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BLOOD AND LIQUOR CYTOKINES AS DIFFERENTIAL-DIAGNOSTIC MARKERS OF ACUTE MENINGITIS IN CHILDREN

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The prospective study covered 93 patients aged 1 mo - 18 y. (47 children with aseptic meningitis, 26 - with purulent meningitis, 20 with neurotoxicosis). The concentration of cytokines (IL-4, IL-1b, IL-10, TNF-α) in cerebrospinal fluid and blood was determined during the first day of hospital stay. It was found that in patients with both aseptic and purulent meningitis, the levels of TNF-α, IL-1ß, IL-10 in the cerebrospinal fluid exceeded the levels of these cytokines in the blood. In patients with aseptic meningitis, activation of the Th2 immune response with active production by tissues of the nervous system of anti-inflammatory cytokines and increased levels in the cerebrospinal fluid, and vice versa, the advantage of Th1 response, characterized by intensive synthesis of proinflammatory cytokines in children with purulent meningitis. The obtained results optimize the differential diagnosis of aseptic and purulent inflammation of the meninges, allow to develop ways of differentiated correction of immune disorders, predict the severity of the disease and increase the effectiveness of treatment of these diseases.

Key words: meningites, children, liquor, cytokines

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ЦИТОКІНИ КРОВІ Й ЛІКВОРУ ЯК ДИФЕРЕНЦІАЛЬНО-ДІАГНОСТИЧНІ МАРКЕРИ ГОСТРИХ МЕНІНГІТІВ У ДІТЕЙ

Проспективним дослідженням охоплено 93 пацієнтів віком від 1-го міс. до 18-ти р., (47 дітей з серозним менінгітом, 26 – з гнійним менінгітом, 20 дітей нейротоксикозом). Концентрацію цитокінів (IL-4, IL-1b, IL-10, TNF-α) у ліквор та крові визначали протягом першої доби перебування хворих у стаціонарі. Встановлено, що у пацієнтів як з серозними так і з гнійними менінгітами рівні TNF-α, IL-1ß, IL-10 у ліквор перевищували рівні цих цитокінів в крові. У хворих з серозними менінгітами виявлено активацію Th2 імунної відповіді з активною продукцією тканинами нервової системи протизапальних цитокінів і підвищенням їх рівнів у спинномозковій рідині, і навпаки, перевагу Th1 відповіді, яка характеризується інтенсивним синтезом прозапальних цитокінів у дітей з гнійними менінгітами. Отримані результати оптимізують диференційну діагностику серозного та гнійного запаленням оболонок мозку, дозволяють розробити шляхи диференційованої корекції імунних порушень, прогнозувати тяжкість перебігу хвороби і підвищити ефективність терапії цих захворювань.

Ключові слова: менінгіти, діти, ліквор, цитокіни

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Meningitis is one of the most serious diseases of the central nervous system caused by an infectious process, and leads to numerous residual effects, complications and frequent deaths [5]. Neuroinfections remain one of the most common forms of central nervous system damage, second only to vascular pathology [2]. The urgency of the problem of meningitis in children is due to the high frequency of severe forms of the disease, high mortality, the complexity of differential diagnosis, a wide range of etiopathogens, increasing resistance of pathogens to antibacterial agents. Therefore, early etiological diagnosis of this pathology in children, especially in the first days of the disease, is one of the main tasks of pediatricians and infectioners. [12]. Routine methods of cerebrospinal fluid examination can not always in the first hours of the disease can give an idea of the pathogens that caused it - bacteria, viruses or others, while diagnosing the etiology of the disease at the earliest possible time to correctly choose empirical therapy [3]. This contributes to the constant search for new diagnostic opportunities to improve the diagnosis and differential diagnosis of meningitis in children. Studies of recent years have shown that not only the state of cellular and humoral immunity, but also the nature of immune responses activation in the CNS affects the course of meningitis. Serum cytokines in children with meningitis of various etiologies have been studied for more than ten years and today, levels of proinflammatory cytokines IL-1β and TNF-a are considered highly effective markers for the differential diagnosis of meningitis of viral and bacterial etiology [4], at the same time the results of studying cytokines levels in cerebrospinal fluid of children with purulent and serous meningitis is reflected only in isolated studies. High levels of cytokines in the cerebrospinal fluid have been found in purulent meningitis, as bacterial components are identified by brain immune cells (astrocytes, microglia), which secrete large amounts of cytokines – hormone-like mediators that involve other immune cells and stimulate other tissues to participate in the immune response. In children with serous meningitis,

