INCIDENCE OF CONFIRMED MANIFEST VIRAL INFECTION IN CASES OF ISCHEMIC STROKE, DEPENDING ON THE SEASON

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Relevance. Seasonal differences in stroke incidence are associated with various physical factors, some associated with pathophysiological changes in the body leading to ischemic stroke. At the same time, there is little research into the differences in the frequency of detection of the genome of herpes virus infection and influenza virus, depending on the season.

Objective. Investigate the existence of associations between the season and the frequency of virologically confirmed herpexoid infection manifested by herpezd virus and influenza virus of patients with ischemic stroke.

Materials and methods. In the period from 01.01.2017 to 31.12.2017, during the year at the bases of the neurological and department of the Alexandrovsky Clinical Hospital Centre. Kyiv conducted a study of 144 cases of cerebral ischemic stroke with medium-severe neurological deficit: 78 (54.2%) women and 66 (45.8%) men, with an average age of 63.1 ± 8.0 years. Neurological examination and identification of the genome of herpes viruses and influenza virus, performed with the help of a polymerase chain reaction (PCR), was carried out monthly in 12 patients hospitalized by ambulance. Account was taken of the presence of a viral manifestation if it preceded the stroke within 2 weeks.

Results. 36 cases were examined each season. The ratio of men to women was 45.8 / 54.2 in total and did not differ between seasons, p = 0.514. 32 (22.2 per cent) patients have demonstrated a viral infection. In winter, 11 (34.4 per cent), in spring, 7 (21.9 per cent), in summer, 4 (12.5 per cent), in autumn, 10 (31.3 per cent) and all p. At the same time, during the summer period, the incidence of viral manifestation was definitely lower than in winter, p = 0.042. Virus genomes were found in 12 (33.3 per cent) patients in winter, 7 (19.4 per cent) in spring, 5 (13.9 per cent) in summer, 12 (33.3 per cent) in autumn and p = 0.131 rice. During the summer period, the frequency of PCR-confirmed herpes virus infection was definitely lower than in the winter-autumn season, p = 0.033.

Conclusions. Viral manifestations of herpes and influenza virus in the blood are more frequent during the winter and autumn periods; the frequency of detection indicated viral infection from October to January is reliably higher than the rate from March to August inclusive.

In 25.0 per cent of patients with ischemic stroke, the genome of herpes viruses and influenza virus in the blood is detected. In the event of a demonstration, the frequency of the above-mentioned viral infection is clearly higher (90.6 per cent as against 9.4 per cent) than that of patients without it.

Keywords: ischemic stroke, herpes virus, flu virus, season.

RELEVANCE

Data from the scientific literature, world experience indicate an increase in the prevalence of cerebrovascular disease (CVD) among the population of most countries, as well as their severe medical, demographic and socio-economic consequences [1-3]. To develop effective strategies for stroke prevention, it is necessary to continue the search for new, non-traditional risk factors, which include viral infection [4].

Numerous foreign studies suggest that latent viral infections may be predictors of ischemic cerebral stroke (ICS) [5-7]. The accumulation of infectious agents is obvious, which can play the role of triggers of the inflammatory process. In most cases, the development of CVD is influenced by the association of viral infection and other risk factors [8-10]. An important contribution in this aspect of environmental factors, climatic factors. Depending on the season, many works are devoted to the frequency of stroke, mainly by foreign authors [11-13].

An in-depth understanding of the role of the infectious factor that precedes a transient ischemic attack and IS, and often determines their development, is important for the development of preventive measures and improvement of treatment of acute cerebrovascular disorders (ACD) [14,15].

In the domestic literature there are isolated works on the influence of seasonality only on the spread of enterovirus infection [16].

Objective: Investigate the existence of associations between the season and the frequency of virologically
confirmed herpezoid infection manifested by herpezoid virus and influenza virus of patients with ischemic stroke.

MATERIALS AND METHODS

The study was conducted during the year from 01.01.2017 to 31.12.2017 on the basis of the neurological department and the department of cerebrovascular pathology of the Alexandrovsky Clinical Hospital Centre in Kyiv. The study included 144 patients with ischemic stroke: 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).

In each season for the presence of viruses were examined 36 patients who were hospitalized by ambulance: 12 patients per month (3 patients per week), in the order of admission to the hospital.

