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CHARACTERISTICS OF WEST NILE ENCEPHALITIS IN THE AUTONOMOUS PROVINCE OF VOJVODINA IN THE PERIOD 2021 TO 2022

KARAKTERISTIKE ENCEFALITISA UZROKOVANIH VIRUSOM ZAPADNOG NILA NA TERITORIJI AUTONOMNE POKRAJINE VOJVODINE U PERIODU OD 2021. DO 2022. GODINE

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Summary

Introduction. Neuroinvasive the West Nile virus disease develops in less than 1% of infected individuals, with a mortality rate of approximately 9%. This scientific research aimed to analyze the epidemiological, clinical, and laboratory characteristics, the presence of comorbidities, and the treatment outcome of West Nile encephalitis in 2021 and 2022. Material and Methods. The retrospective study includes 33 patients treated at the Infectious Diseases Clinic of the University Clinical Center of Vojvodina from January 1, 2021 to December 31, 2022. The diagnosis was confirmed by serological tests and/or real-time reverse transcriptase polymerase chain reaction of cerebrospinal fluid. Results. The study demonstrated a statistically significant predominance of males (57.6%) over females (42.4%) (χ^2 =4.5; p=0.03).Individuals over the age of 65 accounted for 51.52% of cases, with the remaining 48.48% being within the working-age population. The highest concentration of cases was observed in the Novi Sad (24.2%). Upon admission, elevated body temperature was prevalent 97% of patients ($\chi^2 =$ 8.8; p = 0.03), followed by weakness and malaise in 75.8%, and altered consciousness in 66.7%. Meningeal signs were present in only 48% of patients. Infection was confirmed in all patients through serological analysis of cerebrospinal fluid. A fatal outcome was observed in 31.3% of cases. Conclusion. The results of the research indicate that serological analysis is the most reliable method for diagnosing the neuroinvasive form of infection.

Key words: West Nile virus; Encephalitis; Serologic Tests; Signs and Symptoms; Diagnosis; Demography; Treatment Outcome; Risk Factors

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Introduction

Instances of acute encephalitis necessitate prompt intervention to reduce mortality rates and

Sažetak

Uvod. Neuroinvazivna bolest razvija se kod manje od 1% inficiranih osoba, sa stopom mortaliteta oko 9%. Cilj je bio analizirati epidemiološke, kliničke, laboratorijske karakteristike, prisustvo komorbiditeta i ishod lečenja encefalitisa uzrokovanog virusom Zapadnog Nila tokom prethodne dve epidemije 2021. i 2022. godine. Materijal i metode. Istraživanje je koncipirano kao retrospektivna studija i uključuje 33 pacijenta lečena na Klinici za infektivne bolesti Univerzitetskog kliničkog centra Vojvodine u Novom Sadu u periodu od 1. 1. 2021. do 31. 12. 2022. godine. Dijagnoza je potvrđena serološkim testovima i/ili real time reverznom transkripcijom lančane reakcije polimeraze uzorka likvora. Rezultati. Rezultatima je dokazana statistički značajno veća zastupljenost muškog pola (57,6%) u odnosu na ženski pol (42,4%) (χ^2 =4,5; p = 0,03). Tokom navedenih epidemija oboleli su predominantno u uzrasnoj grupi preko 65 godina (51,52%), dok je 16 pacijenata (48,48%) bilo u grupi radno sposobnog stanovništva. Najveća incidencija obolelih je sa prebivalištem na teritoriji opštine Novi Sad (24,2%). Od simptoma na prijemu statistički značajno je dominirala povišena telesna temperatura 97% ($\chi^2 = 8.8$; p = 0.03), slede slabost i malaksalost 75,8% i poremećaj stanja svesti 66,7%. Svega 16 pacijenata (48%) je imalo pozitivne meningealne znake u kliničkom nalazu. Infekcija je kod svih ispitivanih bolesnika potvrđena serološkim analizama likvora. Letalni ishod je registrovan kod 31,3% pacijenata. Zaključak. Rezultati istraživanja su pokazali da serološki testovi predstavljaju najpouzdaniju metodu za postavljanje dijagnoze kod neuroinvazivnog oblika infekcije. Ključne reči: virus Zapadnog Nila; encefalitis; serološki testovi; znaci i simptomi; dijagnoza; demografija; ishod lečenja; faktori rizika