the concentration of cytokines in the cerebrospinal fluid is variable and depends on the pathogen that caused the disease with herpesviruses, measles virus, mumps mice [6].

The purpose of the study was improving the early differential diagnosis of acute serous and purulent meningitis in children based on the determination of cytokine levels in the blood, cerebrospinal fluid and immunological features of the disease.

Materials and methods. The prospective study covered 93 patients aged 1 month. to 18 years, who were hospitalized in the utility unprofitable enterprise UNE ORL "Lviv Regional Clinical Infectious Diseases Hospital" during 2015-2019. Group 1 was included 47 children hospitalized with a diagnosis of "Serous meningitis"; group 2 included 26 children diagnosed with "purulent meningitis". The comparison group consisted of 20 children, randomized by age, who were hospitalized with a diagnosis of "SARS, neurotoxicosis" and negative results of bacteriological and virological examination of cerebrospinal fluid.

Immunological tests of blood and cerebrospinal fluid were performed during the first day of hospitalization. The concentration of cytokines (IL-4, IL-1b, IL-10, TNF-α) in cerebrospinal fluid and blood was carried out by enzyme-linked immunosorbent assay using "Vector Best" kits (Russia). We did not determine the levels of these cytokines in the blood and cerebrospinal fluid of healthy children, but used available in the literature [9, 11] reference values. The Commission on Bioethics of Danylo Halytsky LNMU revealed no violations of moral and ethical norms during the study. The research and treatment methods used in the study are scientifically substantiated and comply with the principles of the Helsinki Declaration of Human Rights (1950), the Council of Europe Convention on Human Rights and Biomedicine (1997) and the requirements of current Ukrainian legislation. All patients were admitted to the study with written informed consent.

For statistical data processing, non-parametric statistics were used using the Mann-Whitney and $\chi 2$ criteria to compare the mean values and frequencies of registration of traits according to the data distribution (differences were considered significant at a significance level of $p<0.05$). Statistical data processing was performed using Microsoft Excel 7, Statistica for Windows v.8.0 (StatSoft, USA).

Results of the study and their discussion. It was found that in patients with purulent meningitis levels of TNFα, IL-1ß, IL-4, IL-10 in the blood were significantly higher than in patients with serous meningitis and children of the comparison group, special attention should be paid to IL-1β, which median concentration in children of group 2 was by 6 times higher than that of patients with serous meningitis and children from the comparison group (table 1).

Table 1

Plasma levels of pro-inflammatory and anti-inflammatory cytokines (in pg/ml) in children with acute meningitis

Index	Group 1 (serous meningitis) (n=47)		Group 2 (purulent meningitis) (n=26)		Comparison group (n=20)		Reference values [5]
	Me	Q_{25}-Q_{75}	Me	Q_{25}-Q_{75}	Me	Q_{25}-Q_{75}	Me, Q_{25}–Q_{75}
IL-1β	6.45* ⊗	1.67-12.04	21.57*	10.93–24.63	3.22	1.92–4.29	1.8 [1.0–3.3]
IL-4	2.63 ⊗	1.03-4.32	3.46*	1.94–5.52	2.67	0.97–5.28	5.2 [4.9–6.1]
IL-10	9.43 ⊗	4.22-15.33	58.17*	9.41–258.54	8.14	3.71–13.23	11.4 [9.5–12.8]
TNF-α	2.06 ⊗	0.27-4.57	24.41*	0.73–12.24	2.35	0.91–3.70	0.4 [0.1–0.6]

Note: * – p<0.05in comparison: group of ratients 1 та 2 with comparison group; ⊗- p<0.05in comparison: group 1 – group 2

