

Original Article

A Study of West Nile Virus Infection in Iranian Blood Donors

Zohreh Sharifi PhD*, Mahmood Mahmoodian Shooshtari PhD*, Ali Talebian MD*

Background: West Nile virus is a mosquito transmitted virus that can cause disease in humans and horses. A majority of people infected with WNV will have no symptoms or may only experience mild symptoms, such as headaches. About 20% of infected humans develop a flu-like illness characterized by fever; while in the elderly and immunocompromised West Nile virus can cause a more serious neurologic disease and may be fatal. West Nile virus infection is endemic in the Middle East. West Nile virus can also be transmitted by transfusion through infected blood components.

The objective of this study is to find the West Nile virus-RNA incidence and anti-West Nile virus prevalence amongst Iranian blood donors in order to determine whether this emerging infection is a possible risk for the blood supply in Iran.

Methods: Serum samples from 500 blood donors who donated blood at the Tehran Blood Transfusion Center were collected between May and October 2005. Serum samples were examined for IgM and IgG antibodies to West Nile virus using the ELISA method. The samples were tested for the presence of West Nile virus RNA by the real-time RT-polymerase chain reaction assay. All data were analyzed statistically using the Chi-Square test.

Results: All 500 donors were negative for West Nile virus-specific IgM antibody at the time of donation. No WNV RNA-positive samples were detected. The percentage of seropositivity of IgG antibodies to WNV was 5% at donation.

Conclusion: No evidence of WNV-specific IgM antibody and WNV RNA in blood donor samples was found. In order to increase the safety of blood donation, it is essential to continue surveillance of this emerging infection in order to protect the blood supply in the future.

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Introduction

West Nile virus (WNV) belongs to the Flaviviridae family in the genus flavivirus.¹ WNV, isolated in 1937 in northern Uganda in a region close to a tributary of the river Nile, is widely distributed in Africa, Asia, the Middle East and Europe.^{2,3} The WNV was isolated from patients, birds and mosquitoes in 1950 in Egypt.⁴

WNV is a small, enveloped, single strand RNA virus that is transmitted from birds to humans via the mosquito vector.⁵ Naturally, most human

infections are asymptomatic (80%).⁵ Approximately 20% of infected human develop a flu-like illness that is characterized by fever. However, in the elderly and immunocompromised, WNV can cause a more serious neurologic disease and possibly be fatal. A smaller proportion (less than 1%) develop neuroinvasive disease.⁵

In the United States (US), WNV transmission to humans was detected in 1999 during an outbreak of encephalitis in New York City⁶ and it was first detected among blood donors in the US in 2002.⁶ Also, WNV nucleic acid testing (NAT) began in 2003 in the US.⁷ WNV can be transmitted by blood transfusion, as well as tissue and organ transplantations.^{5,8,9} In transplant recipients, WNV appears as a cause of neuroinvasive disease (meningitis, encephalitis, and acute flaccid paralysis) and febrile illness.⁸

In a study in 1974 in Iran, 100 sera from

Authors' affiliation: *Research Center, Iranian Blood Transfusion Organization (IBTO), Tehran, Iran.

Corresponding author and reprints: Mahmood Mahmoodian Shooshtari PhD, Research Center, Iranian Blood Transfusion Organization (IBTO), Tehran, Iran.

Tel: +98-218-860-1501-30, Fax: +98-218-860-1555

E-mail: m.shooshtari@ibto.ir

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children 1 – 6 years of age were tested for the prevalence of antibodies against 15 different viruses. A positive reaction was detected in 10% of the sera with WNV.¹⁰ In 1976, Saidi et al. reported the human infection of WNV in Iran. The results of their study showed that out of a total of 698 blood and serum samples examined by the plaque reduction neutralization test for antibodies to WNV, 186 (26.6%) had antibodies. The highest prevalence of antibodies was found in the Dezful-Deigi area.¹¹

On the other hand, although a WNV vector such as the mosquito species of *Culex pipiens* is present in Iran,¹² there are no reports regarding WNV infection in Iranian blood donors.

This study has been conducted to investigate the WNV-RNA incidence and anti-WNV prevalence among Iranian blood donors and to determine whether this emerging infection is a possible risk for the blood supply in Iran.

Patients and Methods

Study design

In this cross-sectional study, a number of individuals who donated blood at the Tehran Blood Transfusion Center between May and October 2005 were enrolled. The blood donors were screened for HBsAg (DADE Behring, Germany), HCV antibody by third generation enzyme immunoassay (Hepanostika HCV Ultra Biomerieux, France) and HIV antibody (Vironostika HIV Uniform II Ag/Ab Biomerieux, the Netherlands) by the ELISA method. Blood donors who were negative for HIV antibody (anti-HIV), hepatitis B surface antigen (HBs Ag) and third generation HCV antibody (anti-HCV) were selected for this study and the questionnaires completed by each of them. A total of 500 serum samples were collected from these donors and stored at -70°C. The study population included 490 males (98%) and 10 females (2%), aged (17 to 65) years. Study approved by the local Institutional Review Board and were informed consents signed by participants.

ELISA tests

For the determination of WNV prevalence, serum specimens were tested for IgG anti-WNV antibody using a commercial enzyme immunoassay (West Nile Virus IgG DxSelect™ Focus Diagnostics, Cypress, CA), according to the

manufacturer's instructions. For the presence of acute WNV infection in serum samples, IgM anti-WNV antibody was performed using a commercial enzyme immunoassay (West Nile Virus IgM DxSelect™ Focus Diagnostics, Cypress, CA).

Real-time RT-PCR

WNV RNA was extracted using a QIAamp Viral RNA Mini kit (QIAGEN) according to the manufacturer's instructions. For the detection of WNV-RNA, real-time PCR was performed with the Artus Real Art™ WNV LC RT RCR kit (QIAGEN, Hilden, Germany).