Inclusion criteria: primary ischemic stroke, confirmed by MRI/CT scan, neurological score according to NIHSS [17] 8-16 points. The pathogenetic subtype was determined according to TOAS criteria [18], namely: atherothrombotic, cardioembolic, lacunar.

Criteria for non-inclusion in the study were: recurrent stroke, inability to collect a history of the patient, NIHSS score over 17, indeterminate pathogenetic subtype of stroke, lack of informed consent for virological examination.

All patients were diagnosed with a history of viral infection (two weeks before ACD), which was assessed by clinical signs of respiratory disease with runny nose, fever and fever, signs of intoxication, herpetic rashes of the lips and nose, available, herpesszoster.

Herpes viruses (HSV1, HSV2, VZV, CMV, EBV, HHV6) and influenza virus were detected.

Detection of herpesvirus DNA was performed by polymerase chain reaction (PCR). Herpesvirus DNA was isolated from cells using a DNA-sorb-BDNAkit reagent kit (AmpliSens, Russia) or innuPREPVirusDNAkit (AnalitykJenaAC, Germany) according to the manufacturer’s instructions. The DNA concentration was determined spectrophotometrically using a Biophotometer (Eppendorf, Germany). DNA detection was performed by semi-quantitative PCR, using a set of reagents «AmpliSens®» (AmpliSens, Russia) according to the manufacturer’s recommendations. Each sample analyzed by PCR contained 50 ng of DNA. The amplification products and 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 Gellmager software (DNA-technology, Russia). In addition, RT-PCR was performed using the AmpliSens kit (AmpliSens, Russia) and EBARPOL (NPF Litech LLC, Russia), according to the manufacturer’s recommendations (qTOWER 2.2 amplifier, Germany). Detection of influenza virus PNA was performed by PCR. Influenza virus RNA from cells was isolated using a set of reagents in real time (Real-TimeRT-PCR), using the technique of multiplex TaqManReal-TimeRT-PCR analysis, primers and TaqMan-probes, part of the domestic test system «DIANfluen».

Statistical processing of the obtained results was performed using the statistical analysis program IBMSPSS Statistics Base v.22. Descriptive statistics were used, comparisons of two independent groups on average values were performed using the Mann-Whitney U-test, on a qualitative basis — using Pearson’s χ². The null hypothesis regarding the equality of variables was rejected at p <0.05.

RESULTS AND THEIR DISCUSSION

The ratio of men and women was generally 45.8/54.2 and did not differ between seasons, p = 0.80 (Table 1).

The mean age of patients was 63.1 ± 0.8 years (from 41 years to 81 years). There were no significant differences in mean age between seasons (Fig. 1).

Table 1

<table>
<thead>
<tr>
<th>Season</th>
<th>Women absolute</th>
<th>Women %</th>
<th>Men absolute</th>
<th>Men %</th>
<th>Total absolute</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>17</td>
<td>47.2</td>
<td>19</td>
<td>52.8</td>
<td>36</td>
<td>100.0</td>
</tr>
<tr>
<td>Spring</td>
<td>20</td>
<td>55.6</td>
<td>16</td>
<td>44.4</td>
<td>36</td>
<td>100.0</td>
</tr>
<tr>
<td>Summer</td>
<td>21</td>
<td>58.3</td>
<td>15</td>
<td>41.7</td>
<td>36</td>
<td>100.0</td>
</tr>
<tr>
<td>Autumn</td>
<td>20</td>
<td>55.6</td>
<td>16</td>
<td>44.4</td>
<td>36</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>54.2</td>
<td>66</td>
<td>45.8</td>
<td>144</td>
<td>100.0</td>
</tr>
</tbody>
</table>

![Fig. 1. The average age of patients with a 95% confidence interval at different times of the year (all p > 0.05)](image)

Atherothrombotic (AT) subtype of stroke had 73 (50.7%) of patients, cardioembolic (CE) – 48 (33.3%), lacunar – 23 (16.0%).

The distribution of patients by stroke subtype did not differ significantly depending on the season (p = 0.886), there was only a tendency to increase the percentage of AT subtype in autumn – 58.3%, and CE – in spring (41.7%), which did not differ from blood pressure (Fig. 2).
Ischemic stroke occurred in the pool of the left middle cerebral artery (LMCA) in 62 (43.1%) patients, the right middle cerebral artery (RMCA) – 46 (31.9%), in the vertebro-basilar pool (VBP) – 36 (25.0%). The distribution of patients in the pool of the affected vessel also did not differ significantly depending on the season (p=0.839) (Fig. 3).