In patients with serous meningitis, the level of pro-inflammatory interleukin-1β in the blood was 6.45 [1.67-12.04] pg/ml, which was twice the rate of patients in the comparison group – 3.22 [1.92–4.29] pg/ml, (p=0.02)

Plasma TNF-α levels in patients with serous meningitis and comparison groups did not differ statistically significantly and were 2.06 [0.27-4.57] pg/ml and 2.35 [0.91–3.70] pg/ml, respectively. At the same time, in patients with purulent meningitis, the level of TNF-α in blood plasma was 24.41 [0.73–12.24] pg/ml and by 10 times higher than in other 2 groups of patients (p< 0.001).

In patients of group 1 and group 2, the levels of cytokines in the cerebrospinal fluid were higher than the age norms. In patients with purulent meningitis, the levels of TNFα, IL-1ß, IL-4, IL-10 in the cerebrospinal fluid were statistically significantly higher than in patients with serous meningitis, in particular the content of interleukin-1β in the cerebrospinal fluid reached 10.97 [3.52–21. 93] pg/ml and five times higher than the comparison group (2.19 [0.76–4.67] pg/ml, table 2). Patients with purulent meningitis showed an even more significant increase in IL–1β levels, which was more than by 30 times higher than in patients with serous meningitis, and by 150 times higher than in the comparison group.

Table 2

Results of determining the level of pro-inflammatory cytokines in the cerebrospinal fluid in patients with acute meningitis (pg/ml)

Index	Group 1 (serous meningitis) (n=47)		Group 2 (purulent meningitis) (n=26)		Comparison group (n=20)		Reference values [7]
	Me	Q_{25}–Q_{75}	Me	Q_{25}–Q_{75}	Me	Q_{25}-Q_{75}	Me. Q_{25}–Q_{75}
IL-1β	10.97* ⊗	3.52–21.93	352.27*	20.56–950.46	2.19	0.76–4.67	0.2 [0.2 – 0.6]
IL-4	3.31* ⊗	1.68–5.92	4.88*	3.03–8.10	2.08	1.20–3.12	4.6 [3.6 – 6.8]
IL-10	65.83* ⊗	21.36–126.63	160.40*	6.20–341.51	6.50	2.74–12.80	3.9 [3.9 – 23.3]
TNF-α	3.68* ⊗	1.31–6.91	79.14*	12.24–252.8	1.29	0.33–2.48	3.3 [1.1 – 3.3]

Note. * - p<0.05 when comparing: group of patients 1 and 2 with the comparison group; □ - p<0.05 when comparing: group 1 - group 2

In 73–89 % of patients in the main group, the levels of proinflammatory cytokines of the cerebrospinal fluid – IL-1β, TNF-a were higher than the age norms, and the concentration of IL-10 exceeded normal values in all patients. The anti-inflammatory cytokine IL-4 in the cerebrospinal fluid was elevated by only 27.3 % of patients.

When comparing the concentration of IL-1β in the blood and cerebrospinal fluid, it was found that only in patients of the comparison group the level of IL1-β in the blood exceeded the level of IL1-β in the cerebrospinal fluid by 1.5 times, and the median levels of this cytokine in cerebrospinal fluid were 3. 22 [1.92–4.29] pg/ml and 2.19 [0.76–4.67] pg/ml, respectively.

In the cerebrospinal fluid of children with serous meningitis, the median concentration of TNF-α was 3.68 [1.31–6.91] pg/ml and was by three times higher than the level of TNF-α in patients from the comparison group (p=0.03). The median TNF levels in the group of patients with purulent meningitis were by 21.5 times higher than in patients with serous meningitis, and by 61.5 times higher in patients from the comparison group and was 79.14 [12.24–252.83] pg/ml (p = 0.002). 79.14 [12.24–252.83] pg/ml (p=0.002).