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minimize the neurological sequelae [1, 2]. Among infectious etiologies, the West Nile virus (WNV) stands out as a significant cause, inciting epidemics characterized by febrile illness, meningitis, en-

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WNV	– West Nile Virus
CNS	 Central nervous system
RNK	- Ribonucleic acid
RT-PCR	- Reverse transcription-polymerase chain reaction
ELISA	 Enzyme-linked immunosorbent assay
UCCV	- University Clinical Center of Vojvodina
CT	- Computed tomography
CRP	 C-reactive protein

cephalitis, and flaccid paralysis [3]. The WNV, an arbovirus of the Flaviviridae family, is globally disseminated via transmission by Culex mosquitoes, primarily Culex pipiens [4]. Birds are the principal reservoir hosts for WNV in natural ecosystems. Certain mosquito species become infected after feeding on birds with high viremia, and these mosquitoes can then transmit the virus to other vertebrates, including horses and the humans [5]. WNV is believed to replicate initially at the site of inoculation, spreading to regional lymph nodes before entering the bloodstream [6]. The virus infiltrates the central nervous system (CNS) after activating toll-like receptors, in which increase blood-brain barrier permeability by elevating tumor necrosis factor levels [7]. WNV primarily targets neurons in the deep nuclei and gray matter of the brain, as well as in the brainstem and spinal cord [8].

Approximately 80% of WNV infections in humans are asymptomatic, while 20% manifest symptoms such as viral fever [9]. Neuroinvasive disease, affecting less than 1% of those infected, is more likely to occur in immunocompromised and elderly individuals, who are at greater risk of long-term sequelae [10]. Other risk factors for neuroinvasive disease include diabetes, hypertension, and cerebrovascular disease [11].

The clinical spectrum of WNV infection ranges from mild fever to severe symptoms lasting several days to weeks, including debilitating fatigue, pain, weakness, and headaches. Gastrointestinal symptoms and a transient macular rash on the trunk and extremities are sometimes observed [12]. Encephalitis from WNV infection can range from mild disorientation to coma, potentially leading to death [9].

rientation to coma, potentially leading to death [9]. Various diagnostic modalities are available for identifying WNV as the causative agent of CNS infection. Diagnosis is typically confirmed through the detection of viral RNA in serum or cerebrospinal fluid using RT-PCR assays [13, 14]. The detection WNV in cerebrospinal fluid or serum during the acute phase of neurological illness is regarded as a confirmatory diagnostic parameter. WNV-specific antibodies can be detected through ELISA, immunofluorescence assays, neutralization tests, or hemagglutination tests. IgM antibodies appear approximately 4-7 days post-exposure and may persist for about a year, while IgG antibodies can be reliably detected from the eighth day following infection [15].

Patients with WNV encephalitis or focal neurological manifestations often experience prolonged neurological deficits lasting for months or years. The overall mortality rate for neuroinvasive WNV disease is approximately 9% [16].

This study aims to assess the incidence, clinical and epidemiological profiles, gender distribution, association with comorbidities, disease progression, outcomes, and diagnostic methodologies used for detecting the virus in patients diagnosed with WNV encephalitis over the past two years.

Material and Methods

The study was designed as a retrospective study. It included 33 patients diagnosed with WNV encephalitis who were hospitalized at the Infectious Diseases Clinic of the University Clinical Center of Vojvodina (UCCV) between May 2021 and September 2022.

The diagnosis of WNV encephalitis was confirmed through cytobiochemical analysis of cerebrospinal fluid, indicative of viral encephalitis. Etiological diagnosis was established in all patients through multiplex polymerase chain reaction detection of the virus or by detecting elevated levels of IgM antibodies in both cerebrospinal fluid and serum samples. All patients included in the study were aged 18 years or older, and both genders were represented.