The severity of neurological deficits on the NIHSS scale averaged 11.1±0.16 points: from 8 to 16 points (Fig. 4).

The severity of neurological deficits of the studied patients on the NIHSS scale did not differ significantly at different times of the year, and was 10.9±0.3 in winter; 10.7±0.2 in spring; 11.4 ±0.2 in summer and 11.2 ±0.2 in autumn (Fig. 5).

Clinical manifestation of acute respiratory viral infection and influenza was found in 32 (22.2%) patients. In winter it was in 11 (30.6%) patients, in spring – in 7 (21.9%), in summer – in 4 (11.1%), in autumn – in 10 (27.8%) (Fig. 6).

There were no statistically significant differences in the frequency of viral manifestations in winter, spring, autumn, all p>0.05. At the same time, in summer the frequency of viral infection was significantly lower.
compared to winter, \( p=0.042 \), and almost significantly lower compared to autumn, \( p=0.074 \).

The distribution of the incidence of viral infection in patients with ICS by months of the year showed approximately the same frequency: 33.3% was from November to January with a decrease to 25% in February, March, April, September, October and the lowest rates in May and summer months – 8.3% (Fig. 7).

Genomes of the herpesvirus family and influenza were detected in 36 (25%) patients with ICS. In particular, in 29 (90.6%) patients among those who had a viral manifestation (i.e., signs of acute viral infection or exacerbation of latent persistent herpes infection), and in 3 (9.4%) patients with ICS without viral manifestation \( (p=0.001) \) (Fig. 8).

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%), in autumn – in 12 (33.3%), \( p = 0.131 \) (Fig. 9).

Although in general there were no significant differences in the frequency of detection of viral infection by season \( (p=0.131) \), at the same time, in summer the frequency of detection of viral infection was significantly lower compared to the winter-autumn season, \( p=0.033 \). In addition, in the winter season there were significantly more patients with two or more types of viruses in associations compared to the summer season: 11 (30.6%) vs. 3 (8.3%), \( p=0.017 \).

The frequency of detection of the genome of viruses in patients with ICS by months of the year was approximately the same from October to January – 33.3% (except February – 25.0%), an average of 35.0% with a decrease to 8.3-25.0% from March to August inclusive, on average 17.9%, \( p=0.019 \) (Fig. 10).

Thus, this work continues a series of studies on the complex interaction of environmental factors, climatic conditions, seasonal influences and insufficiently studied non-traditional risk factors for stroke, with the hope of a significant shift in the prevention of ACD. There are many studies investigating, in particular, the effect of seasonal

**Fig. 7.** Distribution of patients by frequency of viral infection by months of the year

**Fig. 8.** The frequency of detection of the genome of viruses in patients with ACD depending on the presence of a viral manifestation

\[ \text{Manifestation} \]
changes on the incidence of ischemic stroke, but their results are quite different [11-13], which can be partly explained by the lack of mandatory use of the Köppen climate zone classification [20]. As for the countries with a humid continental climate, which includes Ukraine, the latter aims to analyze the increase in the incidence of stroke in winter [21]. The aim of our study was to assess the contribution of infectious agents to the development of ICS, in particular latent herpes viruses, which are characterized by latent persistence, and influenza viruses, whose winter-spring outbreaks cause significant damage to public health and the economy.

We have shown a significantly higher percentage of patients with persistence of herpesviruses in winter compared to the summer season, and in winter the associations of two or more types of viruses were more often detected, which may indicate immunosuppressive tendencies in patients with cerebrovascular pathology. It is noteworthy the high percentage of detection of genomic herpesvirus infection (90.6%) in the case of its clinical manifestation, which may be useful for the selection of groups of patients - candidates for virological examination. Taking into account the common links in the pathogenesis of infectious inflammation and atherothrombotic stroke [22], we can assume a certain connection between the increase in the frequency of the viral trigger in the winter-autumn period and the higher frequency of ICS. Thus, the next question that clearly arises for family physicians is the prevention and treatment of exacerbations of herpesvirus infections to prevent stroke in at-risk groups.

**CONCLUSIONS.**

Viral manifestations of herpes and influenza virus in the blood are more frequent during the winter and autumn periods; the frequency of detection indicated viral infection from October to January is reliably higher than the rate from March to August inclusive.

