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Original Article

Detection of Acute Dengue Infection Using NS1 Rapid Test on Blood Donors of Rawalpindi

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Abstract

Background: During recent years, many cases of dengue virus transmission, through blood transfusion have been reported, including two cases from Karachi, Pakistan. NS1 antigen detection in blood donors can serve as a rapid mean for detection of acute dengue infection thus could prevent transmission through blood donation by affected individuals.

Objectives: The aim of this study was to screen high risk blood donors for active dengue infection during an outbreak in the city of Rawalpindi and rejection of NS1-positive donors to save patients from dengue infected transfusions.

Subjects and Methods: After approval from the IRB Shaikh Zayed Hospital, high risk blood donors during the outbreak were identified in blood banks of selected government hospitals. The objective of the study was explained and an informed consent was obtained from each participant. Blood sample of 3cc was drawn at the time of cross-match. Serum was separated and analyzed for dengue NS1 Ag. Data was entered and analyzed using SPSS version 20.0.

Results: Overall 600 blood donors were included in the survey with the majority of male participants (n=583) than female participants (n=17). Most of the blood donors (57.8%) were falling in the age group of less than 30 years and highest donations were from “B” positive blood group followed by “O” positive and “AB” negative with least donations. None of the sample screened positive for NS1 antigen.

Conclusion: Blood of high risk asymptomatic donors taken were having no dengue NS1 Ag positivity.

Key words: Infection, blood transfusion, dengue, epidemic, asymptomatic.

Introduction

Dengue is the most frequent infectious disease that is distributed all over tropical and subtropical regions of the world. Every year, approximately 1 billion cases of dengue fever and 250,000 cases of dengue hemorrhagic fever are reported with an estimated annual death rate of 25000.[1] Almost one hundred countries had experienced dengue outbreaks at different times and it is estimated that a population of about 2.5 billion lives in dengue endemic zone.[1]

Infectious diseases are more painstaking, especially those which are transmitted by blood transfusions such as hepatitis B & C, HIV, viral leukemia and lymphomas, West Nile Virus, Babesia and Chagas viruses etc, but there is no enough knowledge for dengue virus transmission by blood transfusion.[2]

Dengue cases are frequently reported in Pakistan, specifically in post monsoon period.[3] In past few years, dengue has been reported with a higher incidence rate throughout the world, while approximately 5000 cases had been confirmed from Pakistan after the flood of 2010. In Pakistan, most prevalent serotype of dengue virus is type-2 which is followed by type-1, the fact has been established by the verdicts of a report which included 320 patients from three different districts of Punjab Province; Lahore, Sheikhupura and Gujranwala.[4]

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Authors Contribution

SNM conceptualized the project. SNG & FG did the data collection. SNM & MF did the literature search. MA did the statistical analysis. Drafting, revision and writing of the manuscript were done by SNM & AM.

Pakistan had experienced worst outbreak of dengue infection in 2011, where 20,000 cases of dengue infection with 300 deaths were reported,[5] burden of the disease by different regions was also significant, including 35 cases from federal capital (Islamabad) of the country with one death, 25 cases and 3 deaths from Khyber Pakhtunkhwa, 5 cases from Azad Jammu Kashmir,[6] while highest number of affectants were from Punjab with most cases reported from Lahore followed by Faisalabad, Rawalpindi and Sargodha.[5] Moreover, after an outbreak in 2015, in the province of Punjab, about 4,348 dengue victims were reported, out of which 3303 cases were reported in Rawalpindi district along with 6 casualties.[7] This number was even greater during 2016 outburst, when 3600 cases were reported in Rawalpindi district with 4 lives claimed by the atrocious illness.[7]

In humans, the reported maturation period of dengue virus ranges from 4 to 15 days before the onset of symptoms. The onset of viraemia can occur a day before the onset of symptoms[8] and the duration of viraemia can range from 1 to 7 days of primary infection, with a mean duration of 5.1 days, and slightly shorter mean duration of secondary infection.

In the past, the viraemic period reported in the literature defined was based only on medical observations of symptomatic dengue virus (DENV) infection and there are no data on viraemia during the full incubation period that has been found to last between 4 to 15 days prior to symptoms. Based on the symptomatic cases, data on viraemia up to 7 days and the duration of incubation period that can be as long as 15 days post mosquito bites, the total viraemic period that can be estimated is about 22 days.

