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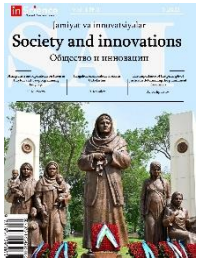

Etiological aspects of viral encephalitis explored using PCR-based cerebrospinal fluid analysis, considering patients' HIV status

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ABSTRACT

This study examined 124 patients suffering from acute viral encephalitis who were admitted to the Center for the Development of Professional Skills of Medical Personnel between 2014 and 2019. The research focused on the outcomes of cerebrospinal fluid PCR diagnostics in viral encephalitis cases, taking into account patients' HIV status. Findings revealed that PCR testing offers a more reliable diagnosis and, when lab resources permit, should be employed for CSF analysis to more accurately identify the etiological agent responsible for SVE. It is advised to repeat real-time PCR diagnostics of CSF in cases where the cause of SVE remains undetermined to improve detection rates of SVE's causative factors and enhance targeted therapy effectiveness, shifting from empirical and symptomatic to etiotropic approaches.

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Patsyentov OIV STATUS hisobga olgan holda PCR asosida miya omurilik suyuqligi tahlil foydalanish virusli ensefalit etiolohycheskyh aspektlari o‘rganildi

Kalit so‘zlar:
OIV,
virusli ensefalit,
PCR,
miya omurilik suyuqligi.

ANNOTATSIYA

Ushbu tadqiqot 2014 va 2019-yillar oralig‘ida RSCEMCga yotqizilgan o‘tkir virusli ensefalit bilan og‘rigan 124 bemorni tekshirdi. Tadqiqot bemorlarning OIV holatini hisobga olgan holda virusli ensefalit holatlarida miya omurilik suyuqligining PCR diagnostikasi natijalariga qaratilgan. Topilmalar shuni ko‘rsatdiki, PCR testi yanada ishonchli tashxisni taklif qiladi va agar laboratoriya resurslari ruxsat bergan bo‘lsa, SVE uchun javobgar bo‘lgan etiologik agentni aniqroq aniqlash uchun CSF tahlilidan foydalanish kerak. SVE qo‘zg‘atuvchi omillarini aniqlash tezligini yaxshilash va maqsadli terapiya samaradorligini oshirish, empirik va simptomatik yondashuvdan etiotropik yondashuvlarga o‘tish uchun, SVE sabablari noma’lum bo‘lgan hollarda CSFning real vaqt rejimida PCR diagnostikasini takrorlash tavsiya etiladi.

Этиологические аспекты вирусного энцефалита, изученные с использованием ПЦР-анализа цереброспинной жидкости, с учетом ВИЧ-статуса пациентов

Ключевые слова:
ВИЧ,
вирусный энцефалит,
ПЦР,
спинномозговая жидкость.

АННОТАЦИЯ

В работе обследовано 124 пациента с острым вирусным энцефалитом, поступивших в РНЦЭМС в период с 2014 по 2019 г. Изучены результаты ПЦР-диагностики ликвора при вирусных энцефалитах с учетом ВИЧ-статуса пациентов. Результаты показали, что ПЦР-тестирование предлагает более надежный диагноз и, когда позволяют лабораторные ресурсы, его следует использовать для анализа спинномозговой жидкости, чтобы более точно идентифицировать этиологический агент, ответственный за ЦСЖ. Для улучшения выявляемости причинных факторов СВЭ и повышения эффективности таргетной терапии, переходя от эмпирических и симптоматических к этиотропным подходам, рекомендуется повторная ПЦР-диагностика ЦСЖ в режиме реального времени в тех случаях, когда причина СВЭ остается неустановленной.

Relevance: Despite the availability of numerous diagnostic methods at the beginning of the 21st century, the cause of encephalitis remains undetermined in 62% of patients, and in 10% of cases, a non-infectious origin is diagnosed (3). According to Lobzin Y.V. and Zhdanov K.V. in their “Manual of Infectious Diseases”, the following

statistics are reported: "...out of confirmed or probable cases, 69% were viral, 20% were bacterial, 7% were related to prions, 3% were parasitic, and 1% were fungal. It is crucial to acknowledge that the inability to determine the cause in many instances may be due to challenging diagnostic cases, as well as limited access to suitable samples and suboptimal sample processing; despite extensive laboratory capabilities, the etiology of encephalitis remained unclear in 64% of patients" (1).

Presently, scientific studies concentrate on the quality of inflammatory and immune responses in cerebrospinal fluid (CSF) patients, drawing correlations between local and systemic immune responses in central nervous system (CNS) infections (2,4). Numerous publications explore neuroinfections during childhood or specifically address the prevention, diagnosis, and treatment of tick-borne encephalitis (5,6). However, there is a scarcity of research focusing on the clinical aspects, diagnosis, inflammatory response, and immune response parameters of CSF in encephalitis among adults, depending on the etiological factor.

