

Seroprevalence of Dengue Antibodies among Healthy Blood Donors from Sabah, Malaysian Borneo: Blood Safety in Dengue-Endemic Communities

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ABSTRACT

Dengue virus (DENV) infection which is an emerging and resurging mosquito-borne infection is a major public health concern in many countries in the tropics and subtropics. It is the most common arbovirus infection globally, and its incidence has increased dramatically in recent decades. Despite several reported transfusion-transmitted cases, the impact of dengue infection on the safety of the blood supply is still controversial. This study aims to determine the seroprevalence of dengue infection among healthy blood donors from Sabah, Malaysia, an area in Southeast Asia that is endemic to the four serotypes of the dengue virus. A cross-sectional study was conducted among 364 eligible blood donors from the Sabah Women and Children Hospital in Sabah State. Serum samples were examined for the presence of dengue-specific immunoglobulin G (IgG) using an enzyme-linked immunosorbent assay (ELISA). Overall, approximately

one-third (36.5%, 133/364) of the participants tested positive for dengue anti-IgG. The prevalence of dengue anti-IgG significantly increased with age, with the lowest prevalence (30.5%) among young adults aged 18 – 26 years and the highest prevalence (73.3%) among those aged 56 – 65 years ($\chi^2 = 10.984$; $P = 0.027$). Likewise, the prevalence was higher among male blood donors (38.9%) compared to female donors (31.3%); however, the difference was not statistically significant ($\chi^2 = 0.295$; $P = 0.267$). The high prevalence of dengue IgG seropositivity among healthy blood donors reflects the high endemicity of dengue disease in this region of Malaysia. The findings suggest the need for blood screening for DENV infection by blood donation services in Malaysia to improve transfusion safety, which is of paramount importance for the recipient.

Keywords: dengue fever, seroprevalence, blood safety, blood donor

INTRODUCTION

Dengue virus (DENV) infection is one of the most prevalent neglected tropical diseases (NTDs) worldwide. It is a vector-borne life-threatening public health problem in many tropical and subtropical countries, particularly in underprivileged rural and urban communities [1]. Dengue fever virus is a single-stranded positive RNA virus with a lipid envelope that belongs to the *Flavivirus* genus and the *Flaviviridae* family. It is a vector-borne viral infection with five distinct, but closely related, genetically identified serotypes (DENV1, DENV2, DENV3, DENV4 and DENV5) [2]. The ability of mosquitoes, specifically *Aedes aegypti* and *Aedes albopictus*, to thrive in and adapt to the rapidly changing urban environment may be one of the factors that explain the increasing incidence of DENV infections in recent years [3]. According to recent estimates, dengue transmission occurs in over 128 countries, with 58.4 million annual symptomatic dengue infections resulting in approximately 10,000 annual deaths, and almost 4 billion people are at risk of infection [1], [4].

The clinical manifestations of DENV infection can be classified as inapparent (also called subclinical or asymptomatic) and apparent cases, with the latter presenting as undifferentiated febrile illness, classic dengue fever or the more severe forms, such as dengue haemorrhagic fever (DHF) or the life-threatening dengue shock syndrome, with which other severe complications can occur, particularly severe liver and/or neurological involvement [5]. A serious challenge to surveillance and control programmes occurs when individuals experience asymptomatic infections, as these cases could be easily missed. Without signs and symptoms, the infected DENV person will not seek any medical attention and it will contribute to the data gaps in true dengue burden. These asymptomatic patients can be diagnosed using IgG dengue investigation and will be beneficial to policymakers for preventive measures to control dengue infection in endemic areas. Previous studies showed that unapparent cases occur more frequently

than apparent ones. For instance, a previous study estimated that approximately 96 million apparent and 294 million inapparent DENV infections occurred globally in 2010 [4]. Hence, it is assumed that inapparent DENV infections could cause new foci of disease transmission or, eventually, an outbreak in non-endemic areas [6]. However, this silent circulation of DENV among humans can be detected by molecular assays such as real-time PCR and virus isolation [7].

Although the major route of transmission occurs through the *Aedes* mosquitoes, dengue infection has also been transmitted through blood transfusions and organ transplantations [8], [9], [10], [11], [12]. The high rate of asymptomatic DENV infection poses a higher risk to transfusion safety, and several transfusion-transmitted (TT) cases have been reported globally. The first cases of probable TT-DENV infections were reported in Hong Kong in 2008 in three recipients of RNA-positive transfusions, followed by another three recipients in Singapore [8], [13]. Subsequently, more cases of TT-DENV infections were reported in recipients from other countries such as Puerto Rico and Pakistan [9], [14]. Similarly, a large-scale study investigated the rates of transmission by DENV RNA-positive blood donors during epidemics of dengue in Brazil and revealed that TT is occurring, with over one-third of the components from RNA-positive donations transmitting infection [15].

