Detection of markers of cytomegalovirus infection in children with hemorrhagic lesions of central nervous system

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Abstract. The purpose of this work was to detect markers of cytomegalovirus infection in children with hemorrhagic lesions of central nervous system (CNS) in neonatal period. The work describes clinical-immunological and laboratory diagnostics of cytomegalovirus infection in infants with a hemorrhagic brain lesion. 60 newborn children in neonatal period with hemorrhagic lesions of central nervous system were examined using the method of polymerase chain reaction and by enzyme immunoassay. 47 cases of cytomegalovirus infection detection were documented.

[Tukbekova B.T., Dyussenova S.B., Maxutova A.K., Kizatova S.T., Sadvakassova S.M., Serikova G.B., Issayeva A.A., Yerimbetova N.A., Alimshaikhina K.Z., Sabiyeva M.M., Babagulovna Z.K. **Detection of markers of cytomegalovirus infection in children with hemorrhagic lesions of central nervous system.** *Life Sci J* 2014;11(10s):260-262] (ISSN:1097-8135). http://www.lifesciencesite.com. 46

Keywords: intraventricular hemorrhage, intracerebral hemorrhage, subarachnoid hemorrhage, cytomegalovirus infection in neonatal period

Introduction

Currently, the issue of hemorrhagic lesions at infants is still pressing, and its relation to infection of viral etiology is not excluded [1,2]. In recent years, infections of viral etiology have become increasingly important within the infectious pathology of infants. Therefore, the study of clinical-immunological markers of cytomegalovirus infection detection, evaluation of the relation between severity of clinical symptoms and paraclinic signs of the current infectious process, which is usually visualized in first instance and is camouflaged by severe hemorrhagic lesions of brain, refer to the category of the most problematic trends of the modern perinatal medicine [3,4,5].

There were assessed 60 sick children up to one month of age, the average age being 13±1.3 days, who were admitted to in-patient treatment in RCCH (Regional Children's Clinical Hospital) of Karaganda, with diagnosis: hemorrhagic lesion of brain, classified under 10 revision of ICD as I62.0. Other nontraumatic intracerebral hemorrhage. Supervision of patients was performed over time, starting from the first days of admittance to in-patient treatment, and included evaluation of clinical signs of hemorrhagic lesion of brain, definition of hematological, biochemical immunogenetic parameters of blood, parameters of hemostasis system, liquor study, ultrasound examination of parenchymal organs and brain.

Patients were assessed by specialists: ophthalmologist, neurologist, and neurosurgeon. Selection of children for study group was made on the basis of clinical signs of hemorrhagic lesion of brain (intraventricular, intracerebral hemorrhage and subarachnoid hemorrhage), severe degree in neonatal period. Laboratory diagnostics of cytomegalovirus infection was performed at early stages of supervision of children in the intensive care unit and units ofthe pathology of infants. Diagnosis of hemorrhagic stroke was verified by performingNSG (neurosonography) of brain, CT (computerized tomography), MRI (magnetic resonance imaging) of brain, and also by verification of subarachnoid hemorrhage by analysis of cerebral-spinal puncture. Verification of cytomegalovirus infection diagnosis was made using the serological and molecular-biological methods with definition of level of immunoglobulins (Ig) M and G to cytomegalovirus (CMV) by the method of enzyme immunoassay, anddetection of DNA of viruses by the method of polymerase chain reaction. Ig M antibodies were detected in 4 (6%) children. Ig G antibodies were detected in 45 (75%) sick children. The DNA of cytomegalovirus was found more frequently in the urinein 44 (73%) sick childrenthan in blood in 38 (63%) sick children.

Determination of IFNγ [gamma], IL-1β[beta] cytokines inblood serum was performed by enzyme immunoassay.

During the analysis of the nature of structural changes in brain, which were obtained from data of instrumental methods of study among patients, the intracerebral hemorrhages with leading

positions of intraventricular hemorrhages (IVH) were prevailing, whichis indicative of current "systemic" vasculitis. Subarachnoid hemorrhages were less frequent. There was also registered the progress (of encephalomeningitis) with formation of multiple cysts, multiple pseudocysts and formation of periventricular leukomalacia (PVL) [6].

Table 1. "Morphological" structure of CNS lesion insick children with hemorrhagic CNS lesions

Type of lesion	n=60	
	abs.	rel.
IVHof 2-3 degree	29	48%
Subarachnoid lesions	17	28%
Multiple cysts	5	8%
Hemorrhage in gray matter Intracerebral hematoma	14	23%
Multiple pseudocysts	13	21%
Formation of PVL	17	28%
Hypertension-hydrocephalic syndrome	33	55%
Congenital hydrocephalic syndrome	2	3%
Hydrocephalic syndrome	15	25%
Cerebral edema	43	71%

Of special interest is the presence of infectious agent, in particular cytomegalovirus infection insick children who were admitted to hospital with hemorrhagic lesions of CNS. The obtained results of the immunogenetic assay (IGA) of patients are provided in Table 2.

