Frequency of West Nile Virus Infection in Iranian Blood Donors

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Abstract West Nile virus (WNV) can be transmitted by blood transfusions and organ transplants. This study was a retrospective study which was performed in Blood Transfusion Center to evaluate the WNV infection in blood donors in Iran. A total of 540 blood samples were taken from volunteer healthy donors who referred for blood donation to Chabahar Blood Center. The presence of WNV was studied by detecting immunoglobulin G (IgG) WNV by enzyme linked immune sorbent assay (ELISA). Demonstration of elevated WNV IgG confirmed by immunofluorescence assay (IFA) Euroimmun kit. Out of the 540 samples 17.96 % (97 cases) were seropositive by ELISA and 1.48 % (8 cases) was seropositive by IFA. This means that 8.24 % of ELISA seropositive samples were confirmed by IFA. Special attention should be paid to criteria of donor selection, albeit positive results may be due to a previous infection in these donors.

Keywords West Nile virus (WNV) · Blood donor · Iran · Donor selection · Epidemiology · Enzyme linked immune sorbent assay (ELISA) · Immunofluorescence assay (IFA)

Introduction

West Nile virus (WNV) is a neurotropic, arthropod-borne flavivirus that is maintained in an enzootic cycle between mosquitoes and birds, but can also infect and cause disease in horses and humans [1].

The virus was first recovered from the blood of a woman in Northern Uganda in a region close to tributary of the river Nile in 1937 [2].

West Nile Virus is distributed nearly worldwide. It is endemic in parts of Africa, Asia, and the Middle East, parts of Europe, the America and Caribbean Islands [1].

Humans usually become infected via mosquito bites, also the virus can be transmitted by blood transfusions and organ transplants. Rare cases of transplacental transmission

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and probable transmission in breast milk have also been reported [3].

There is no human vaccine for WNV yet [3]. WNV infection causes mild symptoms febrile viral illness in 20 % of the infected patients, severe symptoms in less than 1 % with meningoencephalitis or polio-type illness, the most serious manifestation of WNV, and no symptoms in the remaining 79 %. Since approximately 80 % of infections are asymptomatic, asymptomatic infected donors may unknowingly donate blood and transmit infection to blood recipients [4].

West Nile virus has long been considered a mild pathogen causing self-limiting outbreaks [5], but some newer isolates of West Nile virus seem to be particularly virulent; hence an increased incidence of neurological disease and a higher case fatality rate have been associated with these viruses. Consequently, West Nile infection has emerged as a significant human and veterinary health concern in the world [3].

Transmission through blood transfusion was first reported in 2002 [6]. It should be noted that one donor can transmit infection to more than one recipient because multiple blood components are generally prepared from one donation of a whole blood unit [4].

Safety of the blood supply has a great concern, due to most WNV infections are asymptomatic, thus viremic donors could not be identified prior to donation and also a high incidence of mortality was seen in transfusion recipients [4].

West Nile virus infection is endemic in the Middle East, therefore many research have been performed in neighboring countries, but in Iran a few study have been performed for WNV [7].

Sistan and Baluchestan in south East Iran is classified as dry and desert region. Chabahar, a city in Sistan and Baluchestan province, has semi desert climate. Presence of harbour, airport and terminal of Chabahar, makes it a key connection with the other countries due to transit the goods.

Considering climatic and geographical situation in the middle of transit way, the city of Chabahar is selected to study of WNV infection frequency in Iranian blood donors.

Methods

Individuals who have donated blood at the Chabahar Blood Transfusion Center were enrolled for the study. The blood donors were screened for human immunodeficiency virus antibody, hepatitis B surface antigen and hepatitis C virus antibody by the enzyme-linked immunosorbent assay (ELISA). Only blood donors who were negative in these viral tests with completed questionnaires were selected for

this study. The parameters concerning the disease in the questionnaires were consist marital status, education, carrier and travel to endemic country. Age of blood donor, from 17 to 65 years according to Iranian blood transfusion requirements, was also considered in questionnaire. The population in this study included 514 males (95.18 %) and 26 females (4.81 %). A total of 540 serum samples were collected from these donors and stored at -70°C .

The presence of WNV infection was studied by detection of immunoglobulin G (IgG) to WNV by ELISA using Panbio WNV IgG Indirect ELISA kit (PanBio, Australia). Serological screening was performed with ELISA tests because of its high sensitivity. However reactive ELISA results for WNV IgG were confirmed by Immunofluorescence assay (IFA) Euroimmun kit (Euroimmun, Luebeck, Germany) due to high specificity.

Results

Out of the 540 sample tested with ELISA method, 93 samples were seropositive and 4 were equivocal for WNV IgG antibody, although all equivocal samples considered as seropositive.