Demographic data were collected from admission medical records. Clinical findings included neurological assessments, laboratory analyses of blood and cerebrospinal fluid, cerebrospinal fluid cultures, neurological sequelae, and treatment outcomes.

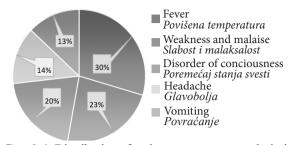
The statistical significance of differences between categorical variables was assessed using the χ^2 test. Continuous variables are presented as the arithmetic mean and standard deviation for normally distributed data, or as the median and interquartile range for data that deviated significantly from normality. The data were retrospectively extracted from medical records. Ethical approval for the study was obtained from the Ethics Committee of the UCCV and the Ethics Board of the Faculty of Medicine in Novi Sad.

Results

The study included 33 patients diagnosed with WNV encephalitis, hospitalized at the Infectious Diseases Clinic of the UCCV from May 2021 to September 2022.

Analyzing gender representation among the examined patients, the total distribution was as follows: 19 out of 33 (57.6%) were male, and 14 out of 33 (42.4%) were female. No statistically significant difference was observed in the distribution of patients by gender (γ^2 test; γ^2 =1.551; p=0.213).

tients by gender (χ^2 test; $\chi^2=1.551$; p=0.213). The distribution of patients by age indicated a slight predominance of individuals within the geriatric population (>65 years old), comprising 17 out of 33 patients (51.52%). The group of non-geriatric patients (aged 31 to 65 years) included 16 out of 33 patients (48.48%), with no patients in the under-30 age group. The mean age of the patients was 63±34 years (range 38-83 years). Among them, 18 out of 33 patients



Graph 1. Distribution of main symptoms upon admission Grafikon 1. Distribucija glavnih simptoma pri prijemu

(54.5%) predominantly living in rural areas, while 15 out of 33 (45.5%) resided in urban settings, often near a river or forest. The average length of hospital stay was 19±9.4 days, with a range from 1 to 44 days.

All patients had symptoms persisting for more than 24 hours before hospital admission (33 out of 33 cases, 100%). During the two-year study period, 32 out of 33 patients (97%) presented with an elevated temperature as a primary initial symptom prompting medical consultation. Weakness and malaise were the second most common symptoms, affecting 25 out of 33 patients (75.8%) ($\chi^2=0.330$; p=0.566). Disturbances in consciousness ranked third, impacting 22 out of 33 patients (66.7%). Additional symptoms included headaches in 15 out of 33 patients (45.5%), nausea in 14 out of 33 patients (42.2%), and vomiting in 15 out of 33 patients (45.5%). The distribution of symptoms is shown in Graph 1. Out of the total number of patients, sixteen exhibited positive meningeal signs upon admission to UCCV, while thirteen out of thirty-three patients displayed neurological signs.

All 33 patients underwent a cranial computed tomography scan upon admission, which revealed no contraindications for performing a lumbar puncture. Cerebrospinal fluid findings consistent with viral encephalitis were observed in all patients. RT-PCR testing was conducted on 31 out of 33 (93.93%) patients, with nine testing positive, accounting for 27.27% of the tests performed. ELISA tests were conducted on all patients using cerebrospinal fluid and serum samples. Positive IgM antibodies were detected in 27 out of 33 patients (81.8%) in both cerebrospinal fluid and blood samples. IgG antibodies were found in the blood samples of 8 out of 33 patients (24.24%) and in the cerebrospinal fluid of 7 out of 33 patients (21.21%). Indicators of the inflammatory response, such as Creactive protein (CRP) levels, total leukocyte count, and lymphocyte count, were assessed. Elevated CRP levels (CRP>5) were observed in 21 out of 33 patients (63.63%) upon admission, and leukocytosis (leukocyte count: $4-10 \ge 10^9/L$) was detected in 18 out of 33 patients (54.54%), primarily due to lymphocytosis.

