of protein, glucose, lactic acid and interleukin-6 in analyzed groups. Statistical significance was set at $p\ (\alpha) < 0.05$ and $p\ (\alpha) < 0.01$. Pearson's correlation coefficient was employed to analyze the correlation between CSF parameters in both groups.

RESULTS

Table I presents the results of CSF analysis in patients with purulent, bacterial meningoencephalitis, obtained on admission.

In group I, mean pleocytosis was 639 cells/mm^3. In all patients, polymorphonuclear neutrophils predominated in cytogram (from 70% to 100% of all cells), mean concentration of protein was 1820 mg/L, glucose – 0.56 mmol/L, lactic acid – 10.75 mmol/L and interleukin-6 – 391.54 pg/mL. On admission, the general health of these patients and the course of disease were assessed as severe. Of these patients, three experienced acute respiratory distress syndrome. It was required to intubate these patients or perform tracheotomy and use respirator

Table 1. Results of CSF analysis in patients with purulent, bacterial meningoencephalitis on admission.

Group of patients	Pleocytosis (cell/mm^3)	Protein (mg/L)*	Glucose (mmol/L)	Lactic acid (mmol/L)**	Interleukin-6 (pg/mL)**
Group I (n = 10)	639 ± 444 (80 – 1210)	1820 ± 760 (831 – 2540)	0.56 ± 0.40 (0 – 1.3)	10.75 ± 4.80 (2.7 – 19.4)	391.54 ± 199.29 (79.02 –1624.31)
Group II (n = 6)	398 ± 244 (78 – 494)	841 ± 303 (470 – 1539)	0.91 ± 0.57 (0.4 – 2.3)	3.24 ± 1.02 (2.6 – 5.6)	110.51 ± 73.28 (43.22 – 611.31)

Table presents the mean values of analyzed parameters,
* - statistically significant difference ($p<0.05$)
** - statistically significant difference ($p>0.01$)

to induce artificial respiration on intensive care unit. Of them, two patients died. A total of 3 fatal cases were reported. In case of other three patients, permanent, neurological sequelae of infection were observed, i.e. deafness or partial deafness. As many as four patients recovered from disease. The highest concentrations of protein, lactic acid and interleukin-6 in CSF were noted in fatal cases.

In group II, mean pleocytosis was 398 cells/1 mm^3. In case of these patients, polymorphonuclear neutrophils also predominated in cytogram (from 65% to 88% of all cells). The mean concentrations of other CSF parameters were as follows: protein-841 mg/L, glucose-0.91 mmol/L, lactic acid-3.24 mmol/L and IL-6-110.5l pg/mL. The general health of these patients and the course of disease were determined as moderately severe or mild. Compared to group I, the outcomes of treatment were significantly better in these patients. A total of 5 cases recovered from disease. Only one patient experienced infection-induced deafness. During hospitalization there were neither patients presenting with respiratory distress nor fatal cases.

Control CSF analysis performed in 5 patients of group I revealed significant decrease of IL-6 concentration in two patients who recovered from disease. Compared to initial results, the reduction of pleocytosis and concentrations of protein and lactic acid were reported in all cases. The results of IL-6 concentration were as follows: testing I- 852.41 pg/mL, testing II-131.09 pg/mL in the first patient and testing I-799.11 pg/mL, testing II-112.00 pg/mL in the second patient. In case of three patients who died, a slight decrease of IL-6 concentration in CSF was reported: testing I-1624.31 pg/mL, testing II-1344.28 pg/mL; testing I-1311.29 pg/mL, testing II-1011.21 pg/mL, testing I-924.68 pg/mL, testing II-624.36 pg/ml, respectively. In all these cases, a high PNM pleocytosis and high concentration of protein and lactic acid in CSF were reported. Table II presents control CSF interleukin-6 concentrations in the course of disease in group I.

Table II. CSF interleukin-6 concentrations in the course of disease in group I.

Patient	Interleukin-6 (pg/mL)		Outcome
	Testing I	Testing II	
1.	852.41	131.09	recovery
2.	779.11	112.00	- „ -
3.	1624.31	1344.28	death
4.	1311.29	1011.21	- „ -
5.	924.68	624.36	- „ -

Differences between mean pleocytosis and concentrations of glucose in CSF in both groups were not statistically significant. However, statistically significant differences in the mean CSF concentration of protein ($p<0.05$), lactic acid ($p<0.01$) and interleukin-6 ($p<0.01$) in these groups were observed.

DISCUSSION

Cerebrospinal fluid analysis is the basic test in the diagnostics of the central nervous system infections. In the majority of laboratories, pleocytosis, cytogram, the concentrations of protein, glucose, chlorides, and to a lesser extent, lactic acid are routinely tested (1,2).

Since many years, attempts aimed at extending the range of diagnostic tests in CNS infections were undertaken. A list of additional CSF parameters included, i.a. the concentration of lysozyme, immunoglobulin, inflammatory cytokines, chemokines, arachidonic acid metabolites (prostaglandins, thromboxanes, leukotrienes), procalcitonin (PCT), lactate dehydrogenase (LDH), creatine kinase (CK), neuron specific enolase (NSE), ciliary neurotrophic factor (CNTF) and S100B protein. Such tests allowed for more accurate assessment of inflammation intensity and course in the subarachnoid space of patient. Such diagnostic tests, however, require considerable financial resources and well-appointed laboratories. Thus, they are not routinely performed (11-16).

