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DETECTION OF WEST NILE VIRUS RNA IN PATIENTS WITH MENINGITIS IN PODLASKIE PROVINCE

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ABSTRACT

AIM. The aim of the study was the detection of WNV RNA in cerebrospinal fluid of patients with lymphocytic meningitis.
MATERIAL AND METHODS. Samples of cerebrospinal fluid from 24 patients hospitalized in the Department of Infectious Diseases and Neuroinfections between May and September because of meningitis were evaluated concerning presence of WNV RNA.
RESULTS. In none of the samples WNV RNA was detected.

Key words: *West Nile Virus, WNV, RNA, meningitis*

INTRODUCTION

West Nile Fever Virus (WNV) is an RNA virus that belongs to Flaviviridae. At least 2 genetic lineages of WNV may be differentiated – lineage 1 present in Europe, Africa, North America, Australia, India, and lineage 2 – mostly in Africa. The main reservoir of WNV are migratory birds that may spread the virus from tropics to areas of moderate climate. Mosquitoes (Culicidae) are the vectors of WNV (1).

WNV infection is usually asymptomatic. Clinical symptoms are present in 20-40% of infected patients (2,3). The most common symptoms are fever, headache, macular rash. In 1 to 150 cases the virus affects central nervous system causing meningitis, encephalitis or flaccid paralysis (4,5). Encephalitis is often accompanied by extrapyramidal symptoms. Other neurological symptoms include tremors, myoclonuses, usually of upper limbs. Flaccid paralysis in the course of WNV is asymmetrical and may be present even in absence of meningitis or encephalitis (6). There are also reports of Guillain-Barré syndrome and brachial plexus palsy in the course of WNV infection (7,8).

The mortality of patients with neurological symptoms accounts of 5-14% (9). After the infection the virus may persist in human body.

In animal models genetic material of WNV was found several months after infection either in central nervous system or in peripheral tissues (kidneys, skin, spleen, heart, lymph nodes). Viral RNA was detected in skin of sparrows and mice 30 days after infection, in kidneys of mice and hamsters – after 60-247 days, in hearts of wild birds – after 30 days, in brains and spinal cords of mice, hamsters and macaques – after 2-6 months (10,11).

In humans with a history of WNV encephalitis, viral RNA was detected in urine 1.6-6.7 years after symptoms remission (12). In 3% of blood donors, with asymptomatic or mild WNV infection, viral RNA was detected 40-104 days after infection (13). There is an antigen similarity between WNV and tick-borne encephalitis virus, which may affect immunoserological tests due to possible cross-reactions, and some WNV infections may be diagnosed as tick-borne encephalitis (5,14).

AIM

The aim of the study was to determine the possibility of occurrence of West Nile virus antibodies in serum and WNV RNA in CSF collected from patients with lymphocytic meningitis.

MATERIAL AND METHODS

Cerebrospinal (CSF) fluid of 24 patients in the mean age of 44 years (range: 19-76 years old), 10 women and 14 men, hospitalized between May and September (time of highest activity of mosquitoes) 2010 in the Department of Infectious Diseases and Neuroinfections in Bialystok, Poland were examined concerning WNV RNA presence by means of NRT-PCR method. All patients were additionally tested for tick borne encephalitis antibodies presence in CSF and serum by means of Enzygnost Anti-TBE/FSME Virus [IgG, IgM] Siemens.

CSF was taken on the day of admission, stored in -20C and transported to National Veterinary Research Institute, Department of Poultry Diseases in Pulawy (BSL III+ class laboratory).

WNV RNA was detected using NRT-PCR method designed in Department of Poultry Diseases (15).

RNA ISOLATION. Genetic material of RNA was extracted from samples by the use of viral RNA Mini Kit (Qiagen) following commercial procedures. The RNA was resuspended in PBS with the addition of the RNay inhibitor (Promega). Isolation probes with RNA were preserved at -70°C.

