SCIENTIFIC VETERINARY INSTITUTE „NOVI SAD“

INSTITUTE OF VETERINARY MEDICINE OF SERBIA

„One Health – New Challenges“

First International Symposium of Veterinary Medicine

(ISVM2015)

PROCEEDINGS



Hotel "Premier Aqua" - Vrdnik
May 21 – 23, 2015

Publisher

Scientific Veterinary Institute „Novi Sad“, Novi Sad, Serbia

For the Publisher

Prof Dr Miroslav Ćirković, Principal Research Felow

Editor in Chief

Dr Tamaš Petrović, Senior Research Associate

Technical Editor

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Printed by

Multidizajn, Novi Sad

250 copies

Novi Sad, 2015

ISBN: 978-86-82871-36-1

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CROSS-REACTIONS IN SEROLOGICAL DIAGNOSIS OF FLAVIVIRUS INFECTIONS

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Abstract

The most important flaviviruses are mosquito-borne viruses responsible for severe encephalitis in humans: Japan encephalitis virus (JEV), Saint Louis encephalitis virus, West Nile virus (WNV) and Dengue viruses (DV-1 through 4). The most important mammalian tick-borne flavivirus is tick-borne encephalitis virus (TBEV). Nowadays, in early illness phase, diagnosis is based on detection of viral RNA by PCR/real time PCR. In the late phase of infection serologic diagnosis is a method of choice to establish the diagnosis of flavivirus infection. Significant problems in serological diagnosis are cross-reactions between members of Flavivirus genus due to antigenic similarity. The aim of the study was to demonstrate the extent to which the use of multiple serological testing is able to contribute solving the cross-reaction issues between some members of genus Flavivirus. Eighteen ELISA IgG-positive sera on WNV were tested on Dengue virus by ELISA as well as on 8 flaviviruses (TBEV, WNV, JEV, YFV, DV 1-4) by indirect immunofluorescent test (IIFT) Flavivirus Profile 2 (Euroimmun, Germany).
All of 18 ELISA IgG- positive sera on WNV were simultaneously ELISA IgG- positive on Dengue virus. Considering the fact that WNV circulates in Serbia among mosquitoes, birds, horses and humans unlike Denga virus, it was assumed that there were cross-reactions and sera from 18 patients were tested on eight flaviviruses by IIFT. In 12 sera specific IgG antibodies to WNV were confirmed by determination of antibody titres. Among those 12 sera, 5 sera were only positive to WNV by IIFT (but not to other flaviviruses), whereas the testing of the other 7 sera showed the presence of both the antibodies against WNV and antibodies against Dengue viruses 1-4 (although the latter in lower titers). Six (33.3%) patients need to be additionally tested for setting the final diagnose. Cross-reaction issues between WNV and Dengue virus can be solved in some cases by IIFT.

Keywords: flaviviruses, cross-reaction, serological diagnosis

Introduction

Flavivirus genus consists of more than 70 viruses. More than thirty of them are pathogens significant for human medicine. Flaviviruses are 40 - 60 nm in diameter and have a single-stranded, positive-sense RNA of 11 kb. There is glycoprotein E inserted in the viral lipid envelope (Jawetz, Melnick & Adelberg's, 2013). The antigenic determinants of glicoprotein E are responsible for production of neutralisation antibodies in the host. On the basis of antigenic characteristics flaviviruses can be divided into eight antigenic groups. The most important antigenic group is the complex of Japan encephalitis (JE) whose members are mosquito - borne viruses that can cause

severe cases of encephalitis in humans: Japan encephalitis virus (JEV), Saint Louis encephalitis virus (SLEV), West Nile virus (WNV), Murray Valley virus and the complex of Denga viruses whose members are DV-1 through 4.

Four denga viruses are widely distributed in the tropics (between 35 north and 35 south latitude) where principal vector *Aedes aegypti* is present. WNV is the only mosquito borne flavivirus which activity in humans and animals is detected in Serbia (Petrović et al., 2014; Petric et al ., 2012). The vast majority of WNV infections in humans are asymptomatic. Approximately 20% of infected humans suffer from WNV fever and less than 1% develops neuroinvasive disease resulting in encephalitis and meningitis. Myocarditis, pancreatitis and fulminant hepatitis have been described in some symptomatic persons infected with WNV (Petersen and Marfin, 2002). Human clinical cases associated with WNV infections have been reported from many countries in Africa, southern Europe, south-western and south-central Asia and Australia (Hubálek and Holouzka, 1999). Cases of yellow fever virus (YFV) infection have been recorded in subtropical areas of Africa and South America (Jerant Patić, 2007). Since 2000, circulation of YFV has been increased significantly in Africa. Epidemics of YFW infection occurred in unimmunized population of Africa in 20th century; morbidity rate was very high, ranging from 51 - 89% (WER, 2005). The most remarkable clinical feature is haemorrhagic syndrome. Neurological disorders, meningitis, kidney and liver failure may also occur.

