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Detection of herpes viruses in patients with classical trigeminal neuralgia

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*A comparison of the presence frequency of herpes viruses HSV1/2, VZV, HHV-6, HHV-7, EBV, CMV was conducted in 430 patients with classical trigeminal neuralgia (TN) using RT PCR method. In the venous blood of patients with paroxysmal pain syndrome (TNP) and persistent background pain (TNB), herpes viruses were detected in 36.3% (97/267) and 80.4% (131/163) cases, respectively. The effectiveness of microvascular decompression and long-term outcomes were assessed depending on the presence of herpes viruses. P*ain syndrome completely regressed in 404 out of 430 patients (93.9%), partially in 25 patients (5.8%), and persisted in 1 patient (0.2%). *In TNB group, pain recurrence and partial regression were observed more frequently (20.2% (34/163) and 12.8% (21/163), respectively) if compared to TNP group (1.8% (5/267) and 1.5% (4/267), respectively). Complete pain regression in TNB group was less than in TNP group: 86.5% (141/163) and 98% (263/267), respectively. The presence of herpes viruses in patients with TN, a higher frequency of detection of herpes viruses, as well as a higher percentage of patients with recurrence of pain in TNB versus TNP group suggest a potential role of herpes viruses in the development of pain syndrome. Thus, studying herpes viruses in the blood could be recommended for improving the diagnostics and therapy of patients with classical TN.*

Key words: classical trigeminal neuralgia; herpes viruses; HSV1/2; VZV; HHV-6; HHV-7; EBV; CMV.

INTRODUCTION

According to the International Classification of Headache Disorders [1], classical trigeminal neuralgia (TN) is caused by compression of the trigeminal nerve root near its point of entry into the pons by tortuous or pathologically altered vessels in 92-94% of cases. In some individuals, TN is not associated with neurovascular conflict, known as idiopathic neuralgia, indicating the heterogeneity of the underlying factors of this pathology [2].

Currently, there is no explanation what is the triggering mechanism of TN in the existing long-term vascular compression of the nerve root. Furthermore, the lack of understanding extends to the question of why neuralgia does not develop in individuals in the presence of vascular compression of the trigeminal nerve root, as verified by MRI and on sections in people who did not suffer from TN [3-5]. The most recognized opinion is that the etiopathogenesis of TN in neurovascular conflict consists in damage to the integumentary oligodendrocytes by mechanical stimuli, the development of local demyelination and remyelination, which makes the entry zone of the trigeminal nerve root a source of aberrant activity that generates pain sensations [6].

It is currently believed that the trigger of TN without neurovascular conflict can be the defeat of oligodendrocytes by viruses, insufficient remyelination and, as a consequence, increased sensitivity of nociceptive sensory fibers of the trigeminal nerve [7]. Postmortem examination of the presence of herpes viruses in the trigeminal ganglion in random individuals showed their presence in 13 of 47 (27%) of examined persons. The most common is HHV6 (19.2%), and

HSV1, EBV and VZV were detected with the same frequency (4.3%). Simultaneous presence of different herpes viruses in the trigeminal ganglion was also shown in this study [8].

The penetration of herpes viruses into neuronal cells is a complex process, which depends on site-specific interaction. Infection of Schwann cells and oligodendrocytes with VZV and HSV-1 herpes viruses occurs via the interaction of viral surface glycoproteins with myelin cell receptors associated with glycoprotein [9]. Direct infusion of herpes viruses HHV-6 and HHV7 into oligodendrocytes has also been demonstrated [10]. Herpes viruses are characterized by direct transmission from infected to uninfected cells (between glial cells as well as from a neuron to glial cell) and the formation of multicellular syncytia. Penetration of viruses into Schwann cells and oligodendrocytes results in the damage of myelin sheaths and TN [11].

Currently, the mechanism of autoimmune damage to oligodendrocytes by cytotoxic T8 lymphocytes is under consideration. For example, HHV-6 U24 protein contains amino acid sequences identical to basic myelin protein [12]. Immunological damage of cells in the presence of molecular mimicry is also observed for other herpes viruses. Thus, damage to oligodendrocytes of myelin membranes by gamma herpes virus EBV is also carried out on the principle of autoimmune process [13]. Infected B cells represent antigen and activate auto-aggressive CD8 T cells with a cytotoxic phenotype against myelin proteins [14].

