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Are We Missing West Nile Virus in Turkish Blood Donors?

Kamuran Ziyaretli Şanlı[1], Nesrin Gareayaghi[2], Nurgül Ceran[3]

[1]University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital, Clinic of Microbiology and Clinical Microbiology, İstanbul, Turkey
[2]University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Microbiology and Clinical Microbiology, İstanbul, Turkey
[3]University of Health Sciences Turkey, Haydarpaşa Numune Training and Research Hospital, Clinic of Infectious Disease and Medical Microbiology, İstanbul, Turkey

What is known on this subject?

While donor selection is made in blood donors (BD) in Turkey, West Nile virus screening tests are not performed. In some countries in the world, this virus screening is mandatory for every donor. Is this screening test necessary for our country?

What this study adds?

Although Istanbul is a city on bird migration routes, the frequency of West Nile virus was found to be negative among BDs.

Therefore, it can be suggested not to perform screening for West Nile viruss in BDs in Turkey.

ABSTRACT

Objective: In countries with the West Nile virus (WNV) presence, blood bank screen donated blood for WNV to reduce contamination risk. Despite surveillance studies in Turkey, which is currently a sporadic region for WNV, it remains unclear whether routine WNV screening is necessary. The aim of this study was to investigate whether WNV screening is necessary in Turkey by analyzing WNV seropositivity in blood donors (BD) in Istanbul, which houses more than one-fifth of the country's population.

Material and Methods: This cross-sectional research was conducted between April 2020 and December 2020 as a joint study by the University of Health Sciences Turkey, Kanuni Sultan Suleyman Training and Research Hospital and University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital, Istanbul, Turkey. A total of 552 healthy BDs who applied to the blood center of these two hospitals and accepted participation were included in the study.

Results: Among the donors, 522 were male and 30 were female, and the median age of the volunteers was 37 (range 22-61) years. The city of residence was Istanbul 507 (91.8%) volunteers, while 45 (8.2%) lived in other cities. The initial WNV immunoglobulin G (IgG) results of 528 (95.7%) volunteers were negative and 24 (4.3%) were equivocal. Tests with equivocal results were repeated and all repeat tests showed negativity.

Conclusion: We did not detect WNV IgG positivity in any BD participating in our study. Our results demonstrate that WNV screening is unnecessary in Turkey. However, to prevent contamination risks, such studies must be conducted and repeated frequently after the emergence of sporadic diseases that can be transmitted by blood.

Keywords: Blood donors, infections transmitted by transfusion, West Nile virus, West Nile virus immunoglobulin G

Address for Correspondence: Kamuran Ziyaretli Şanlı MD, University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital, Clinic of Microbiology and Clinical Microbiology, İstanbul, Turkey
E-mail: kamuran67@gmail.com **ORCID ID:** orcid.org/0000-0003-0814-5637
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Introduction

West Nile virus (WNV) is an enveloped positive- sense RNA virus from the Flavivirus family. It is classified in the Japanese encephalitis serocomplex of the Flavivirus genus (1). The first isolation of the WNV was in the West Nile Region of Uganda in 1937. After this date, it has caused epidemics in different parts of the world (2). WNV circulation is a zoonotic cycle of transmission between various species of birds and primarily Culex mosquitoes (3), whereas mammals are dead-end hosts and demonstrate the clinical symptoms of WNV infection (4). People are often infected with mosquito bites (5). However, scientific studies have shown that WNV can also be transmitted from person to person through intrauterine exposure, breastfeeding, blood transfusion, organ transplant, and occupational exposure (3,4). Since 80% of WNV infections are asymptomatic, there is a high risk of transmission to blood product recipients without screening for WNV (5).

