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DETECTION OF HERPES VIRUSES IN PATIENTS WITH MYALGIC ENCEPHALOMYELITIS /CHRONIC FATIGUE SYNDROME IN BELARUS

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Abstract

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a multifactorial chronic disease. The etiology and pathogenesis of ME/CFS are unknown . There are many theories for the occurrence of this disease. but the most convincing is the infectious or viral theory of the emergence of CFS.
The aim of this study is to detect herpes viruses 6, 7 types and Epstein-Barr to examine the prevalence HHV-6, HHV-7 and EBV infections in Belarusian CFS patients.
We examined 30 patients with CFS who experienced fatigue for more than 2 years (7), more than 1 year (11) and more than 6 months (12). The diagnosis was made on clinical grounds using the Fukuda criteria.
The presence of markers the active forms infection HHV-6 and HHV-7 in CFS patients with a long period of fatigue were detected in 16.6% and 26.6% respectively. IgM antibodies to HHV-6 and EBV. positive, in 16.6% and 6.7% respectively in patients with long-term illness. The detection of IgG antibodies indicates a quiet carrier state, latent phase.

Key words: myalgic encephalomyelitis/chronic fatigue syndrome Human herpesvirus 6, 7, Epstein-Barr, IgM IgG antibodies

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Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a somatic disorder with complex symptoms include extreme fatigue that fails to improve after night sleep. Fatigue lasts 6 months or more, and it is often accompanied by emotional lability, sleep disturbance, headache and other symptoms. According the Fukuda case definition the disease requires severe function-limiting fatigue for at least 6 months, which is often accompanied by emotional lability, sleep disturbance, headache and other symptoms[1-3].

There are different etiological hypotheses which have been proposed implicating the roles for viruses, bacteria, environmental triggers, and immune dysregulation, but the biological pathways leading to this syndrome remain poorly defined. A number of authors define CFS as a multi-cause disorder of neuroimmune mechanisms, which manifests itself in genetically predisposed individuals as a result of the activation of the immune system by infectious agents and dysregulation of the central nervous system. All the data obtained allowed us to consider CFS a multifactorial disease and viral infections are the leading factor. For a long time, the interpretation of this disease was debatable, and in 2009 it was included in the ICD-10 (rubric G 93.3) as a post-viral fatigue syndrome [2,4-6]

The most convincing is the infectious or viral theory of the emergence of CFS, which arose in connection with the fact that the development of the syndrome begins with "flu-like symptoms." Various viruses can serve as trigger factors for CFS:. Epstein-Barr (EBV), cytomegalovirus (CMV), herpes simplex viruses I, II, VI,VII types (HHV-1, HHV-2, HHV-6, HHV-7), Coxsackie virus, hepatitis C, enterovirus, adenoviruses, retrovirus .et al. This is also confirmed by the data on the high frequency of detection of persistent herpes viruses and signs of their reactivation in such patients, which can occur against the background of impaired immune status.(7-9)

Herpes viruses are ubiquitous and most often are triggers of the development of the disease. Herpes human virus type 6 (HHV-6 A B), herpes human virus type 7 (HHV-7) and Epstein-Barr virus, have a close genetic affinity. They are capable of persistence and latency, and after the initial infection they remain for life in the human body. Among them, the most active viruses in this respect are HHV-6 and HHV-7, the least - Epstein-Barr virus [1-3]. Due to this ability to persistence and latency, these caused diseases can have different forms: acute, persistent / latent.

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Recently, studies have appeared that the genome of these viruses can integrate into human chromosomes, while a special form of chromosome-integrated infection develops, characterized by an unusually high viral load [7,9].

Considering the probable viral etiology of CFS, it is possible to recommend the use of viruses, including HHV-6, HHV-7 and EBV, as markers of the development of this pathology.