In 25.0% per cent of patients with ischemic stroke, the genome of herpes viruses and influenza virus in the blood is detected. In the event of a demonstration, the frequency of the above-mentioned viral infection is clearly higher (90.6 per cent as against 9.4 per cent) than that of patients without it.

**REFERENCES**


![Fig. 9. The frequency of detection of viral infection in patients with ACD depending on the season](image)

![Fig. 10. Distribution of patients by frequency of detection of the genome of viruses by months of the year](image)


Conflicts of interest: authors have no conflict of interest to declare.
ЧАСТОТА ВИЯВЛЕНИЯ ПІДТВЕРЖДЕНОЇ МАНИФЕСТАЦIЇ ВIРУСОВIй ІНФЕКЦIЇ У ХВОРИХ З IЩЕМІЧНИМ ІНСУЛЬТОМ В ЗАЛЕЖНОСТІ ВIД СЕЗОНУ РОКУ

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Актуальнiсть. Сезоннi вiдмiнностi у частотi захворюваностi на інсульт пов'язують з рiзними фiзичними природними факторами, деякi з них асоцiюють з патофізiологiчними змiнами в органiзмi, що призводять до розвитку ішемічного інсульта. Водночас практично не дослiджено, чи існують вiдмiнностi в частотi виявлення геному герпесвiрусної iнфекцiї та вiрусу грипу залежно вiд сезону року.

Мети роботи. Дослiдити наявнiсть асоцiацiй мiж порою року та частотою пiдтвердженої манiфестацiї герпесвiрусної iнфекцiї та вiрусу грипу пацiєнтiв з iшемiчним інсультом.

Матерiал та методи. В перiод з 01.01.2017 р. до 31.12.2017 р., протягом року на базi неврологiчного вiддiлення та вiддiлення церебро-васкулярної патологiї Олександрiвської клiнiчної лiкарнi м. Києва виконувалося дослiдження 144 хворих на мозковий ішемiчний інсульт (МІ) iз середньо-також неврологiчнимi дiагнозами: 78 (54,2%) жiнок та 66 (45,8%) чоловiкiв, iз середньо вiком хворих 63,140,8 рокiв. Неврологiчне обстеження та виявлення геному герпесвiрусiв та вirusu грипу, що здiйснювалося за допомогою полiмеразної ланцюгової реакцiї (ПЛР), проводилося шостимiся у 12 хворих, госпiталiзованим за швидкою допомогою. Враховували наявнiсть манiфестацiї вiрусної iнфекцiї, якщо вони передуvalа МІ протягом двох тижнiв.

Результати. В кожний сезон року було обстежено 36 хворих. Спiвiдношування чоловiкiв та жiнок становило в цiлому 45,8/54,2 та не вiдрiзнялося мiж сезонами, p=0,514. Манiфестацiя вiрусної iнфекцiї визначалась у 32 (22,2%) хворих. З них у зимовий перiод — у 11 (34,4%) хворих, у весеннiй — у 7 (21,9%), у лiтнiй — у 4 (12,5%), в осенiй — у 10 (31,3%), вiсім на пiвострою, 5,05. Водночас, у лiтнiй перiод частота манiфестацiї вiрусної iнфекцiї була достовiрно меншою порiвнюючи з зимовим перiодом, p=0,042. Геноми вiрусiв у зимовий перiод виявлено у 12 (33,3%) хворих, у весеннiй — у 7 (19,4%), у лiтнiй — у 5 (13,9%), у осенiй — у 12 (33,3%), p=0,131. У лiтнiй перiод частота пiдтвердженої за допомогою ПЛР грипової iнфекцiї була достовiрно меншою порiвнюючи з зимовим осенiм сезоном, p=0,033.

Висновки. Вiрусологiчна пiдтверджена манiфестацiя герпесвiрусiв та вirusu грипу в кронi частота вiдрiзняється в зимовий та осенiй перiоди; частота виявлення пазовеної вiрусної iнфекцiї з ловка до сiчня достовiрно переживає аналогiчний поки iз зберегає до серпня включаючи.

У 25,0 % хворих з iшемiчним iнсультом вiдзначалась геномна герпесвiрусна iнфекцiя та вiрус грипу в кровi. За умов клiнiчної манiфестацiї (22,2% хворих), частота вiданої вiрусної iнфекцiї достовiрно вище (90,6 % проти 9,4%), нiж у хворих без манiфестацiї.

Ключовi слова: iшемiчний інсульт, герпесвiрусна iнфекцiя, вiрус грипу, сезон року.