In the 1960s, the first studies on the existence of arboviral infections began in Turkey (6). In studies conducted between 2007 and 2010, WNV seropositivity in blood donors (BDs) was found to be 9.4% in the Southeast and 0.56% in the Central Anatolia regions of Turkey (7,8). The first cases of acute human WNV infection in Turkey were documented in 2010 in a cluster of 47 individuals who had presented with encephalitis-like symptoms, which was ultimately defined as an epidemic (6). Before these cases, reporting of WNV infections in Turkey was not mandatory, but this event triggered the inclusion of WNV into the list of notifiable diseases and Turkey was defined as a sporadic region for the disease. Concern about the transmission of WNV by blood transfusion is increasing in the United States (US) and Europe. Today, in some countries where WNV cases are endemic, screening for WNV has started in all blood and organ donors to reduce the risk of transmission of this microorganism through blood and blood products and organ transplantation (3,9). Despite surveillance studies for WNV in Turkey, it remains unclear whether routine WNV screening in BDs is required in the country.

This study aimed to investigate whether WNV screening is necessary in BDs in Turkey, which is currently a sporadic region for WNV, by detecting WNV seropositivity frequency among BDs in Istanbul, which houses more than one-fifth of the country's population.

Material and Methods

Study Design

This cross-sectional study was conducted between April 2020 and December 2020 as joint research by University of Health Sciences Turkey, Kanuni Sultan Suleyman Training and Research Hospital and University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital, Istanbul, Turkey. The study was initiated with the approval of the University of Health Sciences Turkey, Kanuni Sultan Suleyman Training and Research Hospital Ethics Committee (2020.07.136, no: KAEK/2020.07.136) and was performed out according to the BD selection criteria guidelines of the Ministry of Health. Additionally, informed consent was obtained from each volunteer donor included in the study for infectious disease screening in blood products. Routine safety tests, anti HIV-1 antibody, anti HIV-2 antibody, HIV p24 antigen, anti HCV antibody, HBsAg and syphilis total antibody levels were measured.

Study Population

A total of 552 healthy volunteers who applied to the blood center of the University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital or the University of Health Sciences Turkey, Kanuni Sultan Suleyman Training and Research Hospital as BDs were included in the study. The volunteers for the study were selected from the donor group who applied from April 2020 to December 2020, the months during which WNV has been detected most frequently (10).

Serological Screening

The blood samples were centrifuged at 2500 rpm for 15 min and then stored at -70 °C until measurements were performed. All samples were analyzed quantitatively for WNV immunoglobulin G (IgG) using commercial microplate enzyme-linked immunosorbent assay (ELISA) kits (West Nile IgG ELISA, DRG Instruments, Germany, Catalogue number: EIA 4519) according to the manufacturer's instructions as follows. Briefly, all specimens and reagents were brought to room temperature (20-25 °C) and gently mixed. The positive, negative controls were assayed in duplicate and test samples were assayed in duplicate. WNV-derived recombinant antigen (WNRA) and normal cell antigen (NCA) were readily-bound to wells. Serum samples and positive and negative controls were diluted using 1:300 sample dilution buffer. Fifty µL of diluted serum samples and controls were added to the wells. The strips were covered and incubated at 37 °C for one hour (NUVE EN-500, Turkey). After the incubation, the strips were washed 6 times with the wash buffer. An automatic washwell plate washer (Robonik, India) was used for the wash buffer. Then, 50 µL of HRP-conjugated solution was added to each well. A second 1-hour 37 °C incubation was performed and strips were again washed 6 times. Incubation with 150 µL EnWash

(provided in the kit) was performed at room temperature for 5 min. After another washing step, 75 µL TMB substrate was added to each well. Finally, it was incubated for 10 min at dark room temperature. Fifty µL of stop solutionwas then added to each well, and the plates were read immediately at 450 nm (Robonik, India).

Data Analysis and Validity Criteria

The mean optical density of two sample replicates with WNRA was evaluated for each sample and assay control, two sample replicates were calculated with NCA. The WNRA/NCA ratio (immune status ratio, ISR) was then calculated. The same calculation was performed for the positive and negative controls. Any negative control WNRA/NCA ratio greater than 1500 was accepted for repeating the test procedure. Any positive control WNRA/NCA ratio less than 3000 was accepted to indicate that the test procedure must be repeated. We used WNR reverse transcriptase polymerase chain reaction (RT-PCR) for nucleic acid identification as a supplemental test when needed.

