

ORIGINAL ARTICLE

SEASONAL FACTOR AND FREQUENCY OF DETECTION OF HERPES VIRUSES AND INFLUENZA VIRUS IN PATIENTS WITH ISCHEMIC STROKE

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ABSTRACT

The aim: To determine the frequency of HSV1, HSV2, VZV, CMV, EBV, HHV6 and influenza virus detection in patients with ischemic stroke in different seasons.

Materials and methods: 144 patients with ischemic stroke were examined: 78 (54.2%) women and 66 (45.8%) men, mean age of 63.1 ± 0.8 years. Detection of the herpesvirus DNA and the influenza virus RNA was performed using PCR monthly in 12 patients.

Results: A manifestation of a viral infection was detected in 32 (22.2%) and virus genomes were observed in 29 (90.6%) patients. Viral infection frequency is significantly lower in summer, compared to winter-autumn; $p=0.033$. HSV1 and HHV6 were the most common (19 (52.8%) and 16 (44.4%)); VZV was the least common (5 (13.9%)). Influenza virus RNA was detected in 10 (27.8%) patients. In winter-autumn the frequency of HSV1, HSV2, HHV6 viruses detection is significantly higher, compared to the spring-summer ($p<0.05$), and the difference is almost significant for the influenza virus ($p=0.060$) and the EBV ($p=0.060$). Association of stroke occurrence with the presence of two or more types of viruses is more common in winter, compared to the summer season: 11 (30.6%) vs. 3 (8.3%), $p=0.017$.

Conclusions: Prevention and treatment of herpesvirus infections exacerbations, in particular HSV1 and HSV2, which significantly increase in winter, compared to summer, is an important direction of stroke prevention measures in risk groups.

KEY WORDS: ischemic stroke, herpes viruses, influenza virus, seasonal differences

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INTRODUCTION

World stroke statistics remains disappointing due to high morbidity, mortality and disability of the population, and this major problem of both social and economical importance stimulates the continuation of developments, such as measures to prevent acute cerebral pathology [1]. An important direction of research is a study of environmental impact, particularly seasonal fluctuations, on the occurrence of stroke, which becomes particularly important in the context of current climate changes.

Seasonal variability of the ischemic stroke frequency has been shown by numerous studies. However, the conclusions of these scientific works significantly differ. Some authors find a higher incidence of ischemic stroke (IS) in winter [2-4], others – in spring [5, 6], and some studies did not establish a correlation between the season and the incidence of IS at all [1, 7].

A recent meta-analysis showed that existing differences are largely related to different characteristics of the local climate [8]. However, in 29 of the 33 studies included in the meta-analysis, summer was the season with the lowest statistical incidence of IS. When grouped by Köppen climate classification [9] in areas with warm temperate climate, which is typical for most of Eastern European territory and, therefore, Ukraine, the incidence of IS was observed with

the highest frequency in winter months, with a subsequent decrease in frequency in spring, autumn and summer.

Seasonal differences in the incidence of stroke are associated with various physical natural factors, such as changes in the amount of sunlight [10, 11], temperature [12, 13], and humidity [14, 15, 16]. Many previous studies suggest that the winter season and low-temperature weather conditions may be responsible for the pathophysiological changes associated with ischemic stroke. In particular, it was shown that hemodynamic factors and indicators of coagulation hemostasis, such as blood pressure, erythrocyte count, platelet count and function, fibrinogen concentration in blood plasma, and inflammatory markers may increase due to increased activity of the sympathetic nervous system associated with low temperatures [4, 17].

Issues related to the increase of serum lipids and blood glucose are discussed to explain the seasonality of acute vascular diseases [18]. Some studies have found an abrupt increase in total cholesterol and triglycerides in the autumn months [19].

A certain role in IS incidence increase in the cold months is assigned to infection factors, including respiratory infections and influenza, which are activated due to reduced immunity caused by the deficiencies of sunlight, vitamins C and D, seasonal depression, etc. [11, 12, 19, 20, 21]. How-

Table I. Clinical and demographic features of the patients.