All data were analyzed statistically with SPSS 13.5 software using the Chi-Square test.

Results

Of 500 serum samples, 25 (5%) were positive for WNV-IgG antibody at donation. All 500 blood donors were negative for WNV-specific IgM antibody at donation. No WNV RNA-positive samples were detected by WNV LC RT-RCR.

Of the 500 blood donors, 40 males (8%) and 9 females (1.8%) had a flu-like illness and traveled abroad, respectively. No significant association between the presence of IgG anti-WNV, flu-like illness, and international travel was shown ($P>0.4$). Also a significant association between the presence of IgG anti-WNV antibody and specific age groups was not found ($P>0.1$). Based on the percentage of blood donations, 90.6% and 9.4% of the blood donors were repeat donors and first-time donors, respectively. A significant association between the presence of IgG anti-WNV and the number of blood donations was seen ($P<0.05$). The majority of blood donors were repeat donors. Demographic characteristics of volunteer blood donors have been shown in Table 1.

Discussion

WNV emerged as a cause of neuroinvasive disease and febrile illness in the US, in New York City in 1999.⁷ Based on reported evidence in 2002 from the US, it has been shown that WNV could be transmitted by blood transfusions. As a result, screening of donors for WNV was implemented in the US in July, 2003.¹³

The risk of acquiring WNV infection through transfusion varies geographically. WNV infection is naturally transmitted from a mosquito vector to

Table 1. Demographic characteristics of volunteer blood donors

	Number of blood donations	
	n	Percentage
Age		
17 – 20	18	3.6
21 – 30	115	23
31 – 40	153	30.6
41 – 50	165	33
51 – 60	37	7.4
≥60	12	2.4
Sex		
Male	491	98.2
Female	9	1.8

humans. The transmission cycle of WNV depends on the presence of mosquitoes feeding on both humans and birds, and the appropriate climatic situation for the propagation of mosquitoes and migratory birds.⁸ The WNV vector, *Culex pipiens*, is present in Iran. Also, human infection with WNV has been reported in Iran.^{10–12} Saidi et al. have reported the prevalence of human infection with WNV in Iran to be 26.6%. The infection rates in northern Iran were low, while higher rates were observed among residents of central and southwestern Iran. A high prevalence of infection has been found among residents of Kermanshah and Khuzestan provinces, which indicate that the virus is endemic in these areas. Also, they have reported that the higher infection rates in southwestern Iran may be due to the warmer climate in this region and longer season of mosquito activity.¹¹ In our neighboring country, in the Volga Delta of Russia as well as in the Eastern Mediterranean Region including Israel, epidemics have occurred.^{14,15} There are three reports from the Middle East region. A study from Jordan for seroprevalence of WNV found that 8% of the study subjects have had a previous WNV infection. Another study in Egypt determined the seroprevalence for WNV to be 3% among schoolchildren. Also, Alfaresi et al. have reported that acute WNV infection was not present among 500 healthy blood donors in the UAE.^{4,16} According to previous serologic studies in Iran, the presence of WNV in the Middle East region and travel to an area where WNV is endemic may be risk factors in a number of healthy blood donors.¹¹ For these reasons, in order to increase the safety of blood donations, surveillance of WNV in humans, horses, birds and mosquitoes must be performed to safeguard future blood supplies.

In this study, all 500 serum samples were tested for WNV-specific IgM and IgG antibodies. None

of the blood donors tested positive for WNV-specific IgM antibodies. Only 5% of the blood donor samples were positive for WNV IgG antibody. The presence of IgG antibodies without IgM antibody has shown that these donors were previously exposed to WNV or perhaps another flavivirus. A positive WNV IgG antibody result can be found with persons vaccinated for other flaviviruses such as yellow fever, Japanese encephalitis, and dengue. However, based on the questionnaires completed by each of the blood donors; none have been vaccinated against the flaviviruses family. In addition, there are no reports of infection with these viruses in Iran.¹⁰ Therefore, it is possible that these donors have had a previous exposure to WNV.

Positive results must be correlated with the patient's clinical history, epidemiological data and confirmed by tests such as the plaque reduction neutralization test (PRNT).

Unfortunately, in our study WNV was not available for confirming IgG anti-WNV suspicious blood donor samples with the WNV neutralization assay.

A study on healthy blood donors for anti-WNV specific IgG antibodies in Germany revealed that 5.9% of the tested donors were reactive in the focus anti-WNV ELISA test and only 0.03% of donors tested positive for anti-WNV antibodies.¹⁷

The viremic phase is estimated to be from 6 to 11 days, beginning about 2 days before the onset of illness. A study on measuring WNV viral load in the USA has shown that the WNV RNA load in plasma is relatively low.¹⁸ For this reason, we have used the Artus Real Art™ WNV LC RT-PCR kit that contains quantification standards. The results have shown that WNV RNA-positive samples were absent in blood donors by WNV LC RT-PCR. Therefore, there were no viremic blood donors in this study.

In France, a survey of the WNV circulation in the south was initiated due to an equine epizootic in the Camargue in 2000. There was a prevalence of anti-WNV IgG in 0.8% of the blood donors in this area.¹⁹ In another study in 2004, the presence of WNV RNA in Dutch blood donor samples was investigated. WNV infection was not observed among Dutch blood donors.²⁰

In conclusion, acute WNV infection was not observed in this study in blood donors. The blood centers should place greater emphasis on questioning and screening donors for symptoms of illness and those with a history of travel to

endemic areas in order to protect the blood supply. Also, surveillance for this emerging infection is important to protect the future blood supply.

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