Interleukin-6 is one of the most important cytokines involved in the inflammatory response in the central nervous system. It is produced within the central nervous system, i.a. by endothelial cells, astrocytes and glial cells as a response to initiated or ongoing infection. Its production is also affected by other cytokines, i.a. TNF and IL-1. IL-6 has predominantly proinflammatory properties. It induces the synthesis of acute-phase proteins and contributes to blood-brain barrier damage (17,18). Studies of other authors suggest that IL-6 may act as an anti-inflammatory cytokine. It is demonstrated by leukocyte transmission from blood to CSF (19). Nevertheless, the majority of researchers claim that proinflammatory properties of IL-6 predominate in the course of bacterial CNS infections (3,4,17,18).

Concentration of IL-6 was measured in the course of a number of central nervous system diseases. *Aiba et al.* demonstrated that IL-6 concentration in serum is a predictor in the course and outcome of influenza virus-associated encephalopathy (20). Elevated IL-6 concentration was also observed in CSF in the course of traumatic brain injuries (21). Concentration of this cytokine in CSF, as well as other cytokines (IL-1β, TNF-α, IL-8), was an important diagnostic marker in CNS infections in neurosurgical patients (22).

IL-6 role in the course of bacterial meningoencephalitis was mainly analyzed in paediatric population (23-25). In the pathophysiology of bacterial CNS infections, inflammation intensity in the subarachnoid

space is a decisive factor in the course of disease. IL-6, synthesized within CNS, is also involved in this process (2,3,26).

Studies conducted in children with bacterial meningoencephalitis suggested an increase in CSF IL-6 concentration. *Azuma et al.* concluded that IL-6 concentration in CSF is the best diagnostic marker of CNS infections in children (22). The majority of authors claim that this cytokine is used for the differential diagnosis of bacterial and viral meningoencephalitis (23,24). From the studies of *Mukai et al.* exclusively transpires that there is no statistically significant difference in CSF IL-6 concentrations between bacterial and viral meningoencephalitis (25). IL-6 concentration in CSF in the early stage of disease correlated with the severity of patient's condition. Control testing revealed a decrease of IL-6 concentration in CSF in patients who recovered from disease (24).

Similar observations on IL-6 concentration were made for adults with purulent, bacterial meningoencephalitis, especially with regard to the correlation between the severity of patient's condition and reduction of this cytokine concentration in CSF in cases who recovered from disease (27-29).

No correlation was observed between IL-6 concentration in CSF and etiology of bacterial meningoencephalitis (23,24,27-29). Such observations neither were made in studied patients.

No significant correlation was also observed between IL-6 concentration in CSF and other, routinely tested CSF parameters. In case of patients who recovered from disease, decrease of IL-6 concentration was reported concurrently with normalization of other CSF parameters in control testing (pleocytosis, protein and glucose) (23,24,27-29).

In group I, positive correlation was observed between IL-6 concentration and the concentration of lactic acid in CSF. No significant correlation was reported between PNM pleocytosis and the concentrations of protein and glucose. Observation of group II did not reveal the correlation between concentration of this cytokine and other parameters of CSF.

An interesting issue is the relation between IL-6 concentration in CSF and severity of clinical condition of patient with purulent, bacterial meningoencephalitis. The highest concentration of this cytokine in CSF was noted in patients in the most severe conditions. It suggests intense inflammation in the subarachnoid space which leads frequently to irreversible brain damage, resulting from bacterial infection. Changes in the concentration of this cytokine in the course of bacterial meningoencephalitis demonstrate a relation with further progression and course of infection. Control testing during hospitalization, however, showed significant decrease of IL-6 concentration in CSF in patients whose clinical condition was improving and results of routine CSF analysis were subject to normalization. IL-6 concentration in CSF remained at a high level, comparable to its value in the first testing, in patients whose clinical condition did not improve (24,27-29).

From our observations transpires that the highest concentration of IL-6 in CSF was reported in patients in severe clinical condition (group I). No statistically significant differences were observed between average PNM pleocytosis and glucose concentration in CSF in groups I and II. The highest average concentrations of protein and lactic acid were noted in patients in severe condition, especially in fatal cases.

Results demonstrate that there is a significant correlation between IL-6 concentration and the severity of clinical condition of patient on admission and further course of disease. Control testing suggest a relation between IL-6 concentration in CSF and outcome of infection. In case of patients who recovered from disease, IL-6 concentrations were reduced. In majority of cases, it preceded clinical improvement and normalization of other CSF parameters. In fatal cases, however, IL-6 concentrations were slightly reduced compared to the first testing. Concomitantly, CSF inflammatory parameters remained at a high level and clinical condition of patients did not improve. Relatively low number of patients studied hinders a more in-depth statistical analysis of results. Consequently, it does not allow for drawing unequivocal and far-reaching conclusions. Nevertheless, it substantiates a necessity for further studies in this respect.

SUMMARY

To a large extent, IL-6 concentration in CSF seems to demonstrate the severity of brain damage resulting from bacterial infection. Increase of this cytokine in CSF suggest the intensity of inflammation in subarachnoid space of patient, blood-brain barrier damage and consequently brain damage (2-4,18,26,28-30).

Quantitation of IL-6 concentration in CSF in patients with purulent, bacterial meningoencephalitis may be of importance in both the assessment of actual inflammation intensity in subarachnoid space, which affects the course and outcome of disease, as well as infection prognosis. It may also be relevant in monitoring the course and treatment of purulent meningoencephalitis. Furthermore, it is of certain prognostic significance.

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