

Original Article

Seroprevalence of West Nile Virus in Regular Blood Donors Referred to the Blood Bank of Kurdistan Province, Iran

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Abstract

Background: West Nile virus is an infection that is most commonly caused by infected mosquito bites, however, blood transfusions, organ transplants, breast feeding, pregnant mother-to-the fetus transmission, and occupational transmission among laboratory and medical staff are also the less common routes of infection. Given the endemic nature of this virus in the Middle East, the aim of this study was to investigate the presence of this virus in regular blood donors, as the reliable source of blood supply needed for patients in hospitals.

Methods: In this descriptive analytical study, venous blood samples were collected from 259 regular blood donors referred to the Blood Transfusion Organization of Kurdistan. After separating blood serum, the amount of IgM and IgG antibodies against West Nile virus was measured via ELISA test.

Results: Concerning antibodies, IgG and IgM against West Nile virus were positive in 14 patients (5.4%) and 3 patients (1.2%), respectively. Seropositive IgG levels were observed in 11 patients over the age of 40 (12.5%) but only in 3 patients under 40 years of age (1.8%). The difference was statistically significant (OR = 7.95; 95% CI: 2.16–29.32; $p < 0.01$).

Conclusion: Given the value of blood and blood products obtained from regular blood donors for therapeutic purposes and the significant prevalence of the virus and considering the presence of cases with positive IgM, it seems necessary to screen blood donors in blood transfusion centers in the western parts of Iran.

Keywords: seroprevalence, West Nile virus, regular blood donors, blood bank, Kurdistan, Iran

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1. Introduction

West Nile virus (WNV) is a member of *Flaviviridae* family and of the genus of *Flavivirus* that has a small, spherical, coated shape as well as a single-stranded RNA genome with positive polarity (+ssRNA) [1, 2]. The virus proliferates in the tissues of arthropods carrier (*Culex* species mosquitoes) without causing disease or injury [2]. Birds are also considered to be its reservoir hosts. This virus is a virus conserved in nature and its transmission cycle is mosquito–bird–mosquito and its first cycle includes mosquitoes [3, 4]. Most cases are infected via mosquito bites, and other ways of infecting humans are through blood transfusions, organ transplants, breast feeding, pregnant mothers-to-fetus transmission, and occupational transmissions in laboratories and medical centers, which are less common [5]. Horses, like humans, are considered dead-end hosts, that is, their infection does not spread the virus [6]. Migratory birds appear to be the main cause of the spread of the virus, including re-spread of WNV from endemic areas to areas with scattered (single-catch) cases [7].

The virus is historically an endemic infection in Africa, West Asia, and the Middle East, and is the most widespread *Flavivirus* in Africa, Eurasia, Australia, and North America [8, 9]. The virus is not limited to international borders and is observed in all continents with different ecological regions, which indicates the flexibility of the virus [10]. Surveillance data from the US Center for Disease Control and Prevention (CDC) in 2014 showed that the WNV is the most common cause of arboviral neurological diseases in the United States [1, 11].

People infected with the virus (about 80%) do not have any signs or symptoms, but about 20% of people experience signs and symptoms. The virus causes influenza-like symptoms such as muscle aches, fever, headache, weakness, lymphadenopathy, abdominal pain, fatigue, vomiting, and sometimes bloody diarrhea, and severe symptoms such as encephalitis and meningitis or sudden paralysis (polio-like syndrome) and even death (which occurs in about 1 out of 150 people infected with WNV virus in the elderly patients or patients with weakened immune systems or patients with chronic diseases) [6, 12, 13]. Hepatitis, pancreatitis, and myocarditis are also the rare outcomes of the infection with the virus [3, 14]. There is currently no definitive cure for WNV infection, and treatment for this infection is largely focused on supportive acts and controlling the symptoms of the disease, which is a time-consuming process [1]. Although inactivated and recombinant vaccines are available for use in animals, no approved vaccine or antiviral treatment is currently available for humans [15]. Considering the mentioned cases and the spread and endemicity of this virus in the Middle East region and the

proximity of western provinces of Iran with the endemic areas, the aim of this study was to investigate the presence of this virus in regular blood donors as the most important and reliable sources of blood supply for patients. It is hoped that the results of the study would be used to highlight the need for screening this virus in the blood banks in the western provinces of Iran.

2. Materials and Methods

2.1. Study design and setting

In this descriptive analytical cross-sectional study, individuals who were referred to the Blood Transfusion Organization of Kurdistan for blood donation over a period of one year (2018–2019) were investigated.