The aim of this study is to detect the prevalence and forms of herpes viruses 6, 7 types and Epstein-Barr infections in Belarus CFS patients

Materials and methods

Thirty patients with ME/CFS (23 females, 7 males), and 20 randomly selected blood donors (10 females, 10 males) as controls (cohort compared) were investigated

The cohorts were established with the approval of the local Ethics Committee

and all the participants gave informed consent before the examination.

Blood samples

Serum and EDTA blood was collected by venepuncture. Plasma samples were aliquoted and stored at 70 ° C. Blood samples from patients with ME/CFS were taken on the same day, 200 µl was poured into eppendorfs and stored at 40–80 ° C no more than 24 hours. Serum for ELISA, MFA, was stored at 4 ° C for up to 10 days. During longer storage, the samples were frozen to minus 70 ° C. Repeated freezing and thawing are undesirable.

DNA isolation

DNA was isolated from peripheral blood mononuclear cells using the QIAamp DNA Blood mini Kit (QIAGEN). Qualitative analysis of the extracted DNA was checked using globin-PCR followed by agarose gel electrophoresis.1

Real-time PCR

Real-time polymerase chain reaction with hybridization-fluorescence detection was carried out using the Amplisens HHV6 screen titer FL reagent kit (Amplisens, Russia) in accordance with the instructions for use. Amplification and analysis of the results was performed using a Rotor-Gene 3000 or 6000 (Corbet Research; Australia).

Antibodies tests ELISA

ELISA was carried out to detect IgM and IgG antibodies to EBV using commercial test systems manufactured by Vector-Best (Russia) in accordance with the manufacturer's instructions.

ELISA was carried out to detect IgG antibodies to HHV-6 by reagent kit (Amplisens, Russia) in accordance with the instructions for use

Indirect Immunofluorescence Assay (IFA)

Determination of IgM antibodies was carried out by immunofluorescence method in accordance with the instruction "Anti-HHV-6 11FT (IgG or IgM) Instructions for the indirect immunofluorescence test" EUROIMMUNE, Germany. IgG class antibodies were removed from the patient's plasma by immunoabsorption according to the manufacturer's protocol (EUROIMMUN, Germany). A titer of 1:10 or higher was considered positive.

Statistical Methods

Using statistics for an ungrouped data series with a small n value statistical difference in the prevalence viruses between tested and control groups was assessed by student's t-test.

Results

We examined 30 patients with CFS who experienced fatigue for more than 2 years (7), more than 1 year (11) and more than 6 months (12). There were 23 women and 7 men of different ages among them 18 people - 18 and 12 people - 31-56 The diagnosis was made on clinical grounds using the Fukuda criteria.

Most patients (20 and 21) had sleep disturbance and emotional lability. 9 patients had subfebrile temperature for 3 months or more, and 12 patients experienced headache.

Sera from 20 blood donors(BD) as a cohort compared from the Republican Center for Blood Transfusion were included in the analysis (Table 1). Fatigue syndrome and other clinical signs were absent in patients of this group.

To determine the different forms of herpesvirus infections, DNA was detected in serum – active form and peripheral blood leukocytes (PBL) – latent / persistent form.

The incidence of infection caused by one virus and mixed infections caused by two or three viruses in CFS patients were determined. (Table 2) The latent / persistent form of HHV-6 infection was detected in 14 patients (46.7%), and the active form in 16.6% of cases. This was significantly higher, compared with HHV-6 infection, the latent form of HHV-7 infection (73.3%) was determined in CFS patients ($P \leq 0.005$). Viremia HHV-7 was detected in 26.6%. A latent / persistent form of EBV infection was detected in 12 patients (40.0%), and viremia in 2 patients.

Considering the available data on frequently occurring cases of the joint presence of HHV-6 and HHV-7, the DNA of these viruses was determined in CFS patients. A high percentage of the latent / persistent form of dual infection HHV 6 and HHV-7 was shown to be -53.3%, viremia of either both viruses or one was in 4 patients. The presence of HHV-6, HHV-7 and EBV viruses in leukocytes was detected in 2 patients and one of them had an active form of HHV-7.