According to recent estimates, DENV infection is one of the leading causes of hospitalization and mortality in both children and adults in Southeast Asia [16], and over 70% of global dengue infections have been reported in the Asia-Western Pacific region [4], [17]. Within this region, Malaysia is ranked third in terms of the prevalence of DENV infections, after China and Singapore, with over 100,000 suspected cases reported in 2016 [17], [18]. Malaysia has been classified as a country that is “hyper-endemic”. *Ae. aegypti* is the principal vector of dengue, and *Ae. albopictus* is the secondary vector, with four serotypes (DENV1 – DENV4) circulating in the country [19], [20]. It was estimated that approximately 16.5 million people (55% of Malaysia’s total population) had been infected by DENV by 2013 [21]. In 2014, Malaysia exceeded a record when the number of dengue fever cases increased to 314% of the total cases reported in 2013; however, the highest total number of cases ever recorded in Malaysia occurred in 2015, with 120,836 suspected cases and a 50% rise in the number of associated deaths [22], [23].

Since the 1970s, several studies have been conducted on DENV infection in peninsular Malaysia (West Malaysia) concerning the epidemiology, genotyping, and related economic issues [24], [25]. However, data on DENV infection in East Malaysia (Sabah, Sarawak and Labuan) is limited [26]. Similarly, there has only been a single report on the DENV seropositivity status of blood donors in Malaysia, although this issue has recently received increased attention in the media [23], [27]. Within this context, the present study was carried out to determine the DENV seroprevalence among healthy blood donors in Kota Kinabalu, Sabah, Malaysia, enabling a better understanding of blood safety and the potential for transfusion-transmitted DENV infection.

MATERIALS AND METHODS

Ethics Statement

The present study was carried out according to the guidelines laid down in the Declaration of Helsinki, and the protocol was reviewed and approved by the Malaysian Ministry of Health (NMRR-15-2063-27261) and the Medical Ethics Committee of University Malaysia Sabah, Sabah, Malaysia (JKEtika 1/15(1)). Prior to blood sample collection, the participants were informed about the objectives of the research and their involvement in the present study. Then, written and signed informed consent were obtained from the participants.

Study Design

A cross-sectional study was conducted with eligible blood donors at the Sabah Women and Children's Hospital (SWACH) from December 2016 to August 2017. During the interview, all donors were informed about this seroprevalence study, and their signed consent was obtained. Then, the donors went through standard blood donation procedures. No additional blood samples were taken during this study.

Study Area and Subjects

Sabah is located in the Borneo region of Malaysia and is the second-largest state in the country after Sarawak. The capital of Sabah, Kota Kinabalu, is situated on the northwest coast of Borneo facing towards the South China Sea (5°15'N, 117°0'E), with a total population of 452,058 according to a 2010 census. The climate is tropical, with two main seasons, namely, a dry season (March to August) and a rainy season (September to February). The average annual temperature in Kota Kinabalu is 27°C with a mean total annual precipitation of 2,788 mm. Sabah is one of the most popular tourist destinations in Southeast Asia. With regards to dengue infection, Sabah has been reported as having two dengue transmission seasons, from January to March and from August to October every year.

Sabah Women and Children's Hospital (SWACH) is a tertiary centre for obstetrics and gynaecology, and it is also the referral for thalassaemia care for paediatric patients in Sabah, as well as in the Lawas district of Sarawak. The SWACH blood transfusion team is active in conducting blood donation campaigns, especially in the Kota Kinabalu area. From December 2016 to August 2017, a total of 364 eligible blood donors (80 female and 284 male) participated in this study. The subjects included in the study were aged between 18 and 61 years and were healthy and did not have fever, hypertension, G6PD deficiency, diabetes, and thalassaemia. The female subjects were not pregnant, breastfeeding and not in menstruation. All participating donors were tested negative for infections with human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C

virus (HCV) and syphilis. Moreover, all participants were afebrile, meaning that they did not have any symptoms of fever or any signs of dengue infection as per the Malaysian blood transfusion safety guidelines.

Laboratory Tests

Approximately 3 ml of venous blood was collected by a trained phlebotomist who observed strict aseptic precautions. Clotted samples were centrifuged at 3,000 rpm for 10 min to separate the serum. Then, approximately 1 ml of the serum leftover from the infectious disease screening plain tube was collected and transferred to a cryo-preservative tube and stored at -20°C at the SWACH Microbiology Laboratory.