Table 2 shows that the frequency of detection of activator genome in children with hemorrhagic lesions of CNS does not exceed 78%. Different body mediums were used as the material for immunogenetic assay. In particular, 4 mediums (saliva, urine, blood, liquor) from all patients which were included in the group were sampled for performing the PCR study.

Table 2. Frequency of detection of cytomegalovirus genome in children with hemorrhagic lesions of CNS according to PCR data

Activator genome	n=60	
	abs.	rel.
CMV	47	78%
Total	60(100%)	

Altogether, the genome of cytomegalovirus from different mediums was detected in 47 cases. It was also found that the frequency of detection of activator genome in the studied material is defined by the material which was sampled for examination. Thus, the first among the studied mediums became urine, the genome of the virus was detected in 27 (45%) patients, next came blood – here genome of the virus was detected in 20 (33%) patients. Positive results were obtained less frequently at the examination of saliva in 5 (8%) patients, and liquor in 3 (5%) patients.

To confirm the persistence of cytomegalovirus infection,the immunologic study over time was also performed (14 days later). Specifically, the study of level of immunoglobulins Ig G and Ig M was performed. The obtained data on results of primary detection of AB level are provided in Table 3.

Table 3. The frequency of detection of CMV antibodytiters byEIA at examination of blood in children of the study group

AB level	n=60	
	abs.	rel.
Ig M	3	5%
Ig G	28	46%
Ig M after 14 days	4	6%
Ig G after 14 days	45	75%

During immunologic examination of blood, the presence of Ig M antibodies was found only in 3 (5%) patients, and Ig G antibody titer was obtained, in total,in 45 (75%) patients. Rising Ig M antibody titerwas registered in 4 (6%) patients, and Ig G over time, i.e.occurrence of seroconversion, was registered only in 45 (75%) patients.

The study of proinflammatory cytokines in newborn children with hemorrhagic lesion of CNS presented specific interest, due to this,the levels of IFN-γ [gamma] and IL-1β [beta]in blood serum of patients were studied. The results are provided in Table 4. The specified values of indexes of IFN-γ [gamma] cytokines in healthy full-term children are provided according to the data of L.V.Kravchenko, 2008 [7], and those of IL-1β [beta] according to Atici. A., 1996. [8].

Table 4. Characteristics of levels of proinflammatory cytokines in newborn children with hemorrhagic lesions of CNS (M ± m)

Index name	Index level in patients M ± m	Index level in patients, median	Index level in healthy children, median (fluctuations)
IFN-γ,pg/ml	180 ± 7.0	158*	34 (30.75-39.5)
IL-1β,pg/ml	110 ± 9.3*	99.3	27.9 ± 1.7

Note: * - accuracy of difference of values is marked with asterisk: * - $p<0.05$; **- $p<0.01$

While performing analysis of the above mentioned cytokines in infants with hemorrhagic lesions of CNS, it was found that the IFN-γ level was 5 times higher than the index of IFN-γ level in healthy children ($p<0.05$). Speaking of IL-1β level, it was 4 times higher than index of IL-1β level in healthy children ($p<0.05$).

Therefore, the intracerebral hemorrhages with leading positions of IVH, which are the symptom of current "systemic" vasculitis being an integral part of progress of cytomegalovirus infection, because activator is tropic to the endothelium of vessels, were prevailing in patients of the study group during the analysis [2,3,6].

The absolute criterion of diagnostics of CMV infection in infants is the detection of the virus itself or its genome, or its antigens in blood or liquor. In our study, among children with hemorrhagic lesions of CNS, activator genome was found in 47 cases. Rising Ig G antibody titer to CMV, which is a result of infectious process being in stage of active treatment, was found; detection of genome of cytomegalovirus verifies the presence of infectious process in infants which are forwarded to in-patient hospital with hemorrhagic lesions of CNS [3,5].

In the study of proinflammatory cytokines in infants with hemorrhagic lesions of CNS, it was found that the IFN-γ level happened to be 5 times higher than the index of IFN-γ level in healthy children, the IL-1β level was 4 times higher than the IL-1β level in healthy children. It can be assumed that the high IFN-γ titers speak of the presence of infectious process of viral etiology, and the IL-1β level speaks of the actively running infectious process with the possibility of generalization of infectious process into sepsis[11].

Therefore, all the children which come to in-patient hospital with diagnosis «Other nontraumatic intracerebral hemorrhage» should be examined for presence of cytomegalovirus infection, because infection can be viewed as the leading ethiopathogenetic factor of hemorrhagic CNS lesions development [2,6].

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6/7/2014

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