Interpretation of the Results

Results were interpreted as follows:

Negative: WNRA/NCA ratio (ISR) ≤2 are seronegative for WNV IgG,

Equivocal: WNRA/NCA ratio (ISR) of 2-3 is equivocal and should be retested,

Positive: WNRA/NCA ratio (ISR) ≥3 are seropositive for WNV IgG and should be confirmed by supplemental testing.

Statistical Analysis

All analyses were performed on SPSS v25 (SPSS Inc., Chicago, IL, USA). Data are given as median (minimum - maximum) for continuous variables and as frequency (percentage) for categorical variables.

Results

Five hundred and twenty-two male and 30 female volunteers with a median age of 37 (range 22-61) years were included in our study. five hundred and seven (91.8%) volunteers were from Istanbul and 45 (8.2%) volunteers were from other cities. Anti HIV-1 antibody, anti HIV-2 antibody, HIV p24 antigen, anti HCV antibody, HBsAg and syphilis total antibody were negative in all volunteers. All specimens met the assay validity criteria. WNV IgG results of 528 (95.7%) volunteers were negative and 24 (4.3%) were equivocal (Table 1). The tests of 24 volunteer serums with equivocal results were repeated and repeat tests were negative for all of these subjects. Thus, all volunteers included in the study were found to be negative for WNV IgG.

Discussion

WNV is one of the most common arboviruses in the world (2). In the last two decades, there have been many changes in the epidemiology of the WN virus and it has caused endemic outbreaks in many countries. WNV is a Flavivirus that has since emerged as a public health issue (4,8). Interest in WNV has been increasing since 2002, when it was determined that it could be transmitted through blood transfusion and organ transplantation. Efforts to reduce the risk of virus transmission are important in blood recipients, particularly patients who receive multiple transfusions and those who are immunocompromised (11). Our knowledge of its recent epidemiology in Turkey is not Japanese. Tests previously used to distinguish WNV from other Flavivirus strains in Turkey had lower specificity. WNV seroprevalence rates were also based on these experiments (7,12,13). Although blood centers in many countries around the world have made WNV screening a routine the necessity of screening is still unclear for Turkey. To provide data on this topic, we evaluated the presence of WNV IgG antibodies, which is one of the most frequently used

Table 1. Summary of volunteers' characteristics and laboratory measurements

Age	37 (22-61)
Gender	
Male	522 (94.6%)
Female	30 (5.4%)
City	
Istanbul	507 (91.8%)
Other	45 (8.2%)
Anti HIV-1 positivity	0 (0.0%)
Anti HIV-2 positivity	0 (0.0%)
HIV p24 positivity	0 (0.0%)
Anti HCV positivity	0 (0.0%)
HBsAg positivity	0 (0.0%)
Syphilis positivity	0 (0.0%)
WNRA	0.083 (0.062-0.234)
NCA	0.070 (0.051-0.093)
ISR	1.246 (0.710-2.786)
Negative (≤2)	528 (95.7%)
Equivocal (2-3)	24 (4.3%)
Positive (≥3)	0 (0.0%)

Data are given as median (minimum - maximum) for continuous variables and as frequency (percentage) for categorical variables

serological methods for screening WNV, in serum samples taken from BDs who applied to the blood bank of two hospitals in Istanbul. We did not detect WNV IgG positivity in any of BDs that we included in our study.