2.2. Participants

The subjects of this study included regular blood donors referred to the Blood Transfusion Organization (individuals who donated blood at least twice a year) who were 18 to 65 years old; they were selected through census method. Those who did not consent to participate were excluded from the study. The sampling was performed via convenience sampling method; accordingly, one out of three blood donors was randomly examined to reach the required sample size.

2.3. Sampling

After collecting the sociodemographic information of the participants by one of the researchers using a questionnaire, 5 cc of venous blood was collected from the median cubital vein of the participants to measure the level of IgM and IgG antibodies against WNV. Blood samples were kept at room temperature for 15 to 30 min and after centrifugation (3000 rpm) for 15 min, their serum was isolated. Serum samples were stored in a freezer at a temperature of -20°C until testing. The amount of IgM and IgG antibodies against WNV was measured via ELISA method using Euroimmun kits made in Germany with lot numbers of EI2662-9601G and EI2662-9601M following the manufacturer's instructions. When performing the WNV IgG test, a level <16 RU/ml was considered as negative, a level of 16 to 22 was considered as borderline, and a level >22 was considered as positive. When performing the WNV IgM test, a ratio of <0.8

was considered as negative, a ratio from 0.8 to 1.1 was considered as borderline, and a ratio of >1.1 was considered as positive. In cases reported as borderline, antibody titer was checked again after one week.

2.4. Sample size

Based on a previous study [16] and considering $p = 0.20$, a first-type error of 5%, and a test power of 80%, the sample size was calculated to be 245 people and finally 259 people were examined to increase the accuracy of the results.

2.5. Statistical analysis

The results of the study were analyzed using SPSS v.20 software. Qualitative data were reported as frequency and percentage and quantitative data, in case of normal distribution, data were reported as mean \pm standard deviation and, in case of the lack of normal distribution, as median (IQR). Chi-square test was used to compare quantitative variables between seropositive and seronegative individuals. The significance level in this study was set at $p < 0.05$.

3. Results

A total of 246 men (95%) and 13 women (5%) with a mean age of 35 years (within the age range of 20 to 60 years) participated in the study. The sociodemographic characteristics of the participants are presented in Table 1.

Of the 259 serum samples collected from regular blood donors, positive IgG and IgM antibodies against WNV were observed in 14 (5.4%) and 3 cases (1.2%), respectively. Seropositive IgG levels were observed in 11 samples over the age of 40 (12.5%) but only in 3 samples under 40 years of age (1.8%), and the difference was statistically significant (OR = 7.95; 95% CI: 2.16 to 29.32; $p < 0.01$). Concerning the other variables, gender ($p = 0.50$), marital status ($p = 0.20$), place of residence ($p = 0.62$), history of underlying diseases ($p = 0.61$), and contact with animals ($p = 0.06$) did not have a significant relationship with positive IgG against the virus.

Among the cases with seropositive IgG, except for a 37-year-old woman, the rest were male with a mean age \pm standard deviation of 45.5 ± 7.6 years. All the participants were living in the city, and except for one, all were married. Of all, seven were self-employed, five were employees, one was housewife, and one was farmer. Four reported contact

with animals, and half of them ($n = 7$) reported traveling to other countries, of whom six traveled to the Middle East and one to Southeast Asia. None of the participants had a history of underlying disease or neurological disorders. The participants' IgG titers ranged from 49.60 to 424.94. Concerning blood type, it was A in seven cases, O in five cases, and B in two cases. Rh was negative in only two cases, and it was positive in the rest (Table 2).

As shown in Table 3, no history of contact with animals and traveling abroad were reported in three individuals with seropositive IgM. All the cases were male and less than 40 years old. One was living in rural areas and one was employed while two were self-employed.

TABLE 1: Characteristics of the study population.

N (%)		Variables
13 (5.0%)	Female	Gender
246 (95.0%)	Male	
63 (24.3%)	20–29	Age (yr)
108 (41.7%)	30–39	
59 (22.8%)	40–49	
29 (11.2%)	50–60	
198 (76.4%)	Married	Marital status
61 (23.6%)	Single	
237 (91.5%)	Urban	Place of residence
22 (8.5%)	Rural	
90 (34.7%)	Employee	Professions
7 (2.7%)	Farmer	
31 (12.0%)	Housewife/unemployed	
11 (4.3%)	Army	
120 (46.3%)	Self-employed	
90 (34.7%)	A	Blood type
53 (20.5%)	B	
101 (39.0%)	O	
15 (5.8%)	AB	
233 (90.0%)	+	Rh
26 (10.0%)	–	

TABLE 2: The status of studied variables in blood donors with a positive IgG West Nile virus laboratory test.