Among mammalian tick-borne flaviviruses, the most important is tick-borne encephalitis virus (TBEV). TBEV infections are endemic in large parts of Europe (Southern Germany, Austria, Switzerland, Czech Republic, Slovakia, Hungary, Slovenia, Baltic countries, Poland, parts of Scandinavia, European Russia (Heinz et al., 2013). Neighbouring countries like Bosnia, Croatia, Bulgaria, and Romania are at high risk due to high prevalence of the virus in ticks (WHO, 2011.). Japanese encephalitis is zoonosis widely distributed in Asia caused by Japanese encephalitis virus (JEV). Most JEV infections are asymptomatic, but in some cases the virus can cause systemic febrile illness with the central nervous system involvement and possible fatal outcome. Surprisingly, Ravanini reported detection of JEV RNA in mosquitoes collected in northern Italy. Further investigations are needed for confirmation of spreading JEV in Europe (Ravanini et al., 2012).

Nowadays, in early illness phase, diagnosis is based on detection of viral RNA by PCR or real time PCR or serologic tests. However, serologic tests appear to be the method of choice for establishing the diagnosis in the late phase of virus infection. Significant problems in serological diagnosis are cross reactions between members of Flavivirus genus because of antigenic similarity. Viral isolation from blood or cerebrospinal fluid is usually unsuccessful even in the early stage of infection because of low viral loud in humans.

The aim of the study was to examine the value of multiple serological testing in solving the cross-reaction issues between flaviviruses.

Material and methods

Eighteen sera ELISA IgG-positive on WNV were tested on Dengue virus by ELISA. The same sera were tested on 8 flaviviruses (TBEV, WNV, JEV, YFV, DV, 1-4) by indirect immunofluorescent test (IIFT) Flavivirus Profile 2 (Euroimmun, Germany).

For purpose of WNV IgG antibodies detection, commercially available ELISA was used (produced by Euroimmun, Lübeck, Germany). Testing, calculation and interpretation of results were performed strictly on the automatic device Euroimmun Analyzer I-2P following manufacturer instructions. Results were evaluated semiquantitatively by calculating a ratio of the extinction value of patient sample over the extinction value of the calibrator 2 which was included into the test.

Results were considered as positive if ratio was equal to or greater than 1.1, intermediate if ratio was between 0.8 and 1.1 and negative if ratio was less than 0.8. All samples were tested in the Institute of Public Health of Vojvodina and all were positive on WNV IgG antibodies.

Eighteen ELISA WNV IgG-positive sera were tested by commercially available ELISA IgG for denga (Euroimmun, Germany) and IIFT Flavivirus Mosaic 1 (Euroimmun, Germany) against TBEV, WNV, JEV, YFV, DV 1-4. For IIFT, serum samples were diluted 1:10 and applied on the slides coated with TBEV, WNV, JEV, YFV, DV 1-4 antigens. The results were read under a fluorescent microscope (Olympus BH2), objective 40X.

Results

All of 18 ELISA WNV IgG-positive sera were simultaneously ELISA IgG- positive on Dengue virus (table 1). Considering the fact that WNV circulates in Serbia among mosquitoes, birds, horses and humans unlike Dengue virus, it was assumed that there were cross-reactions.