Indicators	Total (n=144)	Winter (n=36)	Spring (n=36)	Summer (n=36)	Autumn (n=36)	p
Sex M/F (%) 45.8/54.2		47.2/52.8	44.4/55.6	41.7/58.3	44.4/55.6	0.80
Age, year (M±m) 63.1±0.8		65.9±1.3	60.1±1.7	62.1±1.8	64.4±1.7	>0.05*
NIHSS, point (M±m) 11.1±0.1		10.9±0.3	10.7±0.2	11.4±0.3	11.2±0.2	>0.05*
Pathogenetic subtype of stroke						
Atherotrombotic (%)	50.7	50.0	44.4	50.0	58.3	0.866
Cardioembolic (%)	33.3	33.3	41.7	30.6	27.8	
Lacunar (%)	16.0	16.7	13.9	16.4	13.9	
Vascular areas						
Left middle cerebral artery (%)	43.1	41.7	47.2	44.4	38.9	0.839
Right middle cerebral artery (%)	31.9	33.3	36.1	30.6	27.8	
Vertebrobasilar arteries (%)	25.9	25.0	16.7	25.0	33.3	

Notes: *statistically unreliable for all pairwise comparisons

ever, the incidence of viral manifestation and persistence of viruses in patients with IS in different seasons has not been studied.

THE AIM

The goal of this study was to determine the frequency of HSV1, HSV2, VZV, CMV, EBV, HHV6 and influenza virus detection in patients with ischemic stroke in different seasons.

MATERIALS AND METHODS

The study took place over the course of one year from 01.01.2017 to 31.12.2017 at Oleksandrivska Hospital and Kyiv City Clinical Hospital №4 departments. The study was conducted in accordance with the requirements of Good Clinical Practice standards. A total of 144 patients with ischemic stroke were included in the study: 78 (54.2%) women and 66 (45.8%) men. The mean age of patients was 63.1 ± 0.8 years (from 41 years to 81 years).

During each season 36 patients hospitalized by ambulance were examined for the presence of viruses; specifically, 12 patients per month (3 patients per week) in order of their admission to the hospital and if the following inclusion criteria were met:

- a primary ischemic stroke confirmed by Magnetic Resonance Imaging / Computed Tomography;
- a neurological score of 8–16 points according to NIHSS [22]
- one of the following pathogenetic subtypes according to TOAST criteria [23]: atherothrombotic (AT), cardioembolic (CE), lacunar.

The patients were tested for herpes viruses (HSV1, HSV2, VZV, CMV, EBV, HHV6) and influenza virus.

There were following exclusion criteria: recurrent stroke, inability to collect a patient's history, NIHSS score above

17, indeterminate pathogenetic type of stroke, and a lack of informed consent for virological examination.

All patients were questioned on the subject of viral infection manifestation in their medical history (in the 2 weeks prior to stroke). The manifestations were evaluated using the clinical indicators of respiratory disease (rhinorrhea or rhinitis, fever and increased temperature, headache, catarrhal signs in upper respiratory tract, and a herpetic rash around lips and nose).

Detection of herpes virus deoxyribonucleic acid (DNA) and influenza virus ribonucleic acid (RNA) was performed by the means of polymerase chain reaction (PCR) monthly in 12 patients who were hospitalized by ambulance. The DNA of herpes viruses from cells was isolated using a set of reagents DNA-sorb-B DNA kit (AmpliSens, Russia) or "innuPREP Virus DNA Kit" (Analytik Jena AC, Germany) according to the manufacturer's instructions. The DNA concentration was determined spectrophotometrically using a Biophotometer (Eppendorf, Germany). The DNA detection was performed by the means of semi-quantitative PCR, using a set of "AmpliSens[®]" (AmpliSens, Russia) reagents, according to the manufacturer's recommendations. Each sample that was analyzed by PCR contained 50 nanograms of DNA. The amplification products of GeneRuler™ DNA Ladder Mix (Fermentas, Lithuania) were analyzed in a 1.7% agarose gel containing 0.01% ethidium bromide. Digital images of PCR products were obtained in UV light of a transilluminator using a Canon Digital IXUS 80IS camera. Analysis of digital images was performed using Gel Imager software (DNA-technology, Russia). In addition, RT-PCR was performed using the AmpliSens kit (AmpliSens, Russia) and EBARPOL (NPF. Litech, Russia), according to the manufacturer's recommendations (qTOWER 2.2 amplifier, Germany).