Dengue infection has a viraemic phase that lasts for 4–8 days,[9] and most infections remains sub-clinical.[10] Viremia can withstand, for a longer period of time in patients who develop clinical disease, prior to onset of symptoms, thus could not be detected during blood transfusions in this phase, as viral RNA load is lower than detection range and may declare the blood as safe for transfusion.[11]

Dengue infection has been transmitted from donor to recipients of renal transplant,[12] bone marrow transplant,[13] through blood transfusion as whole,[14] or through transfusion of blood components from an asymptomatic blood donor.[15,16] Probably, transmission of dengue by blood transfusion was ignored in past despite of higher incidence rate and was attributed simply to dengue outbreaks. The high proportion of asymptomatic infections does not reflect the rate of transmissibility by transfusion.[17] Retrospective studies using nucleotide amplification test (NAT) during outbreaks in Puerto Rico have shown donor prevalence rates ranging from 1:1376 in 2005 [17] to 1:529 in 2007.[18]

American Association of Blood Banks (AABB) has documented transfusion risk models and assessments of Dengue viraemia prevalence among blood donors in dengue endemic areas.[2] Transfusion Transmitted Diseases Committee recently identified DENV as one of three high priority infectious agents with actual or potential risk of transfusion transmission in the US.[2]

In dengue endemic regions, under-reporting of blood transfused dengue cases may also be attributed to the fact that asymptomatic individuals are usually excluded from the studies, while there is clear evidence for an infection period of 1-2 days prior to onset of symptoms. The other elements for dengue transmission include, the amount and stability of the virus, amount of transmitted blood and immunity of host.[19]

Lack of awareness, for transmission of dengue through blood products in addition to vectors, had lead to under-reporting of transfusion based cases for dengue, hence the risk for transfusion-associated dengue had been considered as varying for various countries. Moreover, the regions where dengue mainly affects children, will not report transfusion transmited dengue due to lack of affected blood donors. Whereas, in Pakistan, frequency of dengue infection is higher among adults as compare to children and there is an overlap between infected & blood donating population. Therefore, screening of blood donors i.e. at least high risk population is of paramount significance, so that the risk of this route of transmission of dengue infection could be highlighted.

In this study, screening of high risk blood donors for active dengue infection was done, during an outbreak in the city of Rawalpindi using NS1 Rapid test device.

Subjects and Methods

This was a descriptive study undertaken to screen high risk blood donors for active dengue infection during an outbreak in the city of Rawalpindi and rejection of NS1-positive donors to save patients from dengue infected transfusions. This study was conducted in blood banks of government hospitals of district Rawalpindi selected by Director General Health Services Punjab and Director Institute of Blood Transfusion services Punjab.

High risk blood donors, during the dengue outbreak in Rawalpindi, Pakistan, were identified in blood banks. Further approvals were secured from

Table 1: Distribution of blood donors according to age and gender. (n=600)

Age	*Gender*					
	Male N=583		*Female N=17*		*Total N=600*	
	N	*%*	*N*	*%*	*N*	*%*
≤ 20.0	43	7.4	0	0.0	43	7.2
20.1 - 30.0	334	57.3	13	76.5	347	57.8
30.1 - 40.0	178	30.5	2	11.8	180	30.0
40.1 - 50.0	22	3.8	2	11.8	24	4.0
> 50.0	6	1.0	0	0.0	6	1.0
Total	583	100.0	17	100.0	600	100.0

Table 2: Distribution of blood donors according to blood group.

Blood Group of Donor	*Gender*					
	Male N=583		*Female N=17*		*Total N=600*	
	Count (N)	*%*	*Count (N)*	*%*	*Count (N)*	*%*
A+ve	139	23.85	4	23.5	143	23.83
B+ve	179	30.7	6	35.3	185	30.83
O-ve	20	3.44	0	0.0	20	3.33
AB+ve	46	7.9	2	11.8	48	8
O+ve	166	28.47	3	17.6	169	28.18
B-ve	21	3.6	0	0.0	21	3.5
AB-ve	6	1.02	0	0.0	6	1.0
A-ve	6	1.02	2	11.8	8	1.33
Total	583	100.0	17	100.0	600	100.0

the Medical Superintendent of Holy Family Hospital and Benazir Bhutto Hospital, Rawalpindi to conduct the study. A written informed consent was obtained from volunteers, for their recruitment. Demography and contact details were recorded in a prefixed questionnaire. Blood sample of 3cc was drawn at the time of cross match from each participant and serum was separated. Levels of dengue NS1 antigen in serum samples of study participants were estimated on the same day using commercially available test devices for dengue NS1 Ag. Results of dengue NS1 were communicated to donors within 24 hours of sample collection.

