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## Investigation of West Nile virus among healthy blood donors in the western part of Turkey

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**Background/aim:** The West Nile virus (WNV) is a mosquito-borne flavivirus causing different forms of infection among humans, varying from asymptomatic illness to fetal central nervous system infection. Turkey lies within an endemic region for WNV. Transfusion of infected blood products is another well-documented major route of transmission. The aim of our study was to investigate the presence of WNV viremia among a healthy donor population from the western part of the country.

**Materials and methods:** A total of 438 healthy volunteer blood donors were included in the study. The presence of WNV RNA was investigated by quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR) and anti-WNV IgG was detected by a commercial ELISA test.

**Results:** Ages of volunteer donors were 18–62 years (mean: 34.7) and 34 (7.76%) were women. All samples were negative for WNV RNA by qRT-PCR. Eleven (2.51%) samples, 1 of which was borderline, were positive for anti-WNV IgG. All positive samples were from the western part of the country and 9 of them were from İzmir.

**Conclusion:** Although all donor samples were negative for WNV RNA by qRT-PCR, the risk of WNV transmission via blood products should not be ignored in endemic regions.

**Key words:** West Nile virus, blood donors, quantitative real-time PCR

### 1. Introduction

The West Nile virus (WNV) is a single-stranded, arthropod-borne RNA virus belonging to the genus *Flavivirus* of the family *Flaviviridae*. The virus was first isolated in Uganda in 1937 by Smithburn and colleagues (1). Birds are the natural reservoir of the virus and mammals are incidental hosts. The virus is transmitted to humans by mosquitoes and *Culex* mosquitoes are mainly responsible for viral spread. Humans are mainly infected by mosquito bites, but the virus can also be transmitted via blood transfusion and organ transplantation (2,3). Vertical transmission is rare but possible, either from mother to child transplacentally or through breastfeeding (4). The incubation period of WNV infection ranges from 3 to 14 days and about 80% of human cases are clinically asymptomatic; approximately 20% of the infected produce nonspecific febrile illness and less than 1% develop a neuroinvasive disease (5). Cases with central nervous system (CNS) infection are mostly in the form of encephalitis, but meningitis,

meningoencephalitis, and acute flaccid paralysis can also be seen. Patients who recover from WNV infections can present long-term disabilities, including paralysis and fatigue (6). The possible link of long-term kidney disease with past WNV infection is not yet definitely proven but is still under research (7,8). Until the appearance of the virus in the United States in 1999 (9), WNV was found mainly in the eastern hemisphere (10). Many seasonal outbreaks have been reported from Romania (11), Russia (12), and Israel (13), and more recent outbreaks were also reported from Italy (14) in 2008 and 2009 and from Greece (15) in 2010.

Turkey lies within an endemic geographic region for WNV infection. During the 2010 WNV season, a total of 47 human cases of WNV with 10 deaths (12 laboratory-confirmed and 35 probable cases) were detected from 15 provinces, mainly located in the western part of Turkey, and the incidence was reported as 0.19 per 100,000 population (16). After the first cluster of human cases was reported,

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WNV was accepted as an endemic disease in this region. During July–November 2010, 8 cases were reported from İzmir and the incidence was 0.21 per 100,000 population (16). That same year, human WNV disease was reported to the European Center for Disease Prevention and Control (ECDC) from neighboring countries as follows: Greece, 262 probable and confirmed cases (17); Russian Federation, 448 cases (18); Romania, 57 cases (19); and Italy, 6 cases (20). In 2011, 3 probable and 2 confirmed cases of WNV infections were identified in Turkey from the same provinces that were affected by WNV in 2010 (16). The incidence was 0.0006 per 100,000 population and no cases were reported from İzmir in 2011 (16). The same year, a total of 282 cases, 93 from EU countries and 189 from neighboring countries, were reported to ECDC; 69 were from Greece and 136 were from the Russian Federation (21). The 2012 season was one of the worst for both the United States and European countries since the 1999 US outbreak (22). As of 30 November 2012, 237 human cases of West Nile fever had been reported from the EU, 161 from Greece and 50 from Italy, and 670 from neighboring countries, 447 of which were from the Russian Federation (23). In spite of the hot WNV season in neighboring countries, only 5 human WNV cases were reported to the Turkish Ministry of Health; 4 were from the northwest of Turkey and 1 was from Central Anatolia (data obtained by formal correspondence with the Turkish Institute of Public Health).