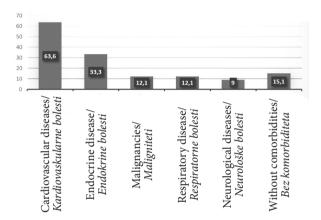
Among the predisposing factors contributing to disease onset, comorbidities were identified (Graph 2). Concurrent diseases were documented in 28 out of 33 patients (84.84%), while five patients presented without comorbidities (15.15%).

Predominant comorbidities included cardiovascular conditions (21 out of 28 patients – 63.6%), endocrine disorders (11 out of 28 patients -33.3%), malignancies (4 out of 28 patients -12.1%), respiratory conditions (4 out of 28 patients -12.1%), and neurological disorders (3 out of 28 patients -9%).

Antibiotic, antiviral, corticosteroid, antiedematous, and symptomatic therapies were continued until the causative agent was identified.

Out of the total number of patients treated at the UCCV Infectious Diseases Clinic during the examined year, 13 (39.4%) were transferred to the Intensive Care Unit due to respiratory complications, insufficient oxygenation, or altered consciousness leading to coma.

During the years 2021 and 2022, ten out of thirtythree patients (31.3%) treated at the UCCV Infectious Diseases Clinic experienced a fatal outcome.



Graph 2. Presence of comorbidities as one of the predisposing factors for the occurrence of WNV encephalitis **Grafikon 2.** Prisustvo komorbiditeta kao jednog od predisponirajućih faktora za nastanak virusa Zapadnog Nila encefalitisa

Discussion

The WNV was first identified in the bloodstream of a febrile patient in Uganda in 1937. Subsequently, the virus spread progressively through migratory birds, originating from Africa and extending to other continents. Before 2004, WNV was associated with sporadic occurrences in humans and equines in Europe. Considering the favorable geographical conditions, appropriate climatic factors, the presence of vectors, and the rising incidence of cases along the Danube River, the first instances of WNV encephalitis occurred in our region [17].

The initial detection of WNV antibodies in clinically asymptomatic individuals in Serbia was documented in 1972, using inhibition and hemagglutination techniques. The first clinical epidemic of WNV infection in humans in the Republic of Serbia occurred in 2012. In 2013, the Republic of Serbia had the highest number of reported WNV infection cases among European countries, with 32 confirmed patients [18].

Considering the demographic profile of our nation, out of the 33 patients included in our study, 18 individuals (54.5%) reside in rural areas. In contrast, during a 1996 epidemic in Romania, the highest infection rate was observed in districts adjacent to the Danube River, with 61% of patients residing in urban areas [19]. Conversely, during the 2010 epidemic in Greece, the affected population was predominantly from rural areas, although data on residence proximity to rivers were also considered [20].

Multiple European and American studies corroborate that over 90% of infections are attributed to the seasonal WNV, with the highest incidence rate occurring between July and September [11].

Both epidemic waves analyzed in our study pertain to the same period of complaints and hospitalizations, with August being the predominant month in both surveyed years.

In a study conducted in Romania, age and gender are identified as the primary predisposing factors for WNV infections. It has been noted that WNV infection is significantly more prevalent in the elderly population compared to younger individuals. During the 2010 epidemic in Romania, where WNV infection was confirmed in 50 cases, the average age of the patients was 59.5 years (ranging from 12 to 81 years). In our study, no confirmed cases were recorded in individuals below the age of 18 years, with an average age of 63 years (ranging from 38 to 83 years), predominantly comprising the geriatric population (17 out of 33 patients). In both studies, males constituted the majority, comprising 57.6% in our case and 68% in the Romanian study [21].

A 2020 American study reported similar findings, with 559 cases of neuroinvasive forms of WNV infection recorded. The incidence escalated with advancing age, with the highest number of patients falling in the age group over 65 years. Additionally, a higher frequency of infection was noted among males [22]. In Europe, specifically in Hungary, a study conducted in 2018 and 2019 revealed that out of a total of 183 confirmed cases of WNV infection, males were more frequently affected [23].

