Original Article

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High Seroprevalence of Dengue Virus Infection in Blood Donors From Delhi: A Single Centre Study

Microbiology Section

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ABSTRACT

Introduction: The risk of transfusion transmitted dengue has been increasingly recognized. Blood donors in an endemic area like Delhi may serve as a potential vehicle for transmission of the infection. Moreover, prevalence of infection in them would be representative of the true picture of dengue in a population.

Aim: To determine the prevalence of dengue virus infection in blood donors in a tertiary care centre.

Materials and Methods: A total of 200 blood donors were recruited in the study after obtaining informed consent in the Institute of Liver and Biliary Sciences, New Delhi in July and August 2012. Data regarding clinical and demographic characteristics was collected using a preformed questionnaire. Blood samples obtained were subjected to anti-dengue IgM and IgG ELISA as well as semi-nested Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for dengue RNA.

Results: Of the study subjects, most were men (97%) with a median age of 28 years (range 19–51 years). Anti-dengue IgG was positive in 116 cases (58%) while IgM was seen in 27cases (13.5%). Of them, in 25 (12.5%) cases both IgG and IgM were positive, while only two (1%) cases tested positive for IgM alone. None of the blood donors were found to be viremic on screening using Nested RT-PCR. A clear increase of IgG seroprevalence with age was evident. No difference in the seroprevalence rates in urban vs. rural areas was seen.

Conclusion: High seroprevalence of dengue infection was seen in healthy asymptomatic blood donors. Though evidence of acute infection was found in some, none were found to be viremic. Larger studies are required to quantify the risk and provide strong evidence for policies to be made.

Keywords: Transfusion Transmitted Dengue, Anti-Dengue IgG, Endemic

INTRODUCTION

Dengue, a major public health problem globally, is caused by an arthropod borne virus belonging to the genus *Flavivirus* in the family *Flaviviridae*. Infection with dengue viruses may produce a spectrum of clinical illness, ranging from asymptomatic infection, undifferentiated fever to Dengue Fever (DF), Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) [1]. Over the last few decades, the transmission of dengue has greatly increased around the globe, with more than 2.5 billion people posed with the risk of infection [1]. Notably, India alone bears one-third of the burden of the apparent dengue infections [2]. With the first major outbreak reported in 1963-1964 in Kolkata [3], India has always remained a hotspot on the global dengue map [1]. Multiple outbreaks of DF/DHF have been reported from various parts of the country ever since [1]. The epidemiology of the disease has been very dynamic in the Indian subcontinent in terms of circulating strains, affected geographical areas and severity of the illness [4]. Delhi, the capital of India, has witnessed several outbreaks, in 1967, 1970, 1982, 1988, 1996, 2003, 2006, 2010 and 2013, with all four serotypes co-circulating presently, hinting towards hyperendemicity of the virus [5,6].

Transmission of dengue occurs most commonly by the bite of an infected *Aedes aegypti* mosquito (sometimes *Aedes albopictus*), with humans serving as the major amplifying host for the virus. However, during the viremic phase of the disease, dengue can become a blood-borne illness in both symptomatic and asymptomatic individuals [7]. Transfusion and transplantation associated dengue cases have been reported, though rare [8–11]. Blood donors in an endemic area like Delhi may serve as a potential vehicle for transmission of infection. However, a mandatory screening of blood donors for dengue infection would be expensive in a developing country, and should only be implemented after evaluation of the risk of the infection posed by apparently healthy blood donors. Blood banks rely on verbal questioning to rule out the risk of transfusion transmitted dengue, although this would not rule out asymptomatic infections.

Prevalence of infection in blood donors will depict true picture of dengue in a population and would be very useful in future implementation of any vaccination policies. We, thereby, undertook this study to determine the seroepidemiology of dengue virus infection in blood donors at our institute.

MATERIALS AND METHODS

This was a longitudinal study conducted at Institute of Liver and Biliary Sciences, a tertiary liver care hospital in New Delhi in July and August 2012. The approval for the study was obtained from the Institutional Review Board and Ethics Committee. The study was conducted according to the official guidelines for blood donation as approved by the Ministry of Health and Family Welfare, Government of India.

A total of 200 blood donors were recruited in the study after informed consent was obtained. A standardized questionnaire was used to enquire whether the donor had experienced any symptoms compatible with a viral illness during the previous one year. Data regarding clinical and demographic characteristics was collected. Four ml blood was obtained from each subject. The samples were centrifuged at 2000rpm for 10minutes and were stored in aliquots at -80^{O}C for further testing. All the samples were screened using a commercially available anti-dengue IgM and IgG capture ELISA (Novatec, Germany). The assays were carried out as per manufacturer's instructions. Test samples with optical density values equal to or greater than the cut-off value (1.1) were considered to be reactive.

