ORIGINAL ARTICLE

NUCLEIC ACID AMPLIFICATION TEST FOR DETECTION OF WEST NILE VIRUS INFECTION IN PAKISTANI BLOOD DONORS

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Background: The study was planned to determine the presence of West Nile Virus (WNV) infection in Pakistani blood donors, using Nucleic Acid Amplification Test (NAT). **Methods:** The blood donors for study were selected on the basis of the standard questionnaire and routine screening results. Six donors were pooled using an automated pipettor and NAT for WNV was performed on Roche Cobas s 201 NAT system. The reactive pools were resolved in Individual Donation-NAT (ID-NAT) format and a sample from FFP bags of reactive donations was retrieved. NAT was again performed on retrieved plasma bag (RPB) sample to confirm the reactive donations. The donors were also recalled and interviewed about history of illness related to recent WNV infection. **Results:** After serological screening of 1929 donors during the study period, 1860 donors were selected for NAT test for WNV detection. The mean age of the donors was 28±8.77 (range: 18–57 years). 1847 (99.3%) donors were male and 13 (0.7%) were female. NAT for WNV identified six initially reactive pools (0.32%). On follow-up testing with RPB samples, 4 donors (0.21%) were found confirmed reactive for WNV RNA (NAT yield of 1 in 465 blood donors). **Conclusion:** WNV is a threat to safety of blood products in Pakistan. A screening strategy can be implemented after a large-scale study and financial considerations. One of the reduced cost screening strategies is seasonal screening of blood donors for WNV, with pooling of samples.

Keywords: Nucleic Acid Amplification Test (NAT); West Nile Virus (WNV); Blood donors

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INTRODUCTION

West Nile Virus (WNV), a mosquito-borne virus and member of flaviviridae family, was isolated in Uganda in 1937.[1] This virus is maintained in nature between the *Culex* mosquitoes and wild birds.[2] WNV infections were initially reported from Africa, Middle East, India and Europe but this virus came to limelight when it was first identified in 1999, in an outbreak from New York, USA.[3,4] In Pakistan, seroepidemiological surveys conducted in the 1980s have described exposure to WNV in both humans and animals.[5–7] A recent cross sectional study was conducted in 2015, to detect flaviviruses as a cause of undifferentiated fever in Sindh Province, Pakistan. Out of 467 subjects tested at 5 different sites, WNV IgM antibody was identified in 16 patients with febrile illness.[8]

Most of the human WNV infections are transmitted due to mosquito bites but other routes of infections including blood transfusion, intrauterine transmission and organ transplants are also well documented.[9,10,11] Transmission of WNV through blood products was identified in 2002 when it was confirmed, after rigorous laboratory tests and follow up investigations, in 23 patients during an epidemic in USA.[10] It was also revealed that transfusion of red cells, platelets as well as fresh frozen plasma can all lead to transmission of this virus. Screening for WNV in blood donors was implemented in both USA and Canada in 2003 using minipool Nucleic acid amplification (MP-NAT) test. Later on, some of the other developed countries also started performing NAT for detection of WNV infection in blood donors.

Based on the facts that transmission of WNV through blood transfusion is well documented and there is evidence of presence of WNV infection in Pakistan in many previous studies, we planned this study to ascertain the level of threat that this virus poses to safety of blood products in our country.

MATERIAL AND METHODS

The study was conducted from 1st September to 30th September, 2016 at Armed Forces Institute of Transfusion (AFIT), Rawalpindi. All the blood donors who provided informed consent were included in the study. Approval was obtained from the Ethical Research Committee (ERC) of the institute. The donors were selected on the basis of the standard questionnaire of our Institute, and samples for serological and NAT screenings were also collected from these donors. Those donors found non-reactive on routine screening for hepatitis B, hepatitis C, HIV and syphilis on Architect i2000 immunoassay system (Abbott Diagnostics, Abbott Park, IL) were selected for WNV-NAT test. A pool of six donors was made using an automated pipettor (Hamilton Microlab Star IVD, Hamilton, Bonaduz,

Switzerland). NAT was performed in pools of six on Roche Cobas s 201 NAT system using Cobas TaqScreen West Nile Virus Test kit (Roche Molecular Diagnostics, Pleasanton, CA, USA). On obtaining the test results, the reactive pools were resolved in Individual Donation-NAT (ID-NAT) format, from the remaining index sample in the primary tubes. The FFP bags of reactive donations were retrieved and a sample from these bags was taken, under aseptic technique, for confirmation of test results. NAT was again performed on retrieved plasma bag (RPB) samples in ID-NAT format to further confirm the reactive donations. The donors were also recalled after a period of 1–2 months after donating blood, and interviewed about history of any febrile illness, flu-like syndrome or neurological symptoms (including weakness in any part of the body, difficulty in speech or difficulty in swallowing) either 2 weeks before or up to 4 weeks after blood donation. A locally prepared algorithm shown in figure-1 was followed for WNV NAT testing in our study. Statistical Package for Social Sciences (SPSS) version 19 was used for statistical analysis of the data. Frequency and percentage was calculated for the qualitative variables like gender and WNV-NAT test reactive status. Mean value±SD was calculated for quantitative variables like age.