The aim of the research is to investigate the outcomes of PCR diagnostics in cerebrospinal fluid for viral encephalitis, considering the HIV status of the patients involved.

Data and Methods. We examined 124 patients with severe viral encephalitis (SVE). The diagnosis of SVE was based on general infectious, general cerebral and focal neurological symptoms, and the detection of infectious agents. HIV infection was diagnosed according to the national clinical protocols for HIV infection in Uzbekistan according to the WHO classification of clinical stages of HIV infection in adults and adolescents (2010). All patients with SVE were treated as inpatients at the RSCEMC from 2014 to 2019.

Based on HIV status, the patients were divided into 2 groups: Group I – 72 (58.1%) patients with SVE with HIV seronegative status (HIV-) and Group II – 52 (41.9%) patients with SVE with HIV seropositive status (HIV+). Out of the number of SVE patients with HIV (+), 41 patients (33.1%) had injecting drug addiction.

In Group I, the average age of the patients was 49.82 ± 2.96 years old; there were 45 (62.5%) and 27 (37.5%) male and female patients, respectively. In Group 2, the average age of patients was 42.28 ± 2.74 years. There were 29 (55.8%) men and 23 (44.2%) women. The age and sex distribution of Group 2 patients are shown in Table 1.

Table 1.

Distribution of patients by sex and age.

Group I – SVE, HIV (-) patients n=72 (58,1%)						
Age (Years)	**men**		**women**		**all**	
	n	**%**	**n**	**%**	**n**	**%**
18-44	21	29,20%	15	20,80%	36	50,00%
45-59	14	19,40%	9	12,50%	23	31,90%
60-74	10	13,90%	3	4,20%	13	18,10%
TOTAL	**45**	**62,5%****	**27**	**37,50%**	**72**	**100,00%**
Group II – SVE, HIV (+) patients n=52 (41,9%)						
Age (Years)	**men**		**women**		**all**	
	n	**%**	**n**	**%**	**n**	**%**
18-44	19	36,50%	15	28,80%	34	65,40%

45-59	8	15,40%	8	15,40%	16	30,80%
60-74	2	3,80%	0	0,00%	2	3,80%
total	29	55,8%*	23	44,20%	52	100,00%
TOTAL	**74**	**59,7%**	**50**	**40,3%**	**124**	**100,00%**

As it was shown in Table 1, the structure of patients in Group I was somehow different from that in Group II. Thus, males prevailed in both groups (62.5% and 55.8% in groups I and II, respectively). It should also be noted that there was a percentage shift toward the younger age of patients in group I compared to group II.

Patients were examined according to the following algorithm: complaints, medical history; general clinical tests; biochemical tests; diagnostic lumbar puncture (LP) with the clinical and biochemical examination; cerebrospinal fluid, diagnostic LP with PCR; MRI of the brain.

Statistical processing of clinical and instrumental materials in accordance with the recommendations for processing the results of biomedical research at the significance level of $p<0.05$ was carried out using a practical statistical package STATISTICA.

Results and discussion. Diagnostic lumbar puncture is one of the first and indicative methods for early diagnosis of meningoencephalitis. When performing a diagnostic lumbar puncture, pleocytosis was determined in all patients under study. With increasing duration of SVE (according to anamnesis) initially neutrophilic or mixed pleocytosis became lymphocytic in 4-5 days, the number of cells decreased, and in some patients there was observed the formation of single erythrocytes in the CSF (Table 2).

Table 2.

CSF study indicators in patients with SVE, (M±σ)

Studied indicator	Group I (n=72)	Group II (n=52)
Pressure (mm. of water column) (M±σ)	198,2±12,6	187,9±16,4*
Cytosis (in 1 μl) (M±σ)	4285±173,6	4762±189,2*
Cellular composition (M±σ)		
Lymphocytes	68,2±12,3%	63,8±17,4%
Monocytes	26,7±9,2%	31,6±11,3%*
Erythrocytes	3,2±1,3%	5,4±1,7%
Protein (g/l) (M±σ)	2,64±0,76	3,17±0,79*
D-Dimer of fibrin mg/ml (M±σ)	8,3±1,6	11,4±2,3
Lactate (mmol/l) (M±σ)	8,7±1,4	10,1±1,6*
Glucose (mmol/l) (M±σ)	2,5±0,6	2,2±0,8

*Note: * significant differences between groups p<0.005.*

The cerebrospinal fluid pressure was measured using a U-shaped manometer tube filled with water in the supine position of the patient. As can be seen from Table 2, the pressure of CSF in group I was higher than in group II – 198.2 mm. of the water column and 187.9 mm. of the water column, respectively.

