Dengue antibodies in blood donors

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Background: *Dengue is an urban arbovirus whose etiologic agent is a virus of the genus Flavorius with four distinct antigen serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) that is transmitted to humans through the bite of the mosquito Aedes aegypti. The Campo Mourão region in Brazil is endemic for dengue fever.*

Obtective: *The aim of this study was to evaluate the presence of IgG and IgM antibodies specific to the four serotypes of dengue in donors of the blood donor service in the city of Campo Mourão.*

Methods: *Epidemiological records were evaluated and 4 mL of peripheral blood from 213 blood donors were collected in tubes without anticoagulant. Serum was then obtained and immunochromatographic tests were undertaken (Imuno-Rápido Dengue IgM/IgG™). Individuals involved in the study answered a social and epidemiological questionnaire on data which included age, gender and diagnosis of dengue.*

Results: *Only three (1.4%) of the 213 blood tests were positive for IgG anti-dengue antibodies. No donors with IgM antibody, which identifies acute infection, were identified.*

Conclusions: *The results of the current analysis show that the introduction of quantitative or molecular serological methods to determine the presence of anti-dengue antibodies or the detection of the dengue virus in blood donors in endemic regions should be established so that the quality of blood transfusions is guaranteed.*

Keywords: *Blood donors; Serology; Dengue; Immunoglobulin M; Immunoglobulin G; blood transfusion*

Introduction

Dengue is a benign urban arbovirus whose etiological agent is a virus of the genus *Flavovirus* transmitted by the mosquito *Aedes aegypti* with four distinct antigenic serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). No indication of crossed immunity exists, that is, infection by one of the serotypes produces immunity for only that particular serotype. When the disease establishes itself in a determined inhabited area, it will remain for a long time[1].

The transmission of infectious and contagious agents through the transfusion of blood derivatives and components is characterized by delayed adverse reactions that increase the risks to recipients. Safe blood is guaranteed by actions that minimize the possibility of transfusion-transmitted diseases including the clinical and epidemiological screening of donors and serological testing[2]. However, serological testing of blood donors does not guarantee total safety from possible transmission of infectious and contagious agents. The Brazilian Ministry of Health requires testing for hepatitis B and C, HIV, syphilis, HTLV I/II and Chagas' disease at all blood donation services in Brazil and malaria in endemic regions. It is expected that the dengue virus will soon be included in the serology of blood banks in regions with tropical and sub-tropical climates[2,3]. This is due to the fact that 40% of patients suffering from dengue fever fail to show any symptoms and thus are neither diagnosed nor are the health authorities notified[3].

Publications have reported on the risks of dengue related to contaminated blood bags. A study in Porto Rico in 2006 calculated the risk of infection through transfusions as one in 1300 donations were infected. It is thought that these data have been surpassed as the Fundação do Hemocentro de São Paulo reported that one in every 1000 blood bags was contaminated. It is believed that approximately 5000 patients were iatrogenically contaminated by the dengue virus during the 2007 dengue epidemic[3].

The mechanisms that determine the disease's clinical presentation (classic or hemorrhagic) remain unknown. The most accepted physical and pathogenic hypotheses for the etiology of the hemorrhagic form of the disease are related to the presence of antiviral antibodies. A person who has been infected by one of the four dengue serotypes has non-neutralizing circulating antiviral antibodies; if a second infection by a different serotype occurs, the virus is recognized by these antibodies, but replication is neither inhibited nor neutralized. An antigen-antibody complex is formed which increases virus replication and makes penetration of macrophages by the virus easier through opsonization. Vessel-active mediators are released by the macrophage and vascular permeability increases with a consequent leakage of plasma through the vessel walls, possibly resulting in hypovolemic shock[4].

The blood transfusion service of Campo Mourão, PR, Brazil, covers 25 municipalities with a total population of 334,254. There were 643 confirmed cases of dengue up to 30th May 2011 which characterized the region as endemic. The aim of this study was to carry out serological screening to detect IgG and IgM antibodies specific to the four dengue serotypes in blood donors of the transfusion service of Campo Mourão using an immunochromatographic test (Imuno-Rápido Dengue IgM/IgG™).

Methods

This is a cross-sectional clinical study of 213 blood donors attended by the transfusion service in Campo Mourão between March and April 2011. The participants completed a socio-epidemiological questionnaire on data including their age, gender and diagnosis of dengue.

Four mL of peripheral blood were collected in tubes without anticoagulant. The tubes were centrifuged at 3000 rpm for 15 minutes to obtain the serum. Sera were then stored in a freezer at -25ºC until the test. The Imuno-Rápido Dengue IgM/IgG Test (Wama Diagnóstica™) was employed in this study following the manufacturer's instructions. The Imuno-Rápido Dengue IgM/IgG test is an immunochromatographic method which identifies dengue-specific IgG and IgM antibodies. These bind to recombinant antigens (DENV-1, DENV-2, DENV-3 and DENV-4) in the viral envelope and conjugate to colloidal gold to form an antigen-antibody complex. This complex migrates by capillarity through the membrane of the test plate and is caught by immobilized human anti-IgG or anti-IgM in two distinct areas, which determine characteristic pink bands in the corresponding areas[5].