No	Gender	Age (yr)	Professions	Location	Marital status	Animal contact	Traveling abroad	BGRh	IgG level (RU/ml)
1	M	41	Self-employment	Urban	Married	No	Yes	O ⁺	295.56
2	M	46	Self-employment	Urban	Married	No	No	O ^{−−}	424.94
3	M	54	Self-employment	Urban	Married	No	No	O ⁺	324.31
4	F	37	Housewife	Urban	Single	Yes	No	A ⁺	252.12
5	M	40	Employee	Urban	Married	No	No	A ⁺	149.85
6	M	37	Employee	Urban	Married	Yes	No	A ⁺	276.69
7	M	43	Self-employment	Urban	Married	No	No	B ⁺	321.01
8	M	37	Farmer	Urban	Married	No	No	A ⁺	168.24
9	M	44	Self-employment	Urban	Married	No	Yes	O ⁺	120.57
10	M	53	Self-employment	Urban	Married	Yes	Yes	O ^{−−}	276.87
11	M	60	Employee	Urban	Married	No	Yes	B ⁺	293.42
12	M	41	Self-employment	Urban	Married	Yes	Yes	A ⁺	265.07
13	M	40	Employee	Urban	Married	No	Yes	A ⁺	49.60
14	M	56	Employee	Urban	Married	No	Yes	A ⁺	243.31

TABLE 3: The status of studied variables in blood donors with a positive IgM West Nile virus laboratory test.

No	Gender	Age (yr)	Professions	Location	Marital status	Animal contact	Traveling abroad	BGRh	IgMRatio
1	M	38	Self-employed	Urban	Married	No	No	B ⁺	1.44
2	M	30	Employee	Urban	Married	No	No	A ⁺	1.47
3	M	24	Self-employed	Rural	Single	No	No	O ⁺	1.12

4. Discussion

Based on the results of this study, the prevalence of positive WNV IgG and WNV IgM antibodies in regular blood donors was 5.4% ($n = 14$) and 1.2% ($n = 3$), respectively. In addition, the prevalence of cases with positive IgG was higher in people over 40 years old than in people under 40 years old and the difference was statistically significant. Other variables such as gender, marital status, place of residence, history of underlying diseases, and contact with animals were not significantly associated with positive IgG antibody against the virus.

Concerning the prevalence of WNV IgG antibody, the results of the present study are in line with many studies conducted in other countries, especially in the Middle East and other parts of the world such as Libya, Algeria, Northern Cyprus, Bolivia, Hungary, Greece and Jordan, as well as in studies carried out in Iran, in which the prevalence of the aforementioned antibody ranged from 2.34% to 8.61% [5, 17–25]. In particular, studies in Northern Cyprus and Hungary had been conducted on blood donors. Nevertheless, contrasting results had also been observed in studies conducted on blood donors in Sudan (44.4%) and Egypt (55%) [26, 27], which are inconsistent with the findings of this study. In a study conducted in Israel on samples collected in Serum Bank of Israel, 11.1% of cases had positive antibody [28]. In the African country of Zambia, the prevalence of this antibody was 10.3% in a population of native farmers [29]. As observed, the prevalence of this antibody is completely different in various populations and regions of the world; it seems that the proximity to endemic centers of this disease (countries and regions around the Nile river), health status in different developing and developed communities, as well as the implementation of health programs such as carrier control programs in different parts of the world are the important and effective factors influencing the prevalence of this infection.

Concerning the WNV IgM antibody, the prevalence of positive cases was 1.2% ($n = 3$) which despite its small amount is consistent with the results of a study by Yousof *et al.* that was conducted in 2018 in Sudan (2.2%) [26]; nevertheless, it is not consistent with the prevalence observed in a study in Hungary (0.14%) [20]. Despite the small prevalence of this antibody, it is an important issue because positive WNV IgM indicates the active presence of the virus in the body and the virus can be transmitted through blood transfusions. It is a more important issue in this study because the population under our study included regular blood donors who donate blood at least twice a year, and each time they are examined fully for clinical symptoms and undergo medical examinations and microbial and viral tests such as hepatitis. B, C, HIV, and RPR. Because of these examinations and controls that are performed at every case of blood donation, they are completely healthy people whose blood is important for the blood transfusion organization as well as hospitals and medical centers and is considered to be reliable and safe.