Several factors contribute to the variability in the clinical presentation of WNV neuroinvasive infection. These factors encompass age, the physiological condition of the affected host, viral strain, tropism, and virus pathogenesis. In our study, the primary symptom upon admission was elevated body temperature. Conversely, in a study conducted in Romania, headache predominated in addition to elevated temperature. The same study described maculopapular and erythematous petechial rashes, which were not observed in patients treated at the UCCV Infectious Diseases Clinic. Gastrointestinal symptoms were reported in patients in both studies. In our patients, nausea was predominantly observed in 42.2% of cases, while vomiting was noted in 45.5% [21].

Literature data suggest that susceptibility to infections, as well as the severity of the clinical presentation and treatment outcomes, are significantly influenced by comorbidities. They are acknowledged as prominent risk factors for patients with cardiovascular, endocrine, malignant, respiratory, and neurological disorders. In our study, five out of 33 patients had no comorbidities (15.1%), while cardiovascular diseases were the most prevalent (63.6%), consistent with an American study conducted in California from 2004 to 2017 [24].

The timely identification and management of complications, which may contribute to an unfavorable disease outcome, can substantially enhance prognosis. In the initial stages of infection, RT-PCR can serve as a valuable diagnostic procedure. Following seroconversion, the diagnosis is more reliably established through serological testing [23]. In our study, 31 out of 33 pa-tients underwent RT-PCR testing, yielding positive results for the presence of the WNV genome in 9 patients (27.27%). This outcome may be attributed to inadequate sampling or patients presenting at a later stage of the disease. In a study by Aslan et al. conducted in 2012, no positive RT-PCR results were ob-tained for the WNV genome in a sample of 23 patients [25]. Serology serves as the cornerstone for confirming WNV infection, involving the detection of antibodies in serum or cerebrospinal fluid, often using ELISA for IgM or IgG [26]. Serological testing was conducted on all patients in our study, revealing positive IgM anti-bodies in serum or cerebrospinal fluid in 78.8% of cases. The obtained data align with studies conducted in 2012 at the UCCV Infectious Diseases Clinic, as well as the Clinics for Tropical and Infectious Diseases of the Clinical Center of Serbia [18, 27]. Numerous European authors emphasize the significance of serological diagnostics in confirming the definitive diagnosis and determining the etiology of the infection [26-28].

In 2018, the largest outbreak ever documented, attributable to WNV, occurred in Central and Southern Europe, resulting in over 2000 symptomatic cases in humans, predominantly reported in Italy, with a mortality rate of approximately 9% [23]. In our country, within the territory of the Autonomous Province of Vojvodina, during the 2012 epidemic, 32 cases of WNV encephalitis were documented with a mortality rate of 3.13% [18]. In contrast, in our study comprising a total of 33 registered patients, the mortality rate was 31.3%. This difference could be attributed to the average age of patients in our study, where the geriatric population predominated, compared to the 2012 study, which included predominantly working-age individuals. Furthermore, comorbidities were documented in as many as 84% of our patients, contrasting with the study by Sević S. et al., where accompanying diseases were registered in 37.5% of the total number of patients included in the mentioned study.

Conclusion

For neuroinvasive infections, the Enzyme-linked Immunosorbent Assay test has proven to be the most reliable diagnostic method during both epidemic years. Research has confirmed that the geriatric population is at a heightened risk of contracting the neuroinvasive form of West Nile Virus infection. Additionally, the presence of comorbidities, particularly cardiovascular disease, significantly influences the severity of clinical presentation, the duration of hospitalization, and the disease outcome. Epidemiologically, it has been verified that West Nile Virus infection is most commonly observed during the summer months in areas situated near large bodies of flowing water. The findings emphasize the importance of ongoing community efforts aimed at eradicating mosquito species in the Danube region and other waterways of Vojvodina.

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