All participants received information on the study and those who agreed to participate, signed consent forms. This study was approved by the Ethics Committee of Research on Human Beings of the Health Department of the state of Paraná, Brazil (# 303/2011).

Results

Two hundred and thirteen patients participated in the study: 77 (36.2%) were female and 136 (63.8%) were male; 148 (69.5%) were Caucasians, 33 (15.5%) were Afro-Brazilians, 24 (11.3%) were Amerindians and eight (3.7%) were Asians. Thirty percent of this population was aged between 18 and 30 years old, 44.2% was between 31 and 45 years old, 23.0% between 46 and 59 years old and 2.8% was over 60. Additionally, 0.5% of participants had no schooling, 41.8% had studied up to primary school, 49.3% had studied up to high school, 7.9% had university degrees and 0.5% had doctoral degrees. Moreover, 62% lived in the rural area and 38% in towns. The prevalence of sera with IgG anti-dengue antibodies amounted to 1.4%, but no samples with IgM anti-dengue antibodies were found (Table 1).

The infected participants had donated blood at least twice a year for more than three years however they had had dengue before being donors without any indication of secondary infection. Table 2 shows that that biological samples analyzed did not present any health risks to the receiver.

Table 1 - Social and epidemiological aspects of the 213 participants of this study

Social and epidemiological aspects	Total patients (%)
Gender	
Male	63.8
Female	36.2
Age	
18 – 30 years	30.0
31 – 45 years	44.2
46 – 59 years	23.0
Over 60 years	2.8
Racial background	
Caucasian	69.5
Afro-Brazilian	15.5
Asian	3.7
Amerindian	11.3
Schooling	
No schooling	0.5
Junior School	41.8
High school	49.3
University	7.9
Doctoral degree	0.5
Residence	
Rural area	62.0
Urban area	38.0
Prior diagnosis of dengue	
Yes	1.4
No	98.6

Table 2 - Characteristics of blood donors with positive serology for dengue

Clinical characteristics	Total patients (%)
Re-infection	
No	100
Period as donor	
3-9 years	33.3
Over 10 years	66.7
Frequency of blood donations	
Twice a year	33.3
Three times a year	66.67
When was diagnosis	
> 5 years ago	33.3
> 10 years ago	33.3
> 20 years ago	33.3
Donors when infected	
No	100

Discussion

The data of this study show that there was an imbalance in the distribution of gender and age in this transfusion service. The predominance of 63.8% of male blood donors in the current study corroborates data reported by the Brazilian Government Sanitary Health Agency, ANVISA (62.4%) for the southern region of Brazil. Thirty percent of donors were within the 30 - 39 year

old age bracket which is very close to the 28.25% reported by ANVISA[6]. Two U.S. studies showed that young female Afro-Americans are, as a rule, the least likely to donate blood[7,8]. A 1.4% of IgG anti-dengue positive blood bags is not an alarming factor as the donors after such a long time would not transmit the virus to the recipient through transfusions. However, it should be stressed that transmission of IgG anti-dengue antibodies may increase the susceptibility of recipients for immunology conditions, with greater risk of hemorrhagic dengue if they are infected by a second dengue serotype within six months after blood transmission[9,10]. Further, the presence of heterophile antibodies of a previous infection may facilitate the entrance of other viral serotypes.

The absence of anti-dengue IgM in the current research suggests that blood donors were not actively infected with the dengue virus. However, it is well known that methodologies for virus detection also include the more efficient viral RNA and NS1 antigen investigations for the dengue virus which eliminate the immunological window period[11]. The current study may not have identified anti-dengue IgM antibodies as these antibodies are only detected by immunochromatography on the 4th day after the onset of symptoms with the highest levels at about the 7th or 8th day; the titer levels gradually decrease until they are undetectable after some months[12]. One of the limiting factors in the current study is the option of investigating antibodies since false-negative results may have possibly occurred[13,14].

In spite of the high specificity (99%) and sensitivity (98%) of the Imuno-Rápido Dengue IgM/IgG Test (Wama Diagnóstica™), it is a qualitative serological screening in which the anti-dengue antibody concentration cannot be determined[7]. It is therefore interesting to study this population further using another methodology to measure the antibody serum or dengue virus antigens. When patients are re-infected, they do not present with detectable IgM antibody titles but IgG antibody titles increase. Consequently, the hypothesis that the three IgG positive individuals may have had re-infection cannot be discarded as the IgG titer remains unknown.

The true frequency of blood bags infected by anti-dengue antibodies was not investigated in the current study. However, when the 5280 blood bags collected in the main transfusion service of Campo Mourão in 2010 are considered, it seems probable that 213 samples is insufficient to discard the hypothesis that there is no risk of the transmission of dengue virus through blood bags. Other studies should be performed in the region and in other endemic regions.

Conclusion

The results from the current analysis show that the introduction of quantitative or molecular serological methods to determine the presence of anti-dengue antibodies or the detection of the dengue virus in blood donors in endemic regions should be established so that the safety of blood transfusions is guaranteed.

Acknowledgements

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