The difference between the occurrence of active and latent forms in the tested groups compared with the group of blood donors is statistically significant $p \leq 0.001$.

The level of specific antibodies IgM and IgG for HHV-6 and for EBV, as a markers of HHV-6 and EBV infection, was determined to assess the form of infection. Specific antibodies IgM were detected in patients with the active form of infection and DNA was in serum (Table 3). IgM antibodies were detected for HHV-6 in 5 and for EBV in 2 patients, which indicated an active form of infection. No active markers of HHV-6 and EBV infection were found in patients of the BD group.

Antibodies Ig G were studied in all CFS patients (30). IgG for HHV-6 and for EBV were detected in 70.0% and in 43.3% of cases respectively. Antibodies Ig G for EBV in 7 patients of the BD group were detected This indicates a previous exposure with pathogens. The presence of IgG means a response to infection, but does not indicate the activity of the infectious process.

Conclusion

Post-infection chronic fatigue syndrome is usually observed in patients in six or more months after the end of an acute viral infection. The presence of markers the active forms infection HHV-6 and HHV-7 in CFS patients with a long period of fatigue were detected in 16.6% and 26.6% respectively. This may be the result of reactivation of viruses under the influence of various provoking factors.

IgM antibodies to HHV-6 and EBV were positive, in 16.6% and 6.7% respectively in patients with long-term illness. They can appear either after the primary infection, or after reactivation of the virus after a long latent period. In our studies, all patients had a fatigue period of -6 months or more.

Detection of IgG antibodies indicates a quiet carrier state, latent phase. Antibody levels may remain elevated for many years after a primary infection, and this may indicate immune memory of a past primary infection.

The high incidence of HHV-6 HHV-7 and EBV in CFS patients suggests that they may be markers and trigger factors for the development of CFS.

Tab. 1: Characteristics of the study cohort. ME/CFS: myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS)

Demographics		ME/CFS (n = 30)	Controls (n = 20)
Sex	Female	23	12
	Male	7	8
Age (years)	20-30	18	20
	31-56	12	-
Clinical measures	Fatigue more than 6 months	30	no
	Headache	12	no
	Low-grade fever for 3 months or more; Subfibril temperature	9	no
	Sleep disturbance	21	2
	Emotional lability	20	2
Duration of ME/CFS	Long duration (>2 years)	7	no
	Duration (<1 year)	11	no
	Duration (<6 month)	12	no

Tab. 2: Prevalence ДНК of latent and active forms HHV-6, HHV-7 and VEB infections in ME/CFS patients

Groups of patients	DNA HHV-6 Mono Infection		DNA HHV-7 Mono Infection		DNA VEB Mono Infection		DNA HHV-6+ HHV-7 Dual Infection		DNA HHV-6+ HHV-7+ VEB Triple Infection	
	PBL	Serum	PBL	Serum	PBL	Serum	PBL	Serum	PBL	Serum
CFS (30)	14* (46.7 %)	5 (16.6 %)	22* (73.3 %)	8 (26.6 %)	12* (40.0 %)	2 (6.7 %)	16* (53.3 %)	4 (13.3 %)	2 (6.6 %)	1/ (3.3 %)
BD* (20)	2/20 (10%)	0/20 (0%)	5/20 (25%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)	0/20 (0%)	0/20 (0%)	0/20 (0%)

* statistically significant $p \leq 0.001$.

Tab. 3: Presence of specific of IgM and IgG antibodies in CFS patients

Groups of patients (n)	Virus	Specific antibodies IgM	IgG
CFS (30)	HHV-6	5 (16.6%)	21 (70.0%)
	VEB	2 (6.7%)	13 (43.3%)
BD× (20)	HHV-6	0 (0%)	7 (35.0%)
	VEB	0 (0.0%)	0 (0.0%)

× blood donors.

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