In patients with serous meningitis, the level of IL-1β in the cerebrospinal fluid was by 1.7 times higher than the corresponding value in blood plasma and was 10.97 [3.52–21.93] pg/ml and was 6.45 [1.67–12, 04] pg/ml, respectively. In children with purulent meningitis, the level of IL-1β in the cerebrospinal fluid was by 22 times higher than the value of IL-1β in the blood – 352.27 [20.56–950.46] pg/ml and 21.57 [10.93–24.63] pg/ml, respectively (fig. 2).

When comparing the level of TNF-α in the blood and cerebrospinal fluid, it was found that only in the comparison group the level of TNF-α in the blood exceeded the level of TNF-α in the cerebrospinal fluid 1.8 times at median levels of this cytokine – 2.36 [0.91 –3.70] pg/ml and 1.29 [0.33–2.48] pg/ml, respectively. In children with both serous and purulent meningitis, the ratio of TNF-α cerebrospinal fluid/blood was significantly higher than in patients of the control group (fig. 2).

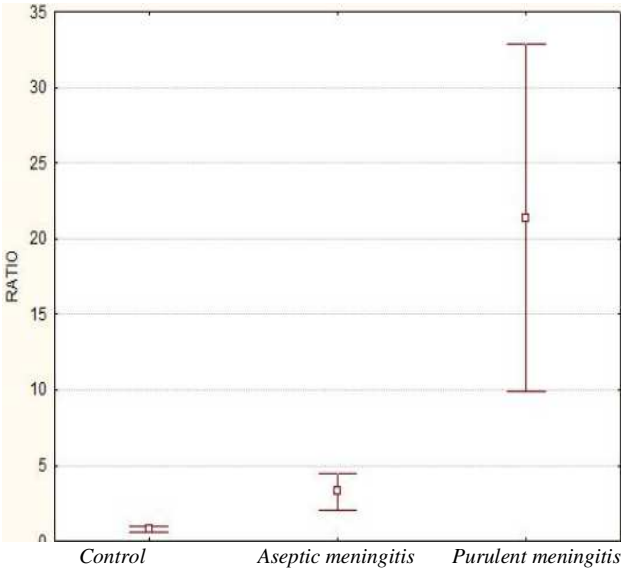

Fig. 1. The relationship between IL-1β levels of cerebrospinal fluid/blood

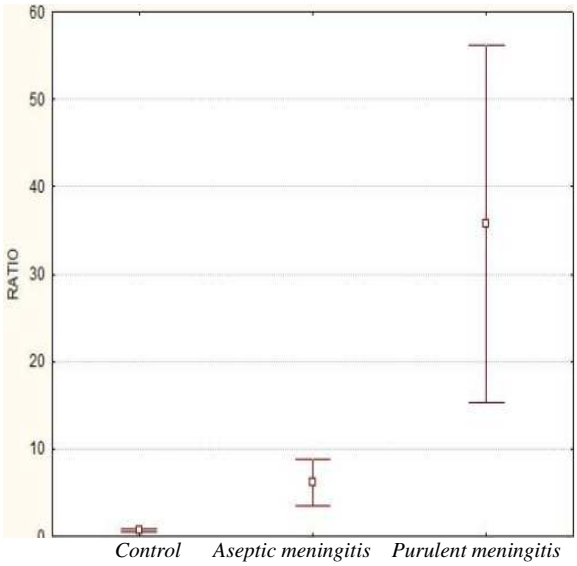

Fig. 2. Relationship between TNF-α levels of cerebrospinal fluid/blood of patients with acute meningitis

Highly informative was the calculated index IL10 $_{liquor}$ / TNF-α $_{liquor}$, which is the ratio between anti-inflammatory and pro-inflammatory cytokines and reflects the state of the T-helper system in conditions caused by infectious factors. It is known that Th1 and Th2 cells cause different pathways of the immune response. Th1 cells provide a type 1 immune response (or so-called "cellular immunity") to fight viruses and other intracellular pathogens, and stimulate delayed-type hypersensitivity skin responses; Th2 cells determine humoral immunity and regulate the production of antibodies to fight extracellular organisms.