The samples were also amplified using semi-nested reverse transcriptase polymerase chain (RT-PCR). Nucleic acid extraction was done using QIAamp Viral RNA Mini Kit (Qiagen, Germany)

as per the manufacturer's protocol. The extracted viral RNA was reverse transcribed to cDNA using transcriptor first strand cDNA synthesis kit (Roche, Germany), and then amplified in the external round of RT-PCR using dengue virus consensus primers (D1: 5' TCAATATGCTGAAACGCGCGAGAAACCG 3'; D2: 5' TTGCACCAACAGTCAATGTCT TCAGGT TC 3') in 25µl reaction volume. The DNA product obtained was of 511bp. Subsequent amplification of cDNA was carried out with the dengue virus consensus forward primer (D1) and four dengue serotype-specific reverse primers; TS (type specific) 1:5' CGTCTCAGTGATCCGGGGA 3'; TS-2: 5' CGCCACAAGGGCCATGAACAG 3'; TS 3: 5' TAACATCATCATGAGACAGAGC 3'; TS 4: 5'CTCTGTTGTCTTAAACAAGAGA 3' [12]. All the 4 serotype-specific primers were added in a single reaction mixture. A 1:20 dilution of the external PCR product was used in the nested PCR reaction. The amplification was carried out for 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes. Dengue virus serotypes were identified by the size of the bands obtained in gel electrophoresis (DENV 1– 482 bp, DENV 2–119 bp, DENV 3–290 bp, DENV 4–392 bp). Results were entered using Microsoft Excel and expressed as mean, median and percentages.

RESULTS

Of the 200 blood donors recruited in the study, most were men (97%). Median age for blood donors was 28 years (range 19–51 years) [Table/Fig-1]. Serologic evidence of past dengue infection (anti-DENV IgG) was identified in 58% of blood donors (n = 116 cases). Twenty seven of 200 serum samples from asymptomatic donors were reactive to anti-DENV IgM by ELISA (13.5%). Twenty five samples (12.5%) tested positive for both IgG and IgM, while only two (1%) tested positive for IgM alone. At the time of blood donation none of the IgM positive patients had any symptoms compatible with the presence of a viral illness. On screening using Nested RT-PCR none of the blood donors were found to be viremic at the time of donation [Table/Fig-2].

DISCUSSION

After entering the body via a mosquito bite, dengue virus replicates in the mononuclear macrophage cells for a period of 3 to 14 days before the symptoms appear. The period of viremia lasts for about 5 to 7 days and roughly corresponds to the period of fever. It is during this phase that dengue can become a blood-borne infection [7]. There are many reports of transmission of dengue through infected blood [8,9]. It has been shown that transmission of the virus can also occur through organ transplant [10,11]. In fact, cases of needlestick injury related nosocomial acquisition of dengue have also been documented [13,14]. Mucocutaneous exposure to infected blood has also led to transmission of the infection [15]. Tambyah et al., described a cluster of cases of dengue associated with blood transfusion in Singapore [9].

Despite the fact that there exists substantial evidence for its transmission by blood and blood products, dengue is not considered a threat to blood safety. The risk of transfusion transmitted dengue varies from country to country as per the geographic endemicity and immunity in the population. The level of viremia, which is expected to be lower and shorter in asymptomatic individuals, may also impact the efficiency of transmission by transfusion [16].

Transfusion of dengue infected blood or even exposed blood with dengue specific antibodies may be of concern in certain groups of patients especially immunocompromised and with chronic liver diseases. Since hepatic dysfunction is a well recognised feature of dengue [17], it is prudent to mention that the transfusion of dengue infected blood may cause deterioration of patients with acute or chronic liver diseases. It is not just the virus, but also the transfer of antibodies which is a matter of concern. It has been proposed that the transfer of non-neutralising or partially neutralizing antibodies while blood transfusion may lead to antibody dependant enhancement in case the recipient is later exposed to heterotypic dengue infection, precipitating dengue haemorrhagic fever and shock [18].

This study has shown that viremia in asymptomatic blood donors was absent, therefore the need to screen blood donors by serological assays for dengue and it may be debatable. But the seroprevalence of infection was 58% which generates concern over the implementation of vaccination in our population. A clear increase in dengue IgG prevalence with age was observed, ranging from 46% in cases less than 30-year-old to 100% in those over 50 years. No difference in the seroprevalence rates in urban versus rural areas was seen, which could have been because of the very low number of donors coming from rural areas (26 out of 200).