Data was entered and analyzed using SPSS version 20. Age of the patients was presented by calculating mean and standard deviation. Data for gender, blood group and NS1 positivity were described by using frequency and percentages.

Ethical approval was taken from the Institutional Review Board (IRB) of the Shaikh Zayed Hospital and Provincial Bioethics Committee (PBC) Punjab. Approval for conducting the study in Rawalpindi was also taken from Director General (DG) Health Punjab.

Results

Holy Family Hospital and Benazir Bhutto Hospital, Rawalpindi were selected due to the availability of blood donors during a dengue outbreak of 2015. A total of 600 participants were considered for the study, including both genders, while the majority of the blood donors were male 583 (97.16%) and only 17 (2.83%) out of total 600 were female. A total of 595 out of 600 participants declared recent exposure to dengue patients, more people were those who have worked together with the affected individuals at home than at outdoor places. About 171 participants exposed to dengue infected patients in hospitals and 135 were those who had exposure to dengue infected individual at their work places. Most of the blood donors were belonging to the age group of 20 years to 30 years while the overall age range of blood donors was ≤20 years to >50 years. Least number of male blood donors were belonging to the age of 50 or more with no female participant belonging to the aforementioned group (Table-1).

On the basis of blood group distribution, highest donations were received from “B” positive 30.83%, followed by “O” positive 28.18%, “A” positive 23.83%, “AB” positive 8%, “B” negative 3.5%, “O” negative 3.33%, “A” negative 1.33% and “AB” negative 1.0% (Table-2).

Furthermore, blood of donors was screened for NS1 antigen by commercially available NS1 device detection method (Table-3). None of the donors was positive for NS1 antigen in their blood, hence approved for blood donation without apparent risk for dengue virus transmission.

Table 3: Detection of NS1 antigen in blood donor.

NS1 Antigen	*Male (%)*	*Female (%)*	*Total (%)*
Positive	0 (0.0)	0 (0.0)	0 (0.0)
Negative	583 (100.0)	17 (100.0)	600 (100)

Discussion

In the present study, high risk blood donors were screened for NS1 antigen by using commercially available NS1 rapid device detection method (Bio Credit Dengue NS1Ag Test Devices, Rapigen Inc.). None of the donors was positive for NS1 antigen in their blood, hence approved for blood donation without apparent risk for dengue virus transmission.

Dengue virus infection is progressively documented as one of the world's emerging infectious diseases. Due to worst dengue outbreaks during 2010 to 2011, Pakistan endured to remain the dengue incipient zone in later years. Dengue is an evolving intimidation to the safety of blood donation. It's viraemia has been identified in blood donors from a number of states as reported by Chuang et al.,[16] the first evidence of transfusion transmitted dengue case from Hong Kong, Tambya et al.,[17] outlined another case of asymptomatic blood donor from Singapore who developed fever soon after donation and screened positive for dengue virus-2, Stramer et al.,[20] delineated a positive blood donor case for dengue virus-2 during 2007 outbreak in Puerto Rico, Karim et al.,[21] reported another instance of infected donor who transmitted dengue in two recipients in Karachi, Pakistan, emphasizing the prospective risk of transfusion transmitted dengue.

This study was designed to assess high risk donors in the dengue epidemic region of Pakistan in order to reduce menace to the safety of blood transfusion. Blood samples of 600 high risk donors were recruited from Holy Family Hospital (HFH) and Benazir Bhutto Hospital, Rawalpindi and screened for NS1 Ag. Our results manifest no NS1 Ag positive case in the experimental group. The ultimate ramification of the above result was in correlation with those procured from a data published from India, where 1709 tested cases were found to be negative for NS1 Ag[22] and other data published from Australia, where no NS1 Ag positive case was disclosed during 2009 and 2012-13 dengue outbreak out of 6,491 donors tested from North Queensland. [23] In the present study, participation of male donors was 97.16%, in contrast to 2.83% female donors with the majority of participants with an average age subsiding within 26-32 years old age group. These results were supported by few recently available data, where the ratio of male/female donors was 98.3/1.7 and 63.8/30 with the majority of participants with 30-36 year age group range.[22-24] During Rawalpindi epidemic, individuals of all age groups were equally receptive to attain dengue viral infection, where most endangered age group was 18-23 and 21-30 years of age, as reported by Ikhlaq et al.,[25] and Afraz et al.,[26] respectively. This age group was well-thought-out as the most appropriate for blood donation so, at this point it would not be a sensible thought to advise NS1 Ag as the only reliable method available for early detection of asymptomatic viraemia in high risk blood donors of Pakistan.