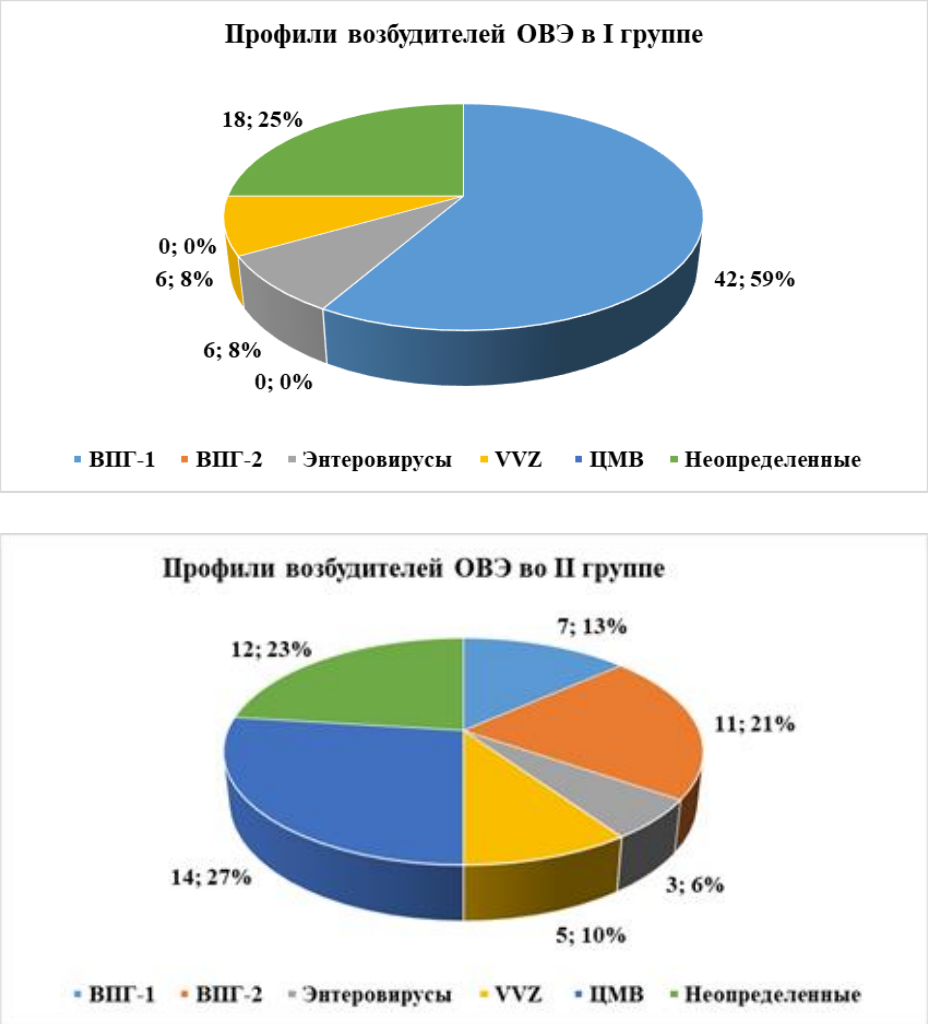


Fig. 1. Etiological factors of SVE in patient groups according to the first PCR-diagnostics of CSF (abs; %).

The severity of reactive changes in the CSF was stronger in the HIV-negative group compared to the HIV-positive group, while the overall neurological status and clinical manifestations were stronger in the HIV-positive group. Thus, in the study of CSF, block content values were higher in group II patients by 18.7%, D-dimer of fibrin prevailed by 27.2%, and lactate was up by 13.9% compared with group I.

To determine the causative agent of SVE, we used PCR diagnostics of CSF to detect genetic material of HSV-1, HSV-2, VZV, enteroviruses, and CMV. PCR testing of CSF was performed in the laboratory of the Research Institute of Virology, in the reference laboratory of the Republic of Uzbekistan.

Table 3.