RT-PCR. Primers used in the RT-PCR were designed to target conservative sequence 3'NCR (non-coding region, GeneBank Accession number: DQ211652) of WNV NY99 strain. Primers for the detection of the mosquito-borne flaviviruses were WNV3 (sense primer): 5'-GCC ACC GGA AGT TGA GTA GA-3' and antisense primer WNV4: 5'-CTG GTT GTG CAG AGC AGA AG-3'. These two primers result in a product measuring 450 bp. Primers were designed with the Primer3 program and were synthesized by The Institute of Biochemistry and Biophysics, Polish Academy of Science in Warsaw, Poland. The amplification procedure was performed by using the Qiagen One Step RT-PCR kit.

NESTED-PCR. Primers for Nested-PCR were designed on the basis of RT-PCR amplicon sequence. The first primer was WNV5 (sense primer): 5' AAA GCC CAA TGT CAG ACC AC 3' and WNV6 (antisense primer): 5' TAG TCC TTT CGC CCT GGT TA 3'. These two primers correspond to a product of the first step of NRTPCR resulting in an amplicon sized 150 bp.

ANALYSIS OF PCR PRODUCTS. Reaction product from RT-PCR and Nested-PCR RNA was submitted to electrophoresis and the results were examined in a 2% agarose gel containing ethidium bromide. The results were visualised on a UV transilluminator and photographed. DNA product was 150 bp in length; Electrophoresis was performed in pH 8.2, voltage 150 V, and intensity 80 mA.

RESULTS

All patients had history of mosquito and tick bites. Mean pleocytosis was 105±121.5 cells and mean protein concentration 73±50.1mg/dl (at admission) and 37±29.6 cells, 48±22.1mg/dl (after 14 days). In 16 patients tick-borne encephalitis was diagnosed. In none of the samples RNA WNV was detected.

DISCUSSION

WNV spreads globally and more and more infections are diagnosed in Europe. In 1996 there was a large outbreak of West Nile Fever in Romania (16), and in 2010 – in Greece (17). In years 2008-2009 infection was identified in Northern Italy (18). Anti WNV antibodies were detected in humans in Austria, Czech Republic and Germany (19), and in Belarus- in birds and mosquitoes (20).

Recently in Italy and Greece Lineage 2 of WNV (so far present only in Africa) was detected in animals (21,22,23). Patients in Poland are not routinely tested for WNV infection. However antibodies against WNV were detected in polish birds. Therefore there might be a possibility of human infections.

In years 1995-96 anti WNV antibodies were detected in *Passer domesticus* (2.8% of examined birds) and *Passer montanus* (12.1%) in Kampinos Forrest region (24). *Wenger* et al. studied population of 10 bird species and detected anti WNV antibodies in 10.6% (25), while *Hubalek* et al. reported antibodies presence in 5.2 % of examined birds (26).

In study conducted by Department of Poultry Diseases, National Veterinary Research Institute on 1912 polish birds no WNV RNA was detected (27). Also a result of study of *Kubica-Biernat et al*, who evaluated WNV RNA presence in mosquitoes from 4 polish regions (kujawsko-pomorskie, podlaskie, warmińsko-mazurskie, mazowieckie) was negative (28).

The possibility of WNV infections in humans in Poland was reported by *Kondrusik* et al. (2007), who examined 93 people in Podlaskie Region and confirmed anti WNV antibodies presence in 5 of them (by means of ELISA and IFA) (1). Earlier, in 2006 *Hermanowska-Szpakowicz* et al. detected anti WNV antibodies in a patient with fever (14).

Serological diagnosis of WNV infections in Poland is difficult because of probability of cross reactions with tick borne encephalitis, an endemic disease e.g. in Podlaskie Region. Therefore positive serologic results should be confirmed by Plaque Reduction Neutralisation Test (PRNT), which is so far not available in Poland.

The results of serologic tests in our patients indicated the possibility of WNV infection. However we

don't publish these results because we were unable to verify them with PRNT test.

Presence of WNV RNA may be detected in serum and blood by means of reverse transcriptase PCR. The sensitivity of PCR in CSF is estimated to ca 55% (3).

CONCLUSIONS

The fact that in none of the samples WNV was detected does not exclude WNV as a causative factor of meningitis in Podlaskie Region. As it was mentioned before WNV rarely affects central nervous system. Additionally PCR is useful only when the virus is present in CSF. Therefore further studies with serological methods (confirmed by PRNT) are planned. This should allow to more accurately estimate incidence of WNV infections in Podlaskie Region and Poland.

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