Serum from the selected blood donors was examined for the presence of dengue-specific immunoglobulins G (IgG) using an enzyme-linked immunosorbent assay (ELISA) KIT from SD BIOSENSOR (South Korea) according to the manufacturer's protocol. A sample was defined as positive for anti-DENV IgG antibody if it had an index value > 22 Panbio Units (PU).

Statistical Analysis

The data analysis was performed using SPSS (version 24; SPSS Inc., Chicago, IL). The frequency distribution and proportions with corresponding 95% confidence intervals (95% CI) were calculated. Pearson's Chi-square test and Fisher's exact test were used, where appropriate, to examine the differences in proportions among groups. A P value of < 0.05 was set as the level of significance.

RESULTS

A total of 364 eligible blood donors, who tested negative for HIV, HCV and HBV infections and who were accepted for blood donation according to the blood transfusion safety policy set by the Ministry of Health (Malaysia), were screened for the seroprevalence of anti-DENV IgG antibodies using commercially available ELISA kits (Panbio). The majority of the blood donors who participated in the study were males (78%), and 22% (80/364) were females. With regards to age, the highest proportion (36.8%) of the participants were aged 26 – 35 years, followed by those aged 36 – 45 years (25%), while only 4.1% of them were aged 56 – 65 years.

Prevalence and Distribution of Anti-Dengue IgG

Among the participants, 133 (36.5%, 95% CI = 0.059 – 0.117%) tested positive for dengue IgG, indicating a history of dengue infection (Figure 1). Table 1 shows the distribution of dengue IgG positive results according to the age and gender of the participants.

The prevalence of dengue IgG was higher among the male participants than the females; however, the difference was not statistically significant (38.9% vs 31.3%; $\chi^2 = 0.295$; $P = 0.267$). Moreover, the results showed that those aged 56 – 65 years had the highest prevalence (73.3%), while participants aged 18 – 25 years had the lowest prevalence (30.5%) compared to other age groups ($\chi^2 = 10.984$; $P = 0.027$). The age-dependent increasing trend of dengue IgG reported among the studied blood donors is further illustrated in Figure 2.

Furthermore, the results showed that male participants were 1.35 times more likely to have been infected with dengue than females (95% CI 0.79 – 2.29). Moreover, the OR of dengue prevalence increased 1.15- to 6.27-fold for every ten-year increment in age. In the OR analysis, although age was found to be strongly associated with dengue-IgG seropositivity, in terms of the odds ratios (OR 6.27; 95% CI. 1.82 – 21.61; $P = 0.004$ for age group 56 – 65), wide 95% confidence intervals indicate low precision in estimation (Table 1).

Table 1 Distribution of dengue seroprevalence among the participants according to their age and gender ($n = 364$)

Variables	No. Examined	No. Positive (%)	χ^2 (P)
Age groups (years)			
18 – 25	82	25 (30.5)	10.984 (0.027) *
26 – 35	134	45 (33.6)	
36 – 45	91	35 (38.5)	
46 – 55	42	17 (40.5)	
56 – 61	15	11 (73.3)	
Gender			
Female	80	25 (31.3)	1.237 (0.267)
Male	284	108 (38.9)	

χ^2 = Chi-square test statistic. * Significant difference between the groups ($P < 0.05$).

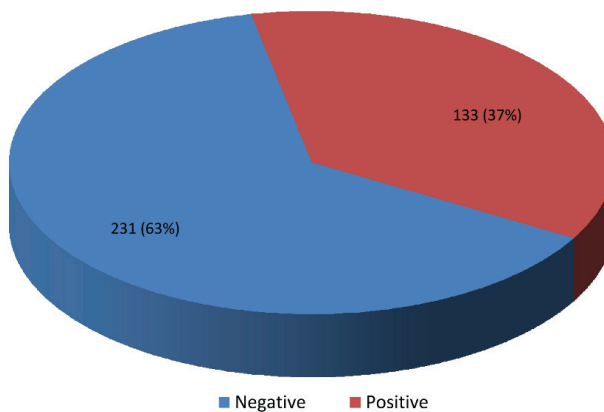


Figure 1 Prevalence of dengue seroprevalence based on the anti-DENV IgG test among blood donors who participated in the study ($n = 364$)