Etiological factors of SVE depending on the presence of HIV infection

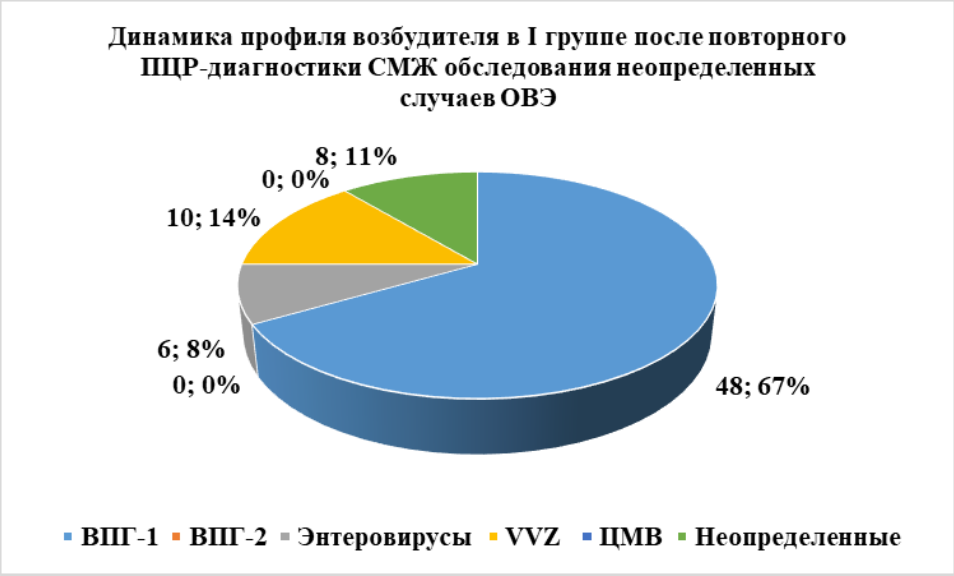
Pathogen	Group I (n=72)		p<	Group II (n=52)	
	n	%		n	%
HSV-1	42	58,3%	0,005	7	13,5%
HSV-2	0	0,0%	0,001	11	21,2%
Enteroviruses	6	8,3%		3	5,8%
VVZ	6	8,3%		5	9,6%
CMV	0	0,0%	0,005	14	26,9%
Non defined	18	25,0%		12	23,1%

PCR diagnostics of cerebrospinal fluid revealed that in Group I HSV-1 prevailed – in 42 patients (58.3%), the pathogen could not be detected in 18 patients (25.0%), VVZ and enteroviruses were detected in 6 patients (8.3%) in each case. In Group II, CMV was found in 14 patients (26.9%), which was significantly more frequent than in Group I patients. In Group II, the frequent causative agents of CVE were also HSV-2 and HSV-1, in 21.2% and 13.5% of patients, respectively. And in 23.1% of patients, the pathogen agent of SVE was not identified (Fig. 1, Table 3).

A repeated real-time PCR diagnosis of CSF was performed to clarify the etiological factor among the unspecified cases of SVE in both groups. The dynamics of the indicators in the pathogen profiles are shown in Figure 2 and Table 4.

As can be seen from Figures 1 and 2, after a repeated PCR study of CSF for the presence of viruses, 14 more cases of SVE were identified overall, 9 cases in Group I and 5 cases in Group II.

Dynamics of pathogen profile in group I after repeated PCR diagnostics of CSF testing of unspecified cases of SVE



Dynamics of pathogen profile in group II after repeated PCR diagnostics of CSF testing of unspecified cases of SVE

Figure 2. The dynamics of the profiles of etiological factors of SVE in the groups of patients after repeated PCR-diagnostics of CSF (abs; %).

As can be seen from Tables 3 and 4, the structure of the etiological factors of SVE in both groups slightly changed. In group I, repeated PCR diagnostics of CSF additionally revealed 4 cases of HSV-1, and 4 cases of ZZV, in group II one patient with CMV and one patient with enterovirus.

Also, in group II, opportunistic infections were detected: cryptococcus – 8 cases (15.3%) and toxoplasma – 5 cases (9.6%), Epstein-Barr virus – 22 cases (42.3%).

Table 4.

Etiological factors of SVE depending on the presence of HIV infection after repeated PCR examination of CSF

Pathogen	Group I (n=72)		p<	Group II (n=52)	
	n	%		n	%
HSV-1	48	66,7%	0,0005	7	13,5%
HSV-2	0	0,0%	0,005	11	21,2%
Enteroviruses	6	8,3%		4	7,7%
VVZ	10	13,9%		5	9,6%
CMV	0	0,0%	0,005	15	28,8%
Non defined	8	11,1%		10	19,2%

In conclusion, PCR-based diagnostics are more dependable, and when lab resources permit, employing PCR analysis of cerebrospinal fluid is recommended to more accurately identify the etiological agent responsible for SVE. In cases where the cause of SVE remains uncertain, repeating real-time PCR diagnostics of CSF is advised to enhance

detection rates of SVE's causative factors and subsequently improve the efficacy of targeted therapy, transitioning from an empirical and symptomatic approach to an etiotropic one.

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