RESULTS

A total of 1929 donors donated blood at AFIT during the study period. After serological screening results, 1860 donors were subjected to NAT test for WNV detection in 310 pools of six. The mean age of the donors was 28±8.77 (range: 18–57 years). Of these 1860 blood donors, 1847 (99.3%) were male and 13(0.7%) were female. Six pools were found initially reactive and resolution of the pools with ID-NAT identified six initially reactive donors (0.32%). On confirmation with FFP samples, 4 donors (0.21%) were found confirmed reactive for WNV RNA by NAT testing; NAT yield of 1 in 465 blood donors for WNV. The mean age of the 4 confirmed reactive donors was 24.5±3.22 years (range: 19–27 years) and all 4 were male. The profile of WNV-NAT reactive donors in our study is shown in table-1

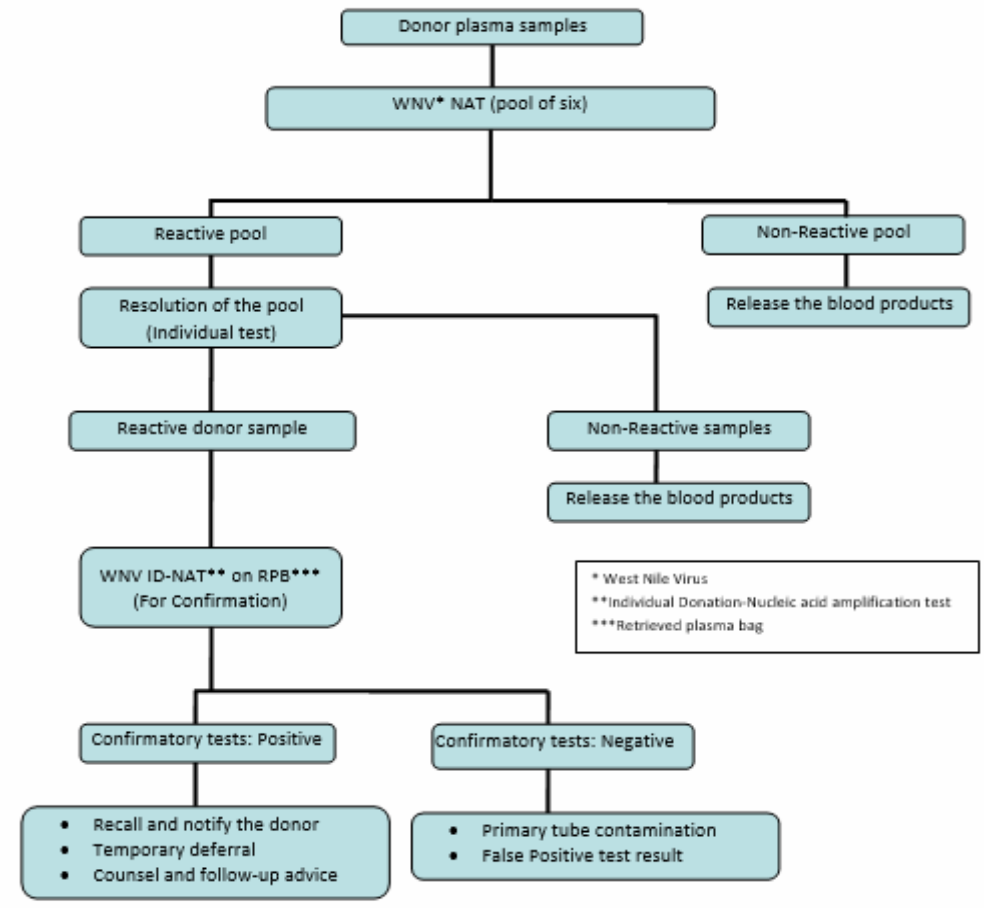

Figure-1: Algorithm for WNV NAT in blood donors

Table-1: Profile of WNV-NAT reactive blood donors

Donor ID	Age	Gender	WNV MP-NAT[1] (Index sample) [2]	WNV ID-NAT[3] (Index sample)	WNV ID-NAT (RPB sample) [4]	History of febrile illness	History of Flu like symptoms	History of Neurological symptoms
WN-01	26	male	Reactive	Reactive	Reactive	Yes	Yes	No
WN-02	19	male	Reactive	Reactive	Reactive	No	No	No
WN-03	27	male	Reactive	Reactive	Non-Reactive	No	No	No
WN-04	26	male	Reactive	Reactive	Reactive	No	No	No
WN-05	21	male	Reactive	Reactive	Non-Reactive	No	No	No
WN-06	27	male	Reactive	Reactive	Reactive	No	No	No