Over the past two decades, the geographic distribution of WNV has significantly expanded, and considerable changes in its epidemiology, virulence, and host species have been observed. This classically self-limiting disease with mild symptoms is showing a change toward being an agent causing major epidemics with morbidity and mortality in thousands of human and animal cases. Therefore, surveillance for this infection is important to ensure a safe blood supply in the future (5). Several studies have been conducted in many countries to estimate WNV seropositivity in the donated blood pool. Some studies conducted around the world reported the incidence of WNV in BDs as 5% in Iran, 3.7% in Italy and 4.4% in Northern Cyprus. However, 8.2% of positives in Iran and 18.1% of positives in Italy were confirmed by immunofluorescence testing (IFA) and plaque reduction neutralization testing (PRNT). Additionally, 78.8% of positives in northern Cyprus were confirmed by PRNT (1,14,15). In studies using serum samples from different blood donation centers in Turkey, the rate of WNV IgG seroprevalence was reported as 1.0%-2.5%. In one study from Turkey, 2821 serum samples from BDs were tested. WNV IgG was positive in 0.9% of them, and 41 of them were found to be indeterminate (16). In a similar study by Sahiner et al. (17) in Ankara, the capital of Turkey. The presence of WNV RNA in serum samples from 729 healthy BBs was investigated by RT-PCR. 702 BDs (96.3%) were located in Ankara. Five hundred ninety six (78%) of the donors had a high probability of contact for arboviral infections (e.g., outdoor activity, mosquito, and tick bites). WNV RNA was not detected in any serum samples (17). Similarly, in another study conducted at Hacettepe University Hospital in Ankara, 1200 BD serum samples were analyzed between April and December 2009. As a result, the positivity rate of WNV and IgG, which was detected by ELISA in BDs had been found to be 1.6%, and half (0.8% of total) had been confirmed via the gold standard method, PRNT (18). Biçeroğlu et al. (19), in their 2015 study, investigated WNV RNA by qRT-PCR and anti-WNV IgG by ELISA in the sera of 438 BDs from the western provinces of Turkey. The WNV RNA test was evaluated as negative in all blood samples. Eleven (2.51%) samples were positive for anti-WNV IgG (19). In another study conducted in 2516 BDs in Central Anatolia, it was found to be positive in 0.5% and was also confirmed by PRNT (8). The first case of WNV originating from Istanbul was diagnosed in 2017 at the Istanbul Faculty of Medicine, and the patient had meningoencephalitis associated with WNV (20). In our study, a significant majority of the donors were from Istanbul. We found WNV IgG was negative in all of these 552 randomly selected participants. The low percentages found in a significant majority of other studies on BD in Turkey and in our study suggest that, currently, routine WNV screening is not required for blood donation in Turkey.

WNV was first identified in humans in the USA in 1999 (21). However, in 2002, the number of WNV increased in humans. Later, WNV was identified in the USA, which was transmitted through transfusions of red cells, platelets, or fresh frozen plasma. This has led to 23 confirmed WNVs being recognized as a viruses that can be transmitted by blood and blood products (3,22). As such, under pressure from the Food and Drug Administration, WNV NAT tests for blood screening were developed by diagnostic kit manufacturers in the summer of 2003 (3). In the European Union (EU) there was a major outbreak of 352 neuroinvasive diseases associated with WNV in 1996, with 17 deaths from the disease in Romania (23). In the European Region; WNV infections, which resulted in death, continued to be seen with the same characteristics, despite precautions being taken. In 2010, there were 262 cases in Greece, of which 197 were neuroinvasive. All 33 resulted in death. All cases were confirmed for WNV (24). Some measures used to prevent contamination of blood products were to delay or restrict blood donation from individuals returning from other countries with human transmission (25). Some EU transfusion centers have decided to perform WNV NAT testing instead of delays or restrictions (3,26). World Health Organization; Apart from European countries, it has been reported that WNV outbreaks have been detected in many Eastern Mediterranean countries such as Turkey, Greece, Palestine, Israel and Egypt (1,3). In a study conducted in Turkey in July-November 2010, a study was conducted on individuals presenting with at least one of the clinical signs or symptoms such as meningitis, encephalitis, meningoencephalitis, myelitis of unknown cause. IgG and IgM antibody tests were performed to identify WNV in patient serum samples. As a result, 47 cases of WNV were identified, of which 12 were confirmed by PRNT and reported as definite and 35 as probable. Fourty were CNS symptoms and 7 were non-neuroinvasive infection cases. The overall incidence of WNV infections was reported as 0.19 cases per 100.000 population (6). This case cluster was defined as an epidemic and WNV became one of the notifiable diseases in Turkey (20). Studies conducted in Turkey show that WNV infection is seen sporadically, and mostly the infection is asymptomatic. Simultaneously, it shows that WNV infection in Turkey mostly circulates in various regions, including central, western, southern and southeastern Anatolia (27).