Detection of influenza virus RNA was performed by the means of PCR. Influenza virus RNA was isolated from cells using reagents kit in real-time (Real-Time RT-PCR kit),

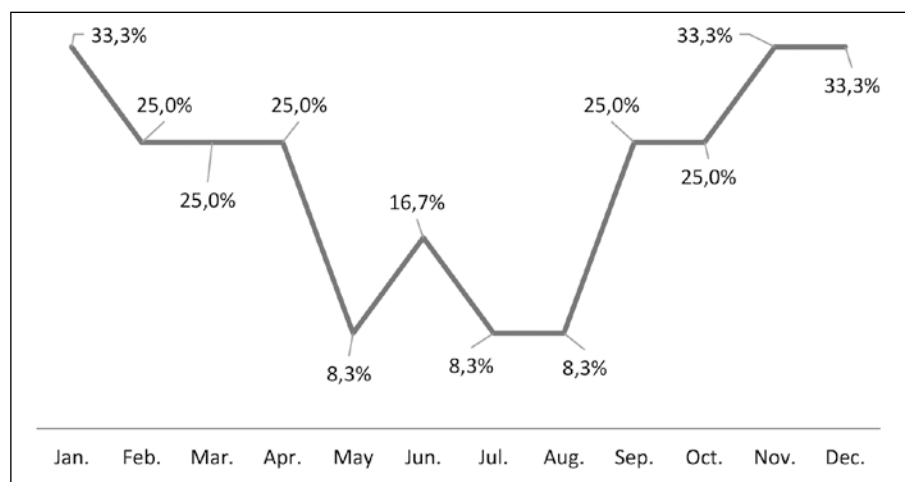


Fig. 1. Distribution of patients by frequency of viral infection by a month of year.

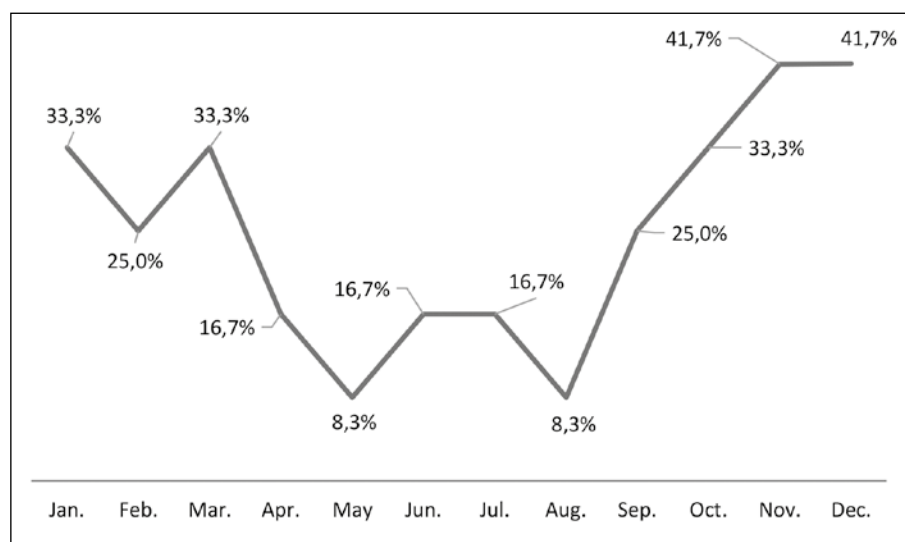


Fig. 2. Distribution of patients by frequency of viral infection by months of the year.

using Real-Time RT-PCR analysis, dPB2-Probe, dPB2-F2 and dPB2-R1 primers.

Statistical processing of the results was performed using the statistical analysis program IBM SPSS Statistics Base v.22. Descriptive statistics were used; comparisons of two independent groups by their average values were performed using the Mann-Whitney U-test, and on a qualitative basis – using Pearson's χ^2 . The null hypothesis regarding the equality of variables was rejected at $p < 0.05$.

RESULTS

The overall ratio of men and women was 45.8/54.2, respectively, and did not differ between seasons, $p = 0.80$ (Table I). The mean age of patients also did not differ significantly from season to season, $p > 0,05$.