Neurons with damaged Schwann cells and oligodendrocytes with various weak effects on peripheral nerve branches produce inadequate efferent impulses. Afferent impulses coming from demyelinated neurons are also activated. This results in an amplified feedback, which is clinically manifested as severe pain with small stimulus. TN is believed to be an inevitable consequence of demyelination [15].

Although there is an ample evidence that the Central Nervous System (CNS) is a location where herpes viruses persist, causal relationship and the pathogenetic role of these infections in CNS diseases, including the development of postherpetic TN, remain to be studied.

The aim of this work was to detect herpes viruses HSV1/2, VZV, HHV-6, HHV-7, EBV, CMV in the whole blood of TN patients with paroxysmal pain syndrome (TNP) and persistent background pain (TNB) in comparision, and to assess the effectiveness of microvascular decompression and long-term results depending on the presence of herpes viruses.

METHODS

In this study we analyzed examination results of 430 patients who applied to the State Institution "A.P. Romodanov Neurosurgery Institute, National Academy of Medical Sciences of Ukraine" in 2006-2021 for TN. These patients underwent a comprehensive clinical examination with an assessment of anamnesis and long-term results of treatment, preoperative magnetic resonance imaging (MRI) in 3D sequences using CISS3D (Siemens 1.5T) or 3Ddrive (Philips 3T) and a standard laboratory preoperative complex. Analysis of the whole venous blood for the presence of herpes viruses HSV1/2, VZV, EBV, HHV6, HHV7, CMV DNA using RT PCR was performed at the stage of outpatient examination, upon admission to the State Institution, or directly in postoperative period.

All patients included in the study were diagnosed with typical TN (all patients met this definition according to the latest classification in 2018) [16]. Based on MRI data, patients with other causes of neuralgia (tumors, cavernomas, venous angiomas) and those without signs of vascular compression, deformation or displacement of the trigeminal nerve root were excluded from the study. All 430 patients underwent surgery (microvascular decompression of the trigeminal nerve root in the cerebellopontine angle), and 11 patients had additional selective proximal rhizotomy. The assessment of surgical outcomes was performed

according to the BNI PS scale [17].

Data on long-term results of treatment were collected on the basis of planned dispensary examinations of patients one year after surgery and treatment at a later date (1-15 years, average 28 months) upon request, or by the method of remote survey with filling in a standardized card.

In this study, the following procedures and materials were employed. DNA extraction was performed using "DNA/RNA-Mag, HEMA, Ukraine" kit. The identification of herpes viruses was carried out using RT PCR method with "HEMA, Ukraine" kits. The reagents utilized included agarose (Amresco), Trilon-B ("Riedel-de Haen"), Tris ("Amresco"), and ethidium bromide ("Sigma"). Real-time PCR (qPCR) was conducted using a "CFX96" thermocycler (Bio-Rad, USA).

Statistical analysis was performed using online software. The results were processed using contingency tables (2x2) with odds ratio (OR) assessment, 95% confidence interval for confirming the hypothesis of sample equality, as well as contingency tables (2xN) and Jamovi software (USA). The statistical tests used included the Pearson's chi-squared test and Fisher's exact test. Differences were considered statistically significant at $P < 0.05$ [18, 19].

The study was conducted in compliance with the rules of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study", the study was approved by the ethics committee of the State Institution "A.P. Romodanov Neurosurgery Institute, National Academy of Medical Sciences of Ukraine".

RESULTS AND DISCUSSION

All 430 patients upon contacting the State Institution "A.P. Romodanov Neurosurgery Institute, National Academy of Medical Sciences of Ukraine" were diagnosed with classical TN on the basis of characteristic complaints of typical pain attacks in projections of the trigeminal nerve innervation on one side of the face. 267 of 430 (62.1%) patients had TNP and 163 of 430 (37.9%) patients − TNB. It was impossible to assess the number of patients for whom background pain was inherent to the disease itself versus those for whom it developed as a result of medical manipulations preceding their visit to the State Institution.

In all 430 patients, MRI examination verified the presence of a vascular loop in the trigeminal nerve root zone and/or deformation and displacement of the nerve root. Microvascular decompression surgery of the trigeminal nerve root was performed on 427 patients, which involved revising the nerve root in the cerebellopontine angle, performing neurolysis if necessary, repositioning the compressing vessel(s) with isolation using shredded teflon, and in 8 cases accomplished by selective proximal rhizotomy (SPR). In 3 cases (all from TNB subgroup) where compressing vessels were not verified, only neurolysis and SPR were conducted.