Studies have shown that WNV infection has an incubation period of 3-14 days. Patients infected with this virus may present with simple nausea, vomiting, headache and fever, and this constitutes only 20% of infected individuals. Additionally, 80% of WNV infections may develop asymptomatically (3). Finally, in 1% of patients, especially the elderly and those with suppressed immune systems, it causes neurological disease with a fatality rate of approximately 10% and high morbidity (2,28). Neurological disease may manifest with severe symptoms such as viral encephalitis, meningitis, seizures and long-term sequelae such as altered mental status, lethargy, cranial nerve palsy, acute flaccid paralysis, and movement disorders (3,29). The early stages of WNV infection have been described in detail. During this period, it can be transmitted through infected donors through blood and blood products. It is therefore important to develop algorithms to screen and confirm infected donors, to decide when it is safe for such donors to continue donating blood and to assess the risk of transfusion (30). Serological tests (IgM, IgG and IgG avidity by ELISA method) are most commonly used when identifying WNV infections in humans. However, this infection can also be detected using methods such as IFA, PRNT, rRT-PCR, immunohistochemistry, and virus isolation from cultured cells (1). Because of short duration of viremia (1-11 days), low level of circulating virus (usually <100 pfu/mL), and late appearance of clinical symptoms, the main method for diagnosing active WNV infection is the detection of specific IgM antibodies in serum or cerebrospinal fluid by serological tests (3,31). IgM antibodies appear shortly after the onset of symptoms. It can be caught if tested by ELISA or hemagglutination inhibition methods during this period. However, IgM-positive samples should be confirmed with PRNT, which is considered the gold standard diagnostic method for Flavivirus serology (32). The PRNT test has also been adopted by the EU WNV infection case definition (33). However, seropositivity should be interpreted with caution due to frequent cross-reactivity with other Flavivirus species (3). IgM cannot be detected in serum for a long time. therefore, it is difficult to detect in serum. Rarely, it can remain positive in the blood for up to 500 days after infection. In these situations, IgG antibody measurements can be used to detect false positives due to persistent IgM antibodies. Otherwise, it may lead to an erroneous BNW diagnosis (3,34). IgG avidity determination has been proposed to distinguish active WNV infection from persistent seropositivity at previous exposures. The serum of individuals who have been exposed to WNV for 6 months or longer demonstrates a high avidity for IgG antibodies (34,35). For these reasons, in our study, as in many similar studies (8,16,18), we used the WNV IgG test to evaluate BDs in terms of WNV infectivity.

Study Limitations

There were some limitations to our study. First, the small number of volunteers and the inclusion of BDs who applied to the blood centers of only 2 hospitals in Istanbul may have limited the generalizability of the results for the whole of Turkey. Secondly, the fact that only WNV IgG was used as a screening test in the study may have caused false negative results in carrier donors who were in active infection period and had asymptomatic disease. Although it seems like a limitation that PRNT, ELISA IgG avidity, RT-PCR tests and IFA were not performed in our study, these tests were not considered necessary since there were no WNV IgG-positive volunteers. Studies with a larger number of volunteers from more centers with more test diversity are necessary for precise assumptions about the subject.

Conclusion

In conclusion, we did not detect WNV IgG positivity in any of the BDs participating in our study. Since WNV is not endemic to Turkey, it is not seen as a risk of blood donation. Our data show that it is not necessary to include WNV in routine tests before blood donation. However, to prevent contamination risks, such studies must be conducted and repeated frequently after the emergence of sporadic diseases that can be transmitted by blood.

Ethics

Ethics Committee Approval: The study was initiated with the approval of the University of Health Sciences Turkey, Kanuni Sultan Suleyman Training and Research Hospital Ethics Committee (2020.07.136, no: KAEK/2020.07.136).

Informed Consent: Informed consent was obtained from each volunteer donor included in the study for infectious disease screening in blood products.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: K.Z.Ş., N.G., N.C., Concept: K.Z.Ş., N.C., Design: K.Z.Ş., N.G., Data Collection or Processing: K.Z.Ş., N.G., N.C., Analysis or Interpretation: K.Z.Ş., N.G., Literature Search: K.Z.Ş., N.G., Writing: K.Z.Ş.

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