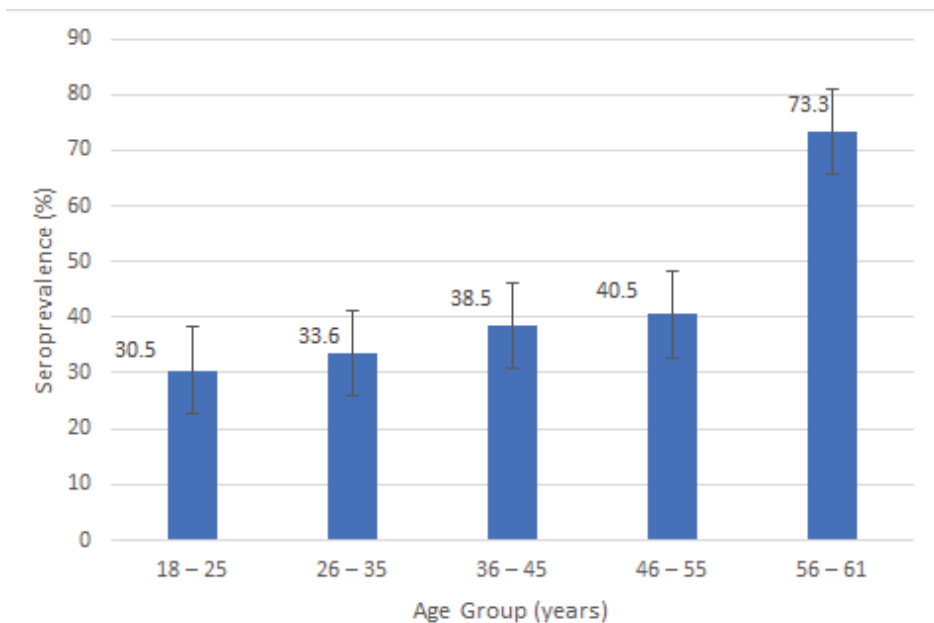


Figure 2 Age-dependent distribution of dengue seroprevalence among the participants ($n = 364$) based on the anti-DENV IgG test

DISCUSSION

Blood transfusion provides unique and life-saving therapeutic benefits to millions of patients every year worldwide and has become an essential part of modern healthcare. It is estimated that approximately 112.5 million blood donations are collected annually worldwide, with one in every ten individuals admitted to a hospital receiving a transfusion [28]. Although blood transfusion is one of the most frequent medical procedures performed in different countries, blood safety remains a serious concern and a WHO global priority, with people in developing countries continuing to face the greatest risks from unsafe blood and blood products [29, [30].

Associations of blood transfusion with several adverse events and adverse consequences such as infections, renal injury, cardiopulmonary complications, cancer recurrence and even mortality are well documented [31], [32], [33]. For instance, approximately 10% of all HIV infections in many countries, including high-income countries during the early 1990s, were attributed to unsafe transfusions, which remain responsible for approximately 5% of HIV infections in Africa today [31], [34]. Currently, the increasing prevalence of many emerging and re-emerging infections such as dengue virus (DENV), West Nile virus (WNV), Zika, chikungunya virus (CHIKV) and yellow fever virus infections has generated continuing global debates on the impact of such infections on blood transfusion safety and availability [35], [36], [37]. However, most of these infections do not receive routine screening during blood transfusion procedures in either developing or developed countries [31], [38], [39].

The present study attempted to bring to light the potential for transfusion-transmitted DENV infection in a well-known dengue-endemic area in East Malaysia. Among 364 blood donors, the current study found that approximately one-third (36.5%) were seropositive for dengue-specific IgG. This high prevalence among healthy blood donors in a dengue-endemic area might be considered a potential threat to blood safety, and therefore, should receive proper attention. This is because asymptomatic cases occur more frequently than symptomatic ones, and those asymptomatic cases could contribute to disease transmission within the same area or to new foci. This includes the transmission of dengue infection from asymptomatic patients to non-infected individuals either naturally through the mosquito vectors or artificially through blood transfusion and organ transplantation [8], [10], [12], [40]. Moreover, it was reported that during infection epidemics, substantial proportions of asymptomatic dengue-infected donors donate blood and that recipients are given RNA-positive blood components [15], [41]. In Australia, to mitigate transfusion-transmitted DENV infection, fresh blood components are not acquired from donors residing in known dengue-endemic areas both nationally and overseas. Also, the transfusion-transmitted DENV (TT-DENV) risk can be substantially mitigated by using an appropriate blood donor screening assay [42]. A lesson learned from the case of West Nile virus (WNV) should be considered. After the first cases of TT-WNV were reported in the U.S. in 2002, WNV nucleic acid testing was added to routine donor screening to mitigate the risk to recipients; that addition prevented thousands of TT-WNV cases [43].