The aim of the present study is to investigate the presence of WNV infection among volunteer blood donors who were admitted to the Ege University Hospital Blood Center located in İzmir, a city in the Aegean region of the west of Turkey very close to Manisa Province, where the first WNV cases were detected in the 2010 outbreak. A quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) strategy was developed to investigate the risk of WNV transmission by blood products and anti-WNV IgG assay (Euroimmun, Germany) was performed to determine the prevalence of past WNV infection among healthy blood donors. This study was approved by the Ege University Medical School ethics committee (No.: 11-4/10).

## 2. Materials and methods

### 2.1. Samples

Blood samples were taken from 438 healthy, randomly selected volunteer blood donors who were admitted to the Ege University Blood Center through August–November 2010. Samples from volunteers, who were accepted as healthy blood donors after assessment of a donor questionnaire, were collected with their informed consent.

### 2.2. Viral RNA extraction

Extraction of viral RNA was performed on an automated QIA-symphony instrument (QIAGEN, Germany) using a

commercially available kit (QIA-symphony Virus/Bacteria Mini Kit, QIAGEN) according to the manufacturer's instructions.

### 2.3. Amplification and quantitation of WNV RNA

A qRT-PCR strategy was developed and optimized for the quantification of WNV RNA. Previously designed WNV-specific primers were used to amplify a 63-bp fragment of the highly conserved nonstructural NS2A region, which was quantified by a highly accurate TaqMan-based real-time PCR analysis (24). As a novel approach, a free circulating nucleic acid in plasma or serum, which can be purified and coamplified with the actual target, was used as an intrinsic internal control. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was chosen to serve as the intrinsic internal control to prevent false negative results and failures in PCR amplifications due to inhibitors, inefficient extraction procedures, or enzymatic reactions. GAPDH labeled with JOE dye gave positive signals in all patients. The 2011 WNV EQA program's West Nile virus test panel, which consisted of 9 coded samples containing WNV viruses of lineages 1 and 2 plus 3 control samples, was used for quantification of the amplicons. Two mixed samples consisting of different other flaviviruses like Japanese encephalitis virus; dengue virus 1, 2, and 4 or dengue 3; yellow fever virus 17D; and tick-borne encephalitis virus were also included in the panel to analyze the specificity of the PCR assay. Reverse transcription and amplification were performed using the One-Step EZ RT PCR Kit (Applied Biosystems, USA) in a 15- $\mu$ L reaction mixture containing 3 mM Mn(OAc)<sub>2</sub>; 0.3 mM dATP, dCTP, and dGTP; 0.6 mM dUTP; 400 nM forward and 400 nM reverse primer; 200 nM TaqMan probe, 0.1 U/ $\mu$ L UNG and 0.1 U/ $\mu$ L rTth DNA polymerase, and 3  $\mu$ L of template RNA. Briefly, UNG was activated at 50 °C for 2 min and WNV RNA was reverse-transcribed at 60 °C for 30 min. UNG inactivation and denaturation was done at 95 °C for 5 min. Afterwards, 40 cycles, each of which consisted of denaturation at 95 °C for 20 s followed by elongation at 60 °C for 1 min, were performed. All reactions and analysis were performed using an ABI PRISM 7500 Sequence Detector (Applied Biosystems). The lower limit of detection of the qRT-PCR was analyzed using probit analysis (Minitab software version 13.1, Minitab Ltd., UK) and the detection limit was estimated to be  $2463 \pm 406$  WNV RNA copies/mL with 95% confidence interval.

### 2.4. Detection of anti-WNV IgG

A semiquantitative anti-WNV IgG ELISA test (Euroimmun) was performed according to manufacturer's instructions.

## 3. Results

Among the 438 donors whose ages were 18–62 years (mean: 34.7), 34 (7.76%) were women. When the donors

were evaluated according to their residency, 369 (84.2%) were from İzmir, 61 (13.9%) were from other cities in the western part of Turkey near İzmir, and 8 (1.8%) were from other regions. All samples were negative for WNV RNA by qRT-PCR. The internal control was shown to be copurified and coamplified in all the samples, excluding the possibility of false negativity. Eleven (2.51%) samples, 1 of which was borderline, were positive for anti-WNV IgG. All positive samples were from the western part of the country and 9 of them were from İzmir.