Table 1.Results of ELISA IgG on Denga virus in IgG WNV positive serum samples

Serum samples	Origin of samples	History of WNV infection	ELISA WNV IgG	ELISA Denga IgG
116	ND	recent	4,46 IgM +	1.28
159	ND	past	5,56	3.28
8	MBD	past	2,98	1,16
14	MBD	past	5,73	3,43
91	MBD	past	IgG+	3,17
31	MBD	past	1,96	2,89
11132	SBD	past	3,25	0,88
5291	SBD	past	2,60	1.70
11582	SBD	recent	5,13 IgM+	2,29
11553	SBD	past	5,97	4,52
11542	SBD	past	5,04	3,23
5409	SBD	past	3,79	4,62
11796	SBD	past	4,60	3,18
688	SBD	past	1,28	1,94
626	SBD	past	5,83	2,97
667	SBD	past	5,85	2,16
11528	SBD	recent	I1,53 IgM+	3,15
11561	SBD	recent	4,69 IgM+	3,35

Legend: ND - Nisava district, MBD - Middle Banat District, SBD - South Backa District

Between WNV and Denga virus by ELISA IgG test independent whether it is recent or past WNV infection. Serum sample No 116, 11582, 11528 and 11561 were positive on WNV IgM antibodies that indicated recent infections. In the other serum samples past WNV infection were detected. Cross reactivity of the WNV positive sera observed in ELISA against DV was 100%.

All 18 sera positive against WNV were tested on eight flaviviruses by IIFT. In 6 (33.3%) sera specific WNV IgG antibodies were confirmed and there were no cross-reactivity with other flaviviruses. In 12 serum samples cross reactivity was observed (table 2). The highest cross-reactivity was observed with DV, in 11/18 (61.11%)

Table 2.Cross-reactivity between flaviviruses

lavivirus	Percentage (number of cross reactive samples/number of total tested)
DV	11/18 (61,11)
YFV	4/18 (22,22)
TBEV	3/18 (16,67)
JEV	1/18 (5,55)

Legend: DV- Denga virus, YFV - Yellow fever virus,
TBEV - tick-borne encephalitis virus, JEV - Japanese encephalitis virus

Cross-reactivity was observed for all denga virus serotypes (table 3). The highest cross-reactivity (33.33%) was observed with DV4. Among those 12 sera, where cross-reactivity was observed in, 11 sera showed the presence of both the antibodies against WNV and antibodies against Dengue viruses 1-4 (although the latter in lower titers).

Table 3. Cross-reactivity between denga virus (1-4) by IgG IIFT in serum samples positive on WNV by ELISA IgG

Flavivirus	Percentage (number of cross reactive samples/number of total samples tested)
DV1	5/18 (27,77)
DV2	5/18 (27,77)
DV3	3/18 (16,67)
DV4	6/18 (33,33)

Legend: DV1-4 Denga virus, serotype 1-4

Discussion and conclusion

Flaviviruses are important human pathogens distributed worldwide. Laboratory diagnosis of flavivirus infection can be made by isolation from CSF or blood on cell culture in BSL 4 in reference laboratories. Neutralisation test, IFT, ELISA as well as PCR test can be applied for identification of isolates. Molecular techniques are the tests of choice to detect viremia in all flavivirus infections. Molecular tests are important because of their sensitivity and specificity but in the late phase of infection they are negative. For this reason, detection of specific antibodies to flaviviruses in late phases of infection by serologic tests is widely used for routine diagnosis of flavivirus infection. The serum and/or CSF (in case of neuroinvasive infections) can be tested by ELISA, IIFT, complement fixation test or hemagglutination inhibition test. ELISA and IIFT are commercially available. The cross-reactivity within the flavivirus group must be considered in setting up the diagnosis (Jawetz, Melnick & Adelberg's, 2013). In areas where many ARBO viruses are present simultaneously, cross-reactivity is important problem in establishing accurate diagnosis.

In this study, cross-reactivity among the Flaviviridae family members was observed using ELISA and IIFT. The study results reported from Makino indicated that the cross-reactivity among flaviviruses has been observed quite often (Makino et al., 1994).

In our study, IIFT showed a better discrimination between specific IgG antibodies to DV and specific IgG antibodies to WNV than did ELISA IgG specific for these viruses. Cross-reactivity among DV and WNV was 100% by ELISA and 66.6% by IIFT. Such results are in agreement with the study results provided by Koraka in which DV antigen was responsible for lower rate of cross-reactions by IIFT than by ELISA (Koraka et al., 2002).

Applying IIFT in our study, WNV, JEV, YFV and TBEV antigens gave lower rate of cross-reactions than DV antigens by the same test. DV4 antigen gave the highest rate of cross-reactivity.

Acknowledgments

The presented work is part of the research done in the project TR31084 and TR43007 granted by the Serbian Ministry of Science and Technological Development and 114-451-2142/2011-01 (2011-2014) by Provincial Secretariat for Science and Technological Development.

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