Furthermore, in this study, it was observed that the prevalence of seropositive IgG in people over 40 years of age was higher than that in people under 40 years of age and the difference was statistically significant ($p < 0.01$) which could be due to the higher level of exposure of people to infectious agents with increasing age. This finding is consistent with the results of Hadjichristodoulou *et al.*'s study in Greece [21]

and Obaidat *et al.*'s in Jordan [22], but it was not consistent with the results of Nagy *et al.*'s study in Hungary [20]. The controversy in the results might be attributed to differences in geographical and health conditions of the two regions as well as the differences between the statistical population and the sample size in the two studies. Other studied variables such as gender, marital status, place of residence, history of underlying diseases, and contact with animals were not significantly associated with positive IgG against the virus in the participants; it is not in line with the results of studies by Hadjichristodoulou *et al.* and Bassal *et al.* [28] which showed that place of residence was associated with positive antibody, and a study by Obaidat *et al.* which reported that positive IgG antibody was significantly associated with gender. The controversy in the results could be due to differences in environmental, geographical, and health conditions in these studies, as well as differences between the statistical populations investigated in the present study and other studies.

As presented in Table 2, all the cases with positive IgG were living in urban areas and all but one were married. Of all, seven were self-employed, five were employees, one was a housewife, and one was a farmer. In addition, four had a history of contact with animals and half (seven persons) had a history of traveling to other countries, of whom six traveled to the Middle East and one to the Southeast Asia. It is worth noting that none of the participants had a history of underlying disease or neurological disorders. Moreover, the IgG antibody titer of seropositive individuals varied between 49.60 and 424.94. Moreover, the blood type was A in seven cases, O in five cases, and B in two cases. Rh was negative in only two cases, and it was positive in the rest. As shown in Table 3, all the people who were seropositive for IgM antibodies were male and aged less than 40 years old. One of them was living in rural areas and one was employee and two were self-employed. Finally, none of them reported contact with animals or traveling abroad.

As strength of the present study was that it was conducted on regular blood donors, that is, those who donate blood at least twice a year, who are the most important and reliable blood donors and the main sources of blood supply to hospitals and medical centers. They undergo full clinical and medical examinations and viral and microbial tests, and because of clinical and laboratory control of these people and their donated blood, they are considered as the most important and reliable source of blood for therapeutic uses. This study also had some limitations; it was conducted only on regular donors referred to the Blood Transfusion Organization of Kurdistan Province. Hence, in order to conduct more comprehensive studies, it is recommended to investigate regular blood donors from other western and border provinces such as Kermanshah, Ilam, etc.

According to the results of this study and given the value of blood products obtained from regular blood donors for therapeutic purposes, since a significant prevalence of this virus and positive cases of IgM were observed among the studied population, it is suggested to take measures to screen blood donors along with virus and microbial tests routinely performed by the Blood Transfusion Organization. It can be performed in a similar way as the testing of blood donors for HTLV in blood transfusion centers in Khorasan Province, which is being performed due to the spread of the virus in the province. The decisions and plans for the western part of the country regarding this issue must be made by the Blood Transfusion Organization and officials and decision makers in the Ministry of Health (this suggestion is especially important for blood donors over the age of 40, and it seems essential to be performed because of the significant association of seropositive cases with the virus antibody in people over the age of 40). It is also worth mentioning that due to the high prevalence of positive cases of IgM in the subjects and the proximity of these areas to the endemic regions of the virus, it seems necessary to take measures for more control and monitoring at the borders. In addition, to control and eliminate carriers, officials and those involved in health and administration fields must make plans for the development and implementation of more strict and serious health programs. Furthermore, given the ignorance and lack of public awareness regarding the virus and its complications, as well as ignorance about ways to control and prevent the infection, people must be informed through the mass media and training workshops.

5. Conclusion

Based on the results of this study, the prevalence of positive WNV IgG antibody and WNV IgM antibody in regular blood donors in Kurdistan province was 5.4% and 1.2%, respectively. Moreover, the prevalence of cases with positive IgG was higher in people over the age of 40 than in those younger than that.

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Ethical Considerations

This study was approved by the ethics committee of Kurdistan University of Medical Sciences (IR.MUK.REC.1396/355). Written informed consent was obtained from all the participants, and the principles of data confidentiality, as recommended by Helsinki Convention, were observed by researchers.

Competing Interests

The authors declare no conflict of interests.

Availability of Data and Material

All relevant data and methodological details pertaining to this study are available to any interested researchers upon reasonable request to corresponding author.

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