In patients with purulent meningitis, the ratio of IL10 $_{liquor}$/TNFα $_{liquor}$ during the first days from the onset of the disease was 4.68±1.02 units, and was significantly lower than in the comparison group (9.02±1.11 units, p=0.02). In patients with serous meningitis, this figure was 23.35±2.08 units. and statistically significantly (p=0.006) exceeded that in the comparison group and in the group of patients with purulent meningitis.

This ratio reflects the significant activation of the Th2 type of immune response and higher production of anti-inflammatory cytokines compared to proinflammatory cytokines in patients with serous meningitis, and conversely, the advantage of Th1 type of immune response, characterized by intensive synthesis of proinflammatory cytokines (TNF-α), IL-1 nervous system and an increase in their level in the cerebrospinal fluid in children with purulent meningitis. Clinically, this is manifested by a long-term normalization of cerebrospinal fluid cytosis in serous meningitis and severe general condition of children with pronounced inflammatory changes in the cerebrospinal fluid in a subgroup of patients with purulent meningitis.

It should be noted that the severity of the disease correlated with the levels of interleukin-1β and tumor necrosis factor in the blood and cerebrospinal fluid: higher concentrations of IL-1β and TNF-α were apparently one of the factors that caused the severity in these patients, as it was found that the result of the biological action of elevated proinflammatory cytokines is drowsiness, loss of appetite, while this cytokine enhances the synthesis of acute phase proteins, stimulation of adhesion molecules, vasodilation and is necessary for the production of γ-interferon.

The importance of early differential diagnosis of meningitis of various etiologies is due to the need for immediate treatment of all patients with acute meningitis, but the basic principles of treatment of purulent meningitis (involving treatment with several antibacterial drugs) are fundamentally different from modern treatment protocols.

It is known that toxins and antigens produced during the inflammatory process activate pro-inflammatory (IL-1β, TNF-α) and anti-inflammatory (IL-4, IL-10) cytokines, thus creating favorable conditions for bacterial growth and penetration of their toxins into the blood [7].

Serum cytokines have been studied in children with meningitis of various etiologies for more than ten years. Today, the levels of pro-inflammatory cytokines IL-1β and TNF-a are considered highly effective markers for the differential diagnosis of meningitis of viral and bacterial etiology. In particular, an IL-1β concentration greater than 20 pg/ml is observed in 80 % of patients with purulent meningitis and only in 4 % of patients with serous meningitis.

Significantly higher levels of cytokines in the cerebrospinal fluid in purulent meningitis can be explained by the presence in this pathology of more powerful inducers of cytokines, primarily they are the components of the bacterial wall and bacterial toxins. If the components of bacteria are identified by immune cells of the brain (astrocytes, microglia), the latter secrete large amounts of cytokines - hormone-like mediators that involve other immune cells and stimulate other tissues to participate in the immune response [1, 11].

The concentration of pro-inflammatory and anti-inflammatory cytokines in bacterial meningitis decreases relatively slowly in the dynamics of the disease and only within the next few weeks returns to the age norm. It is believed that the breakdown time of cytokines in cerebrospinal fluid is much longer than in serum. Experimental studies have shown that cytokines in the blood, such as IL-2, when administered intravenously have a half-life of only a few minutes. This may explain the fact that in diseases in which high levels of cytokines in the CNS are detected, in particular in purulent meningitis, cytokines may not be detected in the blood.

The dynamics of cytokine levels in serous meningitis is reflected only in single studies, in small groups of patients. This may be due to the fact that most patients with serous meningitis do not undergo repeated lumbar punctures [1]. It has been established that in serous meningitis the concentration of cytokines in the cerebrospinal fluid depends on the pathogen that caused the disease. Thus, studies by N. Hikita [6] showed that the content of IL-10 in the cerebrospinal fluid in enterovirus meningitis was 14.3 pg/ml compared with 264.2 pg/ml in patients with meningitis caused by mumps virus. It is quite

interesting to use the determination of cytokines TNF-a, IL-6, IL-8 for the differential diagnosis of purulent meningitis in patients who have already started antibiotic therapy (partially treated meningitis) and serous meningitis.