Accordingly, 97 reactive ELISA samples were further evaluated by IFA. Among these 93 seropositive samples by ELISA, eight were confirmed by IFA and, and none of equivocal samples were confirmed; so totally, eight samples were confirmed seropositive by IFA. This means that from 540 samples, 17.96 % (97 cases) was seropositive by ELISA and 1.48 % (eight cases) was seropositive by IFA. Therefore only 8.24 % of all seropositive ELISA was confirmed by IFA.

All seropositive samples were analyzed by different parameters mentioned in questionnaire. The number of female donors in comparison with male donors was small since only 26 female participated in our study. All of the seropositive samples belong to male donors and no one had illness symptoms and traveling abroad according to their questionnaires. Due to low seropositive samples there was no correlation between marital statuses, professional status, age and education of blood donors.

Discussion

Although the transmission of West Nile virus by blood transfusion had not been reported before 2002, the findings of transient viremia after infection and a high proportion of asymptomatic or infections with mild symptom suggested that this route of transmission might be possible. Finally transmission of West Nile virus through the transfusion of platelets, leukocyte reduced and non-leukocyte reduced red

blood cells, and fresh-frozen plasma has been documented in 2002 [6].

West Nile virus transmission by transfusion was diagnosed by confirming viremia in the blood donor and by demonstration of WNV infection in the recipient of blood products from a viremic donor [4].

In the last two decades WNV has expanded its geographic range dramatically, and is now considered the most widespread arbovirus in the world. In parallel, significant changes have been observed in its epidemiology, virulence and range of host species affected, therefore this self-limiting disease with mild symptoms is now changing as WNV is causing large epidemics with thousands of human and veterinary cases with associated morbidity and mortality [5]. For this reason surveillance for this emerging infection is important to provide safe blood supply in the future.

Whereas the virus is endemic in parts of the Middle East, the frequency of the virus infection has been studied in this area. The virus is almost present in Afghanistan and neighboring countries [8]. In Pakistan military personnel admitted to a hospital for evaluation of febrile illness was evaluated and antibody to WNV was present in 33–41 % [9]. A seroprevalences study for antibodies directed against WNV in Jordan, found that 8 % of the study subjects have a WNV infection previously [10]. In another study conducted in turkey, 20 % of human were positive for WNV neutralizing antibody [11], although recently it was declared that WNV has become endemic in the western part of Turkey [12].

In our study we found that 17.96 % of blood donors have IgG antibodies against WNV, but because of cross reactivity with other Flaviviruses such as Dengue, Japanese Encephalitis, West Nile virus, Murray Valley Encephalitis, Yellow Fever, Tick Born Encephalitis, etc. only 1.48 %-confirmed by IFA test.

The previous study in prevalence of WNV-IgG antibody in blood donor in Tehran showed that 5 % of samples were positive by ELISA [13] which is lower than our finding in blood donor in Sistan and Baluchestan province in Iran. Therefore we consider WNV as a relatively new health threat with increasing importance for the blood transfusion system.

In Iranian Blood Transfusion Organization (IBTO) system all donors are interviewed by physicians, as part of the donor screening process, so only those who are assessed medically healthy and fit are allowed to donate blood. In the interview, donors are asked if they have symptoms of fever in the past 2 weeks. Many different illnesses, have similar flu-like symptoms such as fever, chills, aches, pain, cough and sore throat are also screened. In addition to maintain transfusion safety, haemovigilance system was established in Iran. These programs are monitored any

complications that may have arisen from blood transfusions.

Recently call-back system in blood transfusion services have been established, which has been in place for donors who may have developed symptoms or may be diagnosed with any infection after donation to inform their case to the donation center. This will allow the blood transfusion center to remove the suspicious donation.

Despite all mentioned consideration and according to the obtained results, some of the healthy donors were infected to WNV, which could not been recognized in donor selection.

Therefore special attention should be paid to blood transfusion system. The most effective strategies are implementation of more specific donor selection criteria, serological diagnosis and new developing screening approaches such as minipool testing for viral genome by polymerase chain reaction.

However the presence of WNV IgG antibody by ELISA or IFA means that the donor has infected by West Nile virus in the past. If specific IgM antibodies were detected in the CSF or an increasing titer of WNV-specific IgM was demonstrated in their serum sample, it is the clear indication that the donor has been infected recently. In comparison of the results between ELISA and IFA, it can be concluded that ELISA method due to its high sensitivity, 17.96 %, can be used as primary screening serological tests. In addition, since serological cross-reactivity across the flavivirus group (Dengue, Japanese Encephalitis, West Nile virus, Murray Valley Encephalitis, Yellow Fever, Tick Born Encephalitis, etc.) is common at the level of IgG, additional confirmation tests such as plaque reduction neutralization test is recommended.

Further studies of human population in other parts of Iran are important investigations to evaluate the risk of WNV outbreaks in the future.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest.

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