PCR tests for herpes viruses were positive in 228 of 430 (53.0%) patients. The proportion of positive patients was 80% (131/163) in TNB subgroup, which is significantly higher than that in TNP subgroup – 36.3% (97/267) ($\chi2 = 78.80$; $P < 0.001$; Table 1).

Intraoperatively, the adhesive process of the arachnoid membranes was macro-microscopically observed perifocally around the trigeminal nerve root within the cerebellopontine cistern and was significantly more pronounced in TNB subgroup.

Complete regression of pain after surgery was observed in 98.5% (263/267) of patients with TNP and in 86.5% (141/163) of patients with TNB ($\chi2 = 25.65$, $P < 0.001$).

Partial regression of pain was noticeably more often recorded in TNB than in TNP patients: 12.8% (21/163), 1.5% (4/267), respectively ($\chi2 = 23.96$; $P < 0.001$). Also, the number of patients with pain recurrence in TNB group significantly exceeded that in TNP group: 20.2% (34/163) and 1.8% (5/267), respectively ($\chi2 = 44.24$, $P < 0.001$). One TNP patient with recur-

rence of 5 (20.0%) had positive herpes viruses test, and in TNB group – 33 patients of 34 (97.1%) ($\chi 2$ =23.16, P < 0,001).

The presence frequency of herpes viruses HSV1/2, VZV, HHV6, HHV7, CMV, EBV in the whole blood of patients with TN is presented in Table 2. EBV, HHV6, HHV7 (70.5, 60.0, and 47.6%, respectively) viruses were most frequently detected in patients with TN, while HSV1/2, CMV (23.0, 11.0, 4.5%, respectively) were found less often. This frequency distribution is explained not only by the broad cellular tropism of EBV, HHV6, HHV7, but also by their lymphotropy. EBV is known to persist in B lymphocytes, and HHV6 and HHV7 establish lifelong persistence in T lymphocytes. Two or more herpes viruses were detected in 107 out of 228 (47%) patients.

In 135 out of 228 (59.2%) patients with a positive PCR test and the presence of concomitant clinical symptoms of the infectious process, antiviral treatment was performed (91 out of 228 (39.9%) before and 44 out of 228 (19.3%) after surgery). A standard treatment scheme was used: valacyclovir 42 g per course and interferon alfa-2B (IFNα2B) at a dosage of 3 million IU N15 per course. This resulted in a significant regression of pain syndrome (according to the Barrow Neurological Institute Pain Scale by 1-3 points from the initial level) in 37 out of 91 (40.7%) patients who received antiviral therapy, even on an outpatient basis. These patients temporarily were refrained from surgery, but later relapses of pain caused repeated treatment and microvascular decompression operation of the trigeminal nerve root.

Severe pain syndrome, which could not be controlled with medications (anticonvulsants, antidepressants, antispasmodics and antihypertensive drugs) caused an immediate performing of microvascular decompression operation in 54 of 91 (59.3%) patients with preoperative antiviral treatment.

In the early postoperative period, pain syndrome completely regressed in 404 out of 430 patients (93.9%), partially in 25 patients (5.8%) and persisted in 1 patient (0.2%). When observing patients over extended periods ranging from 1 to 5 years, with an average duration of 28 months, it was observed that relapses of pain syndrome, in varying degrees of severity, occurred in 39 out of 376 cases (10.4%). All patients with relapses underwent PCR tests for the presence of herpes viruses. Positive results were obtained in 34 out of 39 (87.2%) patients, while 33 of them (97.1%) were from TNB group.

Table 1. Herpes viruses in patients with TNP and TNB

Studied groups	TNP	TNB	Total with TN	OR, 95%CI $\chi 2$
Total studied	267	163	430	-
HV + patients, %	97/267 36.3%	131/163* 80.3%	228 53.0%	7.12(4.53-11.97), ($\chi 2$=78.8)
Complete regression of pain after surgery patients, %	263/267 98.5%	141/163* 86.5%	404 94.0%	0.10(0.03-0.29), ($\chi 2$=25.7)
Partial regression of pain after surgery patients, %	4/267 1.5%	21/163* 12.9%	25 5.8%	9.72(3.27-28.88), ($\chi 2$=23.92)
No pain regression	-	1	0.2%	_
Pain recurrence patients, %	5/267 1.9%	34/163* 20.9%	39 9.1%	13.81(6.28-36.15), ($\chi 2$=44.24)
HV+ in case of pain recurrence patients, %	1/5 20.0%	33/34* 97.1%	34 7.9%	132.00(6.84-2546.51), ($\chi 2$=23.2)

*P < 0.001.