Atherothrombotic subtype of stroke occurred in 73 (50.7% of patients), cardioembolic – in 48 (33.3%), lacunar – in 23 (16.0%) of patients. The distribution of patients by stroke subtype did not statistically differ between seasons ($p = 0.886$), although there was a tendency for an increase in the percentage of AT subtype in autumn – 58.3%, and CE subtype in spring (41.7%) with an almost identical percentage of AT in these seasons (Table I).

Ischemic stroke in the left middle cerebral artery territory (MCA) occurred in 62 (43.1%) patients; in the right MCA – 46 (31.9%), in the vessels of vertebrobasilar artery territory – 36 (25.0%). The distribution of patients by the territory of the affected vessel also did not significantly differ depending on the season ($p = 0.839$) (Table I).

A manifestation of a viral infection was found in 32 (22.2%) patients. In winter – in 11 (30.6%) patients, in spring – in 7 (21.9%), in summer – in 4 (11.1%), and in autumn – in 10 (27.8%), $p = 0.485$. Although overall there were no significant differences in the frequency of viral infection by seasons, at the same time in summer the frequency of viral infection was significantly lower than in winter, $p = 0.042$, and almost significantly lower than in autumn, $p = 0.074$.

The distribution of the frequency of viral infection in stroke patients by a month of year showed approximately the same frequency of 33.3% from November to January, with a decrease to 25.0% in February, March, April and September and October, and with the lowest rates in May and in summer months – 8.3% (Fig. 1).

Virus genomes were found in 36 (25%) patients with stroke. In particular, the genomes were found in 29 (90.6%) patients among those who had a viral manifestation (signs

Table II. The types viruses frequency detection and its quantity in the association in different seasons

Indicators	Total (n=144)	Winter n=36	Spring n=36	Summer n=36	Autumn n=36	p
Viruses						
HSV1, n (%)	19 (13.2)	9 (25.0)	3 (8.3)	3 (8.3)	4 (11.1)	0.111
HSV2, n (%)	14 (9.7)	7 (19.4)	3 (8.3)	0 (0.0)	4 (11.1)	0.048
VZV, n (%)	5 (3.5)	1 (2.8)	2 (5.6)	1 (2.8)	1 (2.8)	0.891
EBV, n (%)	10 (6.9)	4 (11.1)	2 (5.6)	1 (2.8)	3 (8.3)	0.542
CMV, n (%)	8 (5.6)	3 (8.3)	1 (2.8)	1 (2.8)	3 (8.3)	0.548
HHV6, n (%)	16 (11.1)	7 (19.4)	1 (2.8)	1 (2.8)	7 (19.4)	0.018
Flu, n (%)	10 (6.9)	5 (13.9)	2 (5.6)	0 (0.0)	3 (8.3)	0.133
Quantity of viruses in the association						
No viruses, n (%)	108 (75.0)	24 (66.7)	29 (80.8)	31 (84.1)	24 (66.7)	0.131
1, n (%)	8 (22.2)	1 (2.8)	2 (5.6)	2 (5.6)	3 (8.3)	0.787
2, n (%)	17 (47.2)	5 (13.9)	3 (8.3)	3 (8.3)	6 (16.7)	0.616
3, n (%)	6 (16.7)	2 (5.6)	2 (5.6)	0 (0.0)	2 (5.6)	0.237
4, n (%)	3 (8.3)	2 (5.6)	0 (0.0)	0 (0.0)	1 (2.8)	0.133
5, n (%)	2 (5.6)	2 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	0.279

of acute viral infection or exacerbation of latent persistent herpes infection) and in 3 (9.4%) patients with stroke without viral manifestation ($p = 0.001$) (Table II).

The genomes of viruses in winter were found in 12 (33.3%) patients, in spring – in 7 (19.4%), in summer – in 5 (13.9%), and in autumn – in 12 (33.3%) patients, $p = 0.131$.

Although in general there were no significant differences in the frequency of viral infection detection by season, at the same time in the summer season the frequency of viral infection detection was significantly lower compared to the winter-autumn period, $p = 0.033$.

The distribution of the frequency of the viruses genome detection in patients with stroke by months of the year showed approximately the same frequency from October to January – 33.3% – 41.7% (excluding February – 25.0%), with an average of 35.0% and a decrease to 8.3 – 25.0% from March through August, an average being 17.9%, $p = 0.019$ (Fig. 2).