In Malaysia, a previous study that investigated the seroprevalence of DENV among blood donors in North Malaysia reported almost similar values (39.2%) [27]. Interestingly, among 166 seropositive donors, Harif et al. found that 141 donors had dengue-specific IgG, while 15 donors had dengue-specific IgM, and 10 donors were positive for both dengue-specific immunoglobulins, IgG and IgM. The presence of IgG may indicate a previous DENV infection, while the presence of IgM may indicate current primary or secondary infections [44], suggesting that the donor is a carrier of the virus and that therefore, blood from the donor may transmit the infection to the recipients. Supporting the present findings, many studies from different countries have shown variable results regarding DENV seroprevalence among blood donors, and cases of TT-DENV have been reported in several endemic settings. For instance, a recent study in India showed that 58% (116/200) of healthy blood donors were positive for dengue IgG, with 27 cases (13.5%) also positive for IgM [12]. Likewise, a previous study in Saudi Arabia found that 39% and 5.5% of 910 eligible Saudi male blood donors were positive for IgG and IgM, respectively [45].

From an epidemiological perspective, the high seroprevalence rate reported by the present study in Sabah agrees with the findings of a previous study among pregnant women attending antenatal care services at a teaching hospital in Kuala Lumpur (35.8%) [46]. Over the past decades, several seroprevalence studies have been carried out in West Malaysia among a variety of population groups, including children, blood donors, patients in private healthcare settings and pregnant women [24], [25]. Overall, higher seroprevalence rates were reported in urban localities (ranging between 61% and 92%) in Malaysia compared to rural localities, where the rates ranged between 28% and 91%

[21]. Higher seropositive rates were reported in a private healthcare setting in Puchong, Selangor; a previous study found a rate of 76.5%, with the seroprevalence ranging from 33% in participants younger than 20 years to 100% in those older than 60 years [47]. Similarly, a national cross-sectional study among the healthy population aged 35–74 years showed that 91.6% of the participants were dengue IgG-positive, with the seroprevalence rate increasing with increasing age [48]. In contrast, a seroprevalence rate of 11% was reported among 1,410 schoolchildren, aged 7-18 years, from nine different states including Sabah, where a rate of 6% was reported [49].

In comparison with findings from other countries, Singapore has reported a consistently higher seroprevalence rate than Malaysia, with the seroprevalence rate of dengue IgG in Singapore ranging from 50.8% to 60% [16], [50], [51]. Moreover, India accounts for 34% of the global dengue infections; however, a lower rate (21%) was reported in 2014 [4], [52]. The present study showed an increasing seroprevalence rate of infection with increasing age, with the seroprevalence ranging from 30.5% to 73.3%, and this agrees with the results of many previous studies in Malaysia and other countries [12], [21], [27], [53], [54]. Furthermore, the present study found a higher seroprevalence among male donors compared to females; however, the difference was not statistically significant. This may indicate similar exposure to infection for males and females in Sabah. These findings were consistent with those of previous reports [55]. In contrast, male gender was found to be significantly associated with dengue infection in Singapore [53]. Dengue virus infection has a perennial transmission and is classified as a significant public health problem in Malaysia, including in Sabah State, which is in Malaysian Borneo. A larger sample size, serial seroprevalence study and wider range of age are required for a better understanding of the real dengue burden in Sabah. Thus, seroprevalence studies are essential to estimate the real burden of dengue infection and to assess the risk factors for dengue transmission that could partially explain the increase in the frequency and burden of recent epidemics in Sabah, despite vector control efforts.

CONCLUSION

The present study revealed a high seroprevalence rate of DENV infection among healthy blood donors in Sabah, Malaysia, reflecting the high endemicity of dengue infection in the study area. The findings highlight the potential for TT-DENV infections among the study population that may affect blood supply availability and safety. Hence, physicians need to be aware of the risk of TT-DENV infections and screening measures to ensure blood safety should be evaluated and implemented, particularly during dengue epidemics. Moreover, the persistence of DENV transmission in Sabah merits further assessment of the epidemiological dynamics of dengue infection.

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AUTHORSHIP CONTRIBUTIONS

MZH, SMA and VKS designed the study. ZM performed the experiments. MZH, ZM, RS, MAA and VKS analyzed and interpreted the data. MZH, HBL and VKS supervised the work. ZM, MZH, SMA and VKS wrote and edited the manuscript. All authors critically reviewed and approved the manuscript.

DISCLOSURE OF CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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