Predominant blood types among the experimental group were B+, O+ and A+ with a total of 185 (30.83%), 169 (28.18%) and 143 (23.83%) reported cases respectively. This data is intrinsically reinforced by Mangawa et al., where they reported predominance of B+ blood type (37.62%, 643, over O+ (27.56%, 471) and A+ (19.92%, 341) blood type.[22]

The method used for detection of NS1 antigen of the high risk donor was NS1 rapid test devices. The rationale behind this device method was the level of NS1 Ag which is evident in the first week of primary infection, following 7 days of viral incubation and collapse later on. A comparative analysis of NS1 Ag assay and RT-PCR was conducted by Pork et al., where they showed specificity of RT-PCR to be 100% in the first three days of infection compared with 81.7% of NS1 antigen assays, representing probable part of NS1 Ag assay, as a cost-effective and expedient substitute method to RT-PCR for the analysis of dengue in a primary health care centres.[27] To add up more about the significance of RT-PCR, data was reported from Indonesia, where the congregate sampling index spotted 8 asymptomatic dengue infection cases out of 785 over a period of 2-year, of which two were validated for viraemia by RT-PCR.[28] Now coming back to an assessment of NS1 Ag level, which is also promising in the secondary infection. The sensitivity of this method was considerably lower in secondary dengue infection compared with primary infection and is in agreement with the declaration of Chaterji et al.,[29] where they signified the importance of NS1 Ag assay in experimental group, ensuring primary dengue infection, evaluation of high titer compared with low viral titer that are considered as asymptomatic susceptible individuals, are few of its drawbacks.

It is during this stage that, a blood transfusion from such a dengue viraemic donor can come to be a blood-borne infection in recipients. In general, choice of diagnosis method for dengue

assessment is dependent on the phase of the infection. Rodríguez et al., reported 59% of blood donors were positive for IgG and 2% for IgM in Mexico.[30] Ribas-Silva and Eid testified 1.4% of blood donors positive for IgG and none for IgM out of 213 donors in Brazil.[27] Ranjan et al.,[31] identified 58% IgG, 13.5% IgM and 12.5% positive donors for both types of antibodies in a study in New Dehli, India. Pacsa et al.,[32] reported 48% donors to be positive for IgG dengue antibodies in Kuwait. Narkwa et al.,[33] revealed 43.6% IgG positive donor cases in Ghana. Mahmood et al.,[34] affirmed that 67.2% healthy population of an experimental group from Lahore, Pakistan, had dengue IgG antibodies in their blood. They revolutionized the world of infectious diseases by putting forward the propositions for the presence of dengue antibodies in asymptomatic blood donors or healthy individuals. Schreiber et al.,[35] stated Transfusion transmitted viral infection as "The greatest threat to the safety of the blood supply is the donation of blood by seronegative donors during the infectious window period when the donors are undergoing seroconversion" Hence the transfusion of antibodies in recipient, that are secreted in response to viral infection in donor's blood, don't fall appropriately under the description of Transfussion Transmitted Infection, but still it has some consequences which made the situation worse in recipient by antibody dependent enhancement of dengue infection.[36]

In conclusion, blood of high risk asymptomatic donors were having no dengue NS1 Ag, although it was collected during the 2015 dengue outbreak. Further studies are warranted with larger sample size and more sensitive method in order to assess probable reasoning of transfusion transmitted dengue in asymptomatic blood donors.

Due to financial constraints, a highly sensitive method (e.g. RT-PCR) cannot be used for the detection of active dengue infection in high risk blood donors during epidemic.

By having negative NS1 Ag results in hand with such a small sample size, we strongly recommend, a cohort study, where NS1 Ag method should be used in combination with RT-PCR to confirm the acute viremia of asymptomatic healthy donor for the authentication of healthy blood transfusion in our country.

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Conflict of interest: None declared.

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