#### 4. Discussion

The 1999 WNV epidemic in Queens, New York City, raised serious concerns about spread of the virus via transfusion. Biggerstaff and Peterson established a mathematical model using the population during this 1999 outbreak to estimate the transfusion transmission risk of WNV (25). Shortly after this model was published, the first cases of WNV transmitted from blood (26) and organ donors (3) were identified. A retrospective study from the 2002 season in the United States documented 23 cases of transfusion-associated WNV infection from 16 blood donors with low virus level and negative IgM antibody (2). Nucleic acid-based screening assays were developed to identify asymptomatic viremic donors and reduce the risk of WNV transmission via transfusion. In the United States, national blood donor screening using minipool (MP) nucleic acid testing (NAT) was initiated in 2003. This rapidly implemented screening strategy prevented the release of presumably infectious blood products from more than 500 donors (27,28). Despite the success of screening, retrospective studies showed that MP-NAT missed some low-level viremic donations and thus could not completely eliminate the risk of acquiring infection by transfusion of infected blood products (27–29). Therefore, screening strategies were investigated and the switch was made from MP-NAT to individual donor-NAT, which has been shown to be the most sensitive method for detecting viremic donations (30). The first studies related to WNV in Turkey were performed in the 1960s. In a study from the western part of Turkey in 1980, WNV seropositivity was reported as 29.1% (31). Until the first cluster of WNV cases occurred in 2010, epidemiologic data regarding human WNV infections in Turkey were limited to clinical studies. Human WNV infection was not a notifiable disease and the most common forms of asymptomatic or mild human infections were ignored by most clinicians. Since April 2011, WNV infection has been included in the 'notifiable disease' list and a case definition was adapted from the EU case definition for reporting communicable diseases to the community network. Most of the WNV human cases that were reported in 2010 and 2011 were from cities located in the western part of Turkey (16). Although the 2012 WNV

season was one of the worst for Europe and the United States, only 5 cases were reported to the Ministry of Health in Turkey. These data might indicate that some WNV cases still remain unrecognized.

Human WNV infections were first detected in the west of the country (16), but previous studies demonstrate the presence of WNV throughout the country, including Central Anatolia and Southeast Turkey. The seroprevalence of WNV IgG among 1200 healthy blood donors from Central Anatolia was 1.6% by ELISA and 0.8% by plaque reduction neutralization test (PRNT) (32). Another study from Central Anatolia reported 0.56% positivity confirmed by PRNT out of the 0.99% ELISA-reactive samples among 2516 blood donors (33). Hizel et al. found 2.4% unconfirmed seropositivity with ELISA among 2821 blood donors from Central Anatolia and all anti-WNV IgG-reactive samples were RNA-negative (34). An in-house real-time PCR method was implemented to investigate WNV presence among 729 healthy blood donors in Ankara and all were negative (35). Clinical cases of CNS infection due to WNV that were seen during the 2010 epidemic were reported from Sakarya (36). CNS infections were also reported from Central Anatolia (37,38) and WNV lineage 1 was identified as the cause of encephalitis from the same region (38). The samples of the present study were collected during the 2010 WNV epidemic at the time when 8 confirmed cases were reported from İzmir. The anti-WNV IgG positivity rate among 438 healthy blood donors was 2.5% and all were WNV-RNA-negative by qRT-PCR. A seropositivity study was conducted in October 2010 following the peak of epidemics in 3 provinces. Out of 104 samples collected from Manisa, 4 (3.8%) were confirmed as positive with ELISA and IFA by the national reference laboratory (16). The unconfirmed IgG positivity rate of 2.5% found in our study is similar to other seropositivity rates reported from neighboring provinces, as well as the results from Central Anatolia. The likelihood of obtaining a viremic individual among a small donor population is quite low, and this is a limitation of the present study. Nevertheless, the presence of positive samples for WNV antibodies is an important finding to emphasize the fact that the virus has circulated among the population of the region. Although all donor samples were negative for WNV RNA by qRT-PCR in a small donor population, the risk of WNV transmission via blood products should not be ignored. Since WNV screening among healthy blood donors is not mandatory in Turkey, detailed donor questionnaires with WNV infection awareness during the season of high viral activity may help to eliminate viremic donors with mild symptoms. After acute WNV infections in humans were documented for the first time in 2010, enhanced surveillance in humans and animals and mosquito control measures were

implemented in Turkey. Field epidemiological surveys on vectors and blood donors, a seroprevalence study, and a case surveillance study were initiated by the Public Health Agency of Turkey in 4 chosen provinces (Edirne, Sakarya, Manisa, and Muğla). The results of these ongoing studies will make serious contributions to the present

epidemiologic data of WNV infections and new strategies regarding blood safety will be implemented if needed according to the results of these surveys.

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