Large community based seroprevalence studies are required to estimate the true burden of infection in a population. Periodically conducted serosurveys would also serve as a measure to evaluate the efficacy of control programs. Additionally, the sero-epidemiological prevalence of infection in general population/blood donors may also serve as an important feeder of information before implementation of any vaccination policies. Notably, the Sanofi Pasteur dengue vaccine recently licensed in Mexico is meant for areas that are highly endemic, with a seroprevalence over 60% [19]. There is definite lack of literature regarding seroepidemiology of dengue in healthy population from our country. In a population based seroprevalence study from Pakistan, 67.2% dengue IgG positivity was seen [20]. A few studies have been done across the globe to estimate the burden of dengue infection in blood donors [Table/Fig-3] [18,21-28]. While studies from Brazil, Puerto Rico and Australia have shown very low prevalence in blood donors [21–25], high seroprevalence has been reported from Malaysia [26] and Singapore [27] (42% and 52% respectively). However, the two studies did not screen the donors for active viremia. In another study from Delhi [27] blood donors were tested for NS1 antigen, but no positivity was seen.

Characteristic of donor	Total sample n (%)
Gender	
Male	194 (97)
Female	6 (3)
Age	
19-30	123 (61.5)
31-40	57 (28.5
41-50	19 (9.5)
>51	1 (0.5)
Residence	
Rural area	26 (13)
Urban area	174 (87)

[Table/Fig-1]: Baseline characteristics of the blood donors.

Age group (years)	Total number of donors tested	Anti-Dengue Antibody positivity			RNA positivity
		IgG only (%)	IgM only (%)	IgG+IgM (%)	
<30	123	57 (46.3)	2	12 (9.8)	0
31-40	57	40 (70.2)	0	9 (15.8)	0
41-50	19	18 (94.7)	0	4 (21)	0
>50	1	1(100)	0	0	0
Overall	200	116 (58)	2 (1)	25 (12.5)	0

[Table/Fig-2]: Age wise distribution of anti-dengue antibody and dengue RNA positivity in blood donors.

LIMITATION

The study has been conducted on a small sample size which may not be depictive of seroprevalence in the general polulation. Studies of larger magnitude are required to quantify the risk and provide strong evidence for policies to be made.

Study	Place	Sample size	Test Done	Methodology	Results
Ashshi, 2015 [18]	Saudi Arabia	100	NS1 IgM IgG	ELISA	NS1: 1% IgM: 6% IgG: 7%
Linnen et al., 2008 [21]	Honduras, Brazil and Australia	13,731	RNA detection	Transcription mediated amplification	0.30%-Honduras 0.06%-Brazil 0%-Australia
Mohammed et al.,2008 [22]	Peurto Rico	16,521	RNA detection	Transcription mediated amplification	0.07%
Leny lobo Dias et al.,2012 [23]	Brazil	500	RNA detection	RealTime Reverse Transcriptase PCR	0.4%
Ribas-Silva et al.,2012 [24]	Brazil	213	IgM IgG	Immunochromato-graphic test	IgM: 0 % IgG: 1.4%
Faddy et al., 2013 [25]	North Queensland, Australia	5453	IgM IgG*	ELISA	IgM: 0.22% IgG: 9.43%
Harif et al., 2014 [26]	Malaysia	360	IgM IgG	ELISA	IgM: 4.2% IgG: 42.0%
Low et al., 2015 [27]	Singapore	3,995	IgG IgM	ELISA	IgG: 52% IgM: 2.83%
Mangwana, 2015 [28]	New Delhi, India	1709	NS1 Antigen	ELISA	0%
This study	New Delhi, India	200	IgG IgM RNA	ELISA, Reverse Transcriptase PCR	IgG: 58% IgM: 13.5% RNA: 0%

[Table/Fig-3]: Studies for estimation of dengue seroprevalence in blood donors [18,21-28].
* IgG testing was done on selective samples

CONCLUSION

In the present era where transfusion safety in terms of HBV, HCV and HIV is given paramount importance, the risk of transmission of the dengue virus and/or its antibodies has been increasingly perceived. Despite high seroprevalence of anti-dengue antibodies in the study subjects, active viremia was not seen. Mandatory screening for dengue before blood transfusion may not be feasible in a resource limited setting. Stringent questioning and deferral of donors with history compatible with a viral illness is crucial.

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