[1] West Nile Virus Mini Pool- Nucleic Acid Amplification Test. [2] Index sample: The first sample collected in primary tube.[3] West Nile Virus Individual Donation - Nucleic Acid Amplification Test. [4] Retrieved Plasma bag sample

DISCUSSION

This is the first study conducted in Pakistan for detection of WNV infection in blood donors using NAT. The high level of infection in healthy blood donors (1 in 465) suggests that the situation is quite alarming. Availability of previous data about high level exposure to WNV infection; in up to 41% of the tested subjects, and the more recent data from Sindh Province support our findings.[8,12] In addition to this, the high prevalence of WNV in horses (55.4%) also suggests that this infection is not uncommon in Pakistan.[12] In the neighbouring India, there have been reports of detection of WNV-RNA by PCR in the cerebrospinal fluid (CSF) of a patient with acute flaccid paralysis (AFP) as well as about the replication potential and transmission by the local mosquito vector.[14,15] In Iran, 5 % of the blood donors had shown previous exposure to WNV but no active infection with WNV-RNA reactive results was reported.[16] This further augments the available data about the ongoing transmission of the virus in the region.

The study was planned in the month of September as a study from USA has documented higher risks of WNV transmission during the months of August and September.[17] A similar kind of seasonality has been observed in Pakistan also where the WNV infection rates increase during the months of September and October; this also correlates with abundance of mosquito vector during these months.[6] Upon testing the index sample, a total of six blood donors were detected as reactive for WNV by MP-NAT and then by ID-NAT (resolution of pool). On follow-up testing, running ID-NAT on RPB samples identified only 4 donors as confirmed NAT-reactive. As NAT is a highly sensitive test, the reason for this could be contamination of index sample during sample collection or contamination during sample processing in NAT lab. However, the strategy to follow-up on samples from frozen plasma of the same donors and repeating ID-NAT, rectified this issue and identified only the confirm-reactive donors. In the context of NAT testing, the importance of follow-up on frozen plasma samples (RPB) of donors has been stressed upon in many other studies to rule out false-positive results.[18,19] As the viral load in WNV infections detected in blood donors is usually quite low, an optimized and robust molecular assay with highest possible sensitivity is needed. As most of the in-house PCR assays are not well-optimized and also have much lower sensitivity than the Roche assay, we decided to follow-up testing on RPB by repeating ID-NAT (Roche), due to its higher sensitivity (95% limit of detection (LOD): 40.3 copies/ml for cobas TaqScreen WNV test kit).

Most of the WNV infections (80%) remain asymptomatic and only 20% of the infected people show any kind of symptom. The most common symptoms are mild, self-limiting febrile illness with flu like symptoms, and only 1 in 150 infected individuals will develop any kind of neuroinvasive disease.[20] In our study, only one of the reactive donors reported a mild fever with flu-like syndrome, about two weeks before blood donation. None of the donors provided any history about the neurological symptoms during the specified time period. This depicts that many acute WNV infections with viremia may remain undetected and therefore, have a strong potential to be transmitted by transfusion. When extrapolated for 55000 blood donors that we receive on average in our institute per annum, we may be able to detect 118 WNV infected donors with help of NAT testing.

However, after taking the seasonal variation into account this number is going to be lower than this expected rate, but still significant enough to implement a screening strategy. The WNV screening data collected by the American Red Cross from 2003 to 2012, shows that out of a total 27 million donations tested by WNV NAT, 1576 reactive donations were identified (1 in 17,132).[21] Although, this NAT yield is far less that that reported by our study, the transfusion services in USA still continues to screen blood donations for WNV by NAT testing. The main hindrance in implementing a screening program for WNV in our country is the financial constraints and limited health resources.

CONCLUSION

WNV is an emerging threat towards safety of blood transfusion in Pakistan and the major blood banks should not only be aware of its presence, but should also formulate strategies to limit its possible transmission via blood products. After a large-scale study and cost-based analysis, a screening program for WNV can be implemented. One of the reduced cost screening strategies is seasonal screening of blood donors for WNV using MP-NAT. After perceiving the threat of WNV, Italian blood services implemented seasonal screening by doing WNV-NAT from 15 June to 15 November, in north-eastern parts of the country, since 2010.[22] Apart from screening, pathogen reduction technology (PRT) can also reduce the risk of transmission of not only WNV but other arboviruses, which pose threat to transfusion safety.

AUTHORS' CONTRIBUTION

SKN conceived the idea, collected data and wrote the manuscript. MA and MSY searched the literature, analysed data and revised the manuscript. EG and MAR interpreted the data and did the critical analysis and proof reading of the manuscript.

Conflict of interest: The authors declare that they have no conflict of interest regarding this study.

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