Table 2. Herpes viruses in patients with classic TN

Type of herpes virus	HSV1/2	VZV	HHV-6	HHV-7	EBV	CMV
Patients, %	66/228	13/228	137/228	173/228	201/228	31/228
	28.9%	5.7%	47.6%	60.1%	88.2%	13.6%

Note: Df = 5, $\chi 2 = 536.331$ $P = 10^{-5}$

Currently, there is no complete picture of the pathophysiological mechanism of spontaneous pain and pain induced underlying TN as a result of herpes virus infection. Integumentary Schwann cells and oligodendrocytes are of critical importance to maintain the functioning of neurons. Demyelination of TN neurons is a key aspect of their dysfunction due to the formation of aberrant excitability [6]. It has been shown that herpes viruses HSV1/2, VZV, HHV-6, HHV-7 directly infect oligodendrocytes [9, 10]. It is assumed that the cause of postherpetic neuralgia may be infection of oligodendrocytes during the reverse transport of the virus after reactivation [11]. Damage to oligodendrocytes by herpes viruses by autoimmune mechanism has been shown [12-14].

The high detection rate of herpes viruses in patients with classical trigeminal neuralgia and a particularly high percentage in the subgroup with persistent background pain syndrome may indicate the likelihood of their participation in pathogenesis of the disease [16].

The difference in characteristics of pain syndrome with short-term paroxysms, neuralgic type, from persistent background pain syndrome of neuropathic type can also be caused by participation of herpes viruses in the damage to the structural elements of the nerve root or ganglion. There is reason to consider the formation of an adhesive process around the root as perifocal arachnoiditis [20]. The influence of herpes viruses on pain syndrome in patients with classic TN may be suggested by the observed regression of pain during antiviral therapy.

The presence of reactivated herpes viruses in the whole blood in a large number of patients with classic TN (53.0%) indicates the probable role of herpes viruses in the development of pain, which leads to the need for further research in this direction. In patients with TNB, herpes viruses are found more often (80.4%) than in TNP group (36.3%), this may indicate the likelihood of their participation in the disease pathogenesis. In TNB subgroup, partial regression of pain and pain relapses is observed more often than in TNP subgroup (12.8; 20.2, and 1.5; 1.8%, respectively), which may indicate the participation of herpes viruses in the cell damage and pathogenesis of the disease.

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ДОСЛІДЖЕННЯ НАЯВНОСТІ ВІРУСІВ ГЕРПЕСУ У ПАЦІЄНТІВ З КЛАСИЧНОЮ НЕВРАЛГІЄЮ ТРІЙЧАСТОГО НЕРВА

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Проведено порівняння методом полімеразної ланцюгової реакції частоти наявності вірусів герпесу HSV1/2, VZV, HHV-6, HHV-7, EBV, CMV серед 430 пацієнтів з діагнозом класична невралгія трійчастого нерва. При пароксизмальному больовому синдромі та персистуючому фоновому болю віруси герпесу виявлено у 36,3% (97/267) та у 80,4% (131/163) відповідно. Оцінено ефективність мікроваскулярної декомпресії та віддалених результатів залежно від

наявності вірусів герпесу у венозній крові. Повна елімінація болю в результаті мікроваскулярної декомпресії становила 94% (404/430), часткова – 5,8% (25/430), у одного пацієнта (0,2%) біль не регресував. У разі персистуючого фонового болю рецидиви та часткова регресія спостерігалися значно частіше (20,2% (34/163), 12,8% (21/163) відповідно), ніж у пацієнтів з пароксизмальним больовим синдромом (1,8% (5/267), 1,5% (4/267) відповідно). Повна регресія у разі персистуючого фонового болю була рідше, ніж при пароксизмальному больовому синдромі (86,5% (141/163) та 98% (263/267) відповідно). Наявність вірусів герпесу в крові пацієнтів з класичною невралгією трійчастого нерва, більша частота їх виявлення, а також вищий відсоток пацієнтів з рецидивом у разі персистуючого фонового болю порівняно з пароксизмальним больовим синдромом вказують на ймовірну роль вірусів герпесу у розвитку больового синдрому, і саме персистуючого типу. Таким чином, дослідження вірусів герпесу в крові є доцільним для вдосконалення діагностики та терапії пацієнтів з діагнозом класична невралгія трійчастого нерва.

Ключові слова: класична невралгія трійчастого нерва; віруси герпесу; HSV1/2; VZV; HHV-6; HHV-7; EBV; CMV.

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