In the virus-positive group, HSV1 was the most common, occurring in 19 (52.8%) patients (13.2% of the total) and HHV6 – in 16 (44.4%) patients (11.1%), with VZV-5 occurring least often (13.9%). Influenza virus RNA was found in 10 (27.8%) patients.

Despite the fact that there was a tendency toward a higher frequency of the viruses genome detection in winter and autumn compared to spring, and, most of all, summer, the differences did not reach statistical significance, $p = 0.052$. At the same time, there were significantly more patients with two or more viruses in the winter season compared to the summer season: 11 (30.6%) against 3 (8.3%), $p = 0.017$.

The frequency of viruses detection (except for HSV2 and HHV6) did not significantly differ depending on the season, when comparing the distribution of the frequency of detection of certain types of viruses in different seasons (Table II).

At the same time there is a significantly higher ($p < 0.05$) frequency of HSV1, HSV2, HHV6 viruses detection in the winter-autumn period, compared to the spring-summer period, and an almost significant difference for influenza viruses ($p = 0.060$) and EBV ($p = 0.060$).

There was no significant correlation between viral manifestations, the presence of the viruses genome and severe neurological deficits: $r = 0.037$ and $r = 0.039$, respectively.

There also was no significant correlation between viral manifestations, the presence of the herpes or influenza viruses genome and the age of patients: $r = 0.033$ and $r = 0.096$, respectively.

DISCUSSION

In our research we continued to study the issue of seasonal variability of the ischemic stroke frequency, which has been the subject of a number of foreign authors' studies that in particular at the role of a viral infection as a potential risk factor for increased frequency of IS in winter compared to summer [4, 5, 7]. This applies to countries with a warm continental climate, including Ukraine. [8]. The goal of our study was to determine whether there were any seasonal differences in the frequency of detection of the most common types of viruses – herpes viruses and influenza virus in patients after one stroke with moderate and severe neurological deficits. It should be noted that each season groups with the same number of patients were studied; further, the patients also did not significantly differ in age and gender characteristics. Our attention has been drawn to herpes viruses, because of their high prevalence at 30-40% of the population in developing countries [24], as well as toward the influenza virus, the epidemic outbreaks of which continue to regularly damage the health of the population and the economies of countries around the world.

The genome of herpes viruses and influenza virus was found in a total of 36 (25%) patients with stroke. The most prevalent was HSV1 found in 13.2% of patients and HHV6 found in 11.1% of patients; influenza virus was discovered in 6.9% of patients in the pre-stroke period. In most cases (77.8%) viral pathogens were in associations (2, 3, rarely 4 – 5 viruses), $p < 0,01$.

The study has demonstrated a significantly higher percentage of patients with herpesviruses persistence, specifically, with two or more viruses, in winter, compared to the summer season. Thus, according to the literature, a more frequent development of strokes in the winter season (compared to summer in our climate zone), is associated, according to our findings, with a higher frequency of detection of herpes and influenza viruses genomes in patients' blood during the winter season.

It is known that infection and stroke have common pathogenetic pathways and mechanisms: inflammation, hypercoagulation and thrombosis [25]. Therefore, it is logical to assume the existence of an indirect connection between an increase in the frequency of strokes and the increase in the frequency of detection of viral factors that activate during the winter season – a period of reduced immune activity due to a deficiency of sunlight, vitamins D and C, seasonal depression, cold sympathetic systems activation, etc. The obtained data may help to improve the prevention of ischemic stroke by conducting a virological examination in instances of respiratory viral infection manifestation in patients in stroke risk groups in the autumn-winter period, as well as by an active use of influenza vaccinations.

CONCLUSIONS

Detection of the herpes viruses genome in the blood of patients with ischemic stroke with moderate to severe neurological deficits, both with and without the clinical manifestations of viral infection, is more common in winter and autumn.

During the winter-autumn period there is a significantly higher frequency of HSV1, HSV2, HHV6 viruses detection compared to the spring-summer season ($p < 0,05$).

In the winter season, the frequency of patients with two or more types of viruses in an association is significantly higher compared to the summer season: 11 (30.6%) against 3 (8.3%), $p = 0.017$.

The frequency of herpes viruses detection does not correlate with the age of patients and the severity of neurological deficits: $r = 0,096$ and $r = 0,039$, respectively.

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Conflict of interest:

The Authors declare no conflict of interest.

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