This is a particularly pressing problem for meningitis caused by meningococci. These pathogens are sensitive to antibiotics that cross the blood-brain barrier. Therefore, after one or several days of treatment in such patients, the cerebrospinal fluid is rapidly sanitized and in the cerebrospinal fluid mostly changes are detected that are usually observed in serous meningitis – low lymphocytic pleocytosis, slightly elevated protein content. At the same time, the levels of cytokines in the cerebrospinal fluid, in particular TNF-a, IL-6, IL-8, remain high and are significantly higher than in patients with serous meningitis [8, 10].

Conclusion

Summarizing the data obtained, it can be stated that in patients with both serous and purulent meningitis, the levels of TNFα, IL-1ß, IL-10 in the cerebrospinal fluid exceeded the levels of these cytokines in the blood; similar patterns were found for the ratio of proinflammatory cytokines IL-1ß, TNFα in cerebrospinal fluid and blood.

Patients with serous meningitis also showed significant activation of the Th2 immune response with active production of anti-inflammatory cytokines by nervous system tissues and increased levels in the cerebrospinal fluid, and vice versa, the predominance of Th1 response, which is characterized by intensive synthesis of proinflammatory cytokines in children with purulent meningitis.

Determining the immunopathogenetic features of neuroinfections will improve the differential diagnosis of serous and purulent meningitis, develop ways of differentiated correction of immune disorders, predict the severity of the disease and increase the efficacy of these diseases treatment.

References

1. Venherov YJ, Nahybyna MV. Lykvorologicheskie issledovaniya pri bakteryalnykh gnoynykh meningitakh. Zhurnal infektologii. 2016. 8(2): 29–30. [in Russian]

2. Voloha AP. Serozni meninhity u ditey. Sovremennaya pediatriya. 2014;1:39–43. [in Ukrainian]

3. Shostakovich-Koreckaja LR, Slatvitskiy VG, Budaeva IV. Enterovirusnyj meningit osobennosti techeniya i diagnostiki na sovremennom etape. Zdorovye rebenka. 2016;8:78–81 [in Russian]

4. Al-Obaidi MMJ, Desa MNM. Mechanisms of Blood Brain Barrier Disruption by Different Types of Bacteria, and Bacterial-Host Interactions Facilitate the Bacterial Pathogen Invading the Brain. Cell Mol Neurobiol. 2018 Oct; 38(7):1349–1368. doi: 10.1007/s10571-018-0609-2.

5. Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's principles and practice of infectious diseases, Vol. 1. 9th edn. 2019:1178–1180.

6. Hikita N, Seto T, Yamashita K, Iritani N, Aata M, Ogura H, Shintaku H. Relationship between Severity of Aseptic Meningitis and Cerebrospinal Fluid Cytokine Levels. Osaka City Med J. 2015; 61(2): 63–71.

7. Kany S, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. Int J Mol Sci. 2019; 20(23):6008 doi: 10.3390/ijms20236008.

8. Nadraga A, Khomyn O. Cerebrospinal fluid inflammatory markers in children with aseptic meningitis. Curr Issues Pharm Med Sci, 2020, 33(1), 6–9 doi.org/10.2478/cipms-2020-0002

9. Pranzatelli MR, Tate ED, McGee NR, Colliver JA. Pediatric reference ranges for proinflammatory and anti-inflammatory cytokines in cerebrospinal fluid and serum by multiplexed immunoassay. J Interferon Cytokine Res. 2013; 33(9): 523–528. DOI: 10.1089/jir.2012.0132.

10. 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): 60–65 DOI: 10.1016/j.pediatrneurol.2013.08.016.

11. Srinivasan L, Kilpatrick L, Shah SS, Abbasi S, Harris MC. Elevations of novel cytokines in bacterial meningitis in infants. PLoS One. 2018; 13(2): e0181449. doi.org/10.1371/journal.pone.0181449.

12. Suthar R, Sankhyan N. Bacterial Infections of the Central Nervous System. Indian J Pediatr. 2019 Jan;86(1):60–69. doi: 10.1007/s12098-017-2477-z.

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