

Serological and molecular evaluation of parvovirus B19 (B19V) in blood donors from the Blood Center of Brasília, Brazil: focus on women of childbearing age

Avaliação sorológica e molecular do parvovírus B19 (B19V) em doadores de sangue da Fundação Hemocentro de Brasília, Brasil: foco em mulheres em idade fértil

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ABSTRACT

Parvovirus B19 (B19V) can be transmitted by the respiratory route, vertically – from the mother to the fetus – and via blood transfusion or organ transplantation. Infection by transfusion of blood or blood products occurs due to the resistance of B19V to viral inactivation methods. Our study evaluated the presence of B19V deoxyribonucleic acid (DNA) and the prevalence of anti-B19V class G immunoglobulin (IgG) in women of childbearing age blood donors of the Federal District, Brazil. Our results demonstrated the absence of B19V DNA in these blood donors. However, the seroprevalence for anti-B19V IgG was observed in 60.7% of this population. This study provides important data of B19V circulation in the Center-West of Brazil.

Key words: erythrovirus; human parvovirus B19; prevalence; seroepidemiological studies.

BRIEFING COMMUNICATION

Parvovirus B19 (B19V) belongs to the family *Parvoviridae* (genus *Erythrovirus*). It is a small (19-25 nm), non-enveloped, icosahedral and single-stranded deoxyribonucleic acid (DNA) genome virus⁽¹⁾. The viral capsid consists of two structural proteins: VP1 and VP2. The viral genome also encodes a NS1 non-structural protein, which participates in viral replication and apoptosis of the host cell⁽²⁾.

In most cases, B19V causes a benign infection in children known as infectious erythema or fifth disease. However, the virus can cause a number of symptoms, including lethal outcome. The symptoms related to B19V infection depend on the age, immunological condition and mainly the hematological condition of the infected individual⁽³⁾. When infection is acquired in adulthood, the clinical presentation is compatible with arthritis

or arthralgia, especially in the large joints, and may persist for years. In individuals with a high erythrocyte turnover rate – for example, in individuals with hemoglobinopathies – acute B19V infection may lead to transient aplastic crisis⁽⁴⁾. In pregnancy, B19V represents a great danger to the fetus by crossing the placental barrier and infecting erythroid progenitor cells in the bone marrow and liver of the fetus, which can lead to blockage of erythropoiesis, severe anemia, fetal hydrops, and death⁽⁵⁾.

B19V has multiple transmission mechanisms, including respiratory route transmission, vertical transmission from mother to fetus, and by bone marrow and solid organ transplantation⁽⁶⁾. Due to the high rates of persistence of the virus in the blood, the resistance to pathogen inactivation processes and the fact that it can cause an asymptomatic infection, there is a possibility of transmission also through transfusion of blood and blood components, mainly with the therapeutic use of coagulation

factors derived from human plasma⁽⁷⁾. Considering the possibility of B19V transmission by blood transfusion, this study aimed to evaluate the presence of B19V DNA circulating plasma obtained from a group of blood donors from the Blood Center of Brasília, Federal District, Brazil, more specifically in women of childbearing age. There is still little information on the circulation of B19V in the Center-West of Brazil, and no information in the Federal District. The project was approved by the Human Research Ethics Committee (CAAE: 62718016.0.0000.5553).

A total of 89 serum samples were randomly collected from women aged between 20-38 years (mean of 28.5 years), previously stored in serum bank at -80°C in December 2015. Viral DNA was extracted from 200 µl of serum, using PureLink™ Viral RNA/DNA Mini Kit (Invitrogen – ThermoFisher Scientific, São Paulo, Brazil), following the manufacturer’s recommendations. After extraction of the viral DNA, the molecular detection of B19V was performed by the real-time polymerase chain reaction (PCR) methodology (TaqMan® technology) as described in the literature⁽⁸⁾. In addition to the 1X TaqMan Real-time PCR MasterMix reagent (ThermoFisher Scientific, São Paulo, Brazil), the following primers were used at the concentration of 300 nM: VP1F (5'-GGCCATTTC AAGGAAGTT-3') and VP1R (5'-GAAGCCAGCACTGGTGCA-3'). The VP1P probe (5'-FAM-TACAACGCCTCAGAAAA-3'-MGB) at the concentration of 150 nM was also used. The primers and probes were designed for a highly conserved portion of the VP1 gene, ensuring the detection of all genotypes. The final volume of the reaction was 25 µl. All reactions were performed in ABI 7500 thermal cycler, following standard amplification protocol (50°C for 5 min, 95°C denaturation for 5 min and 40 cycles at 95°C for 15s and 60°C for 1 min). The sensitivity of this reaction was 10 copies/reaction, evaluated by the use of a plasmid⁽⁹⁾ containing the sequence of interest (VP1 gene fragment of the B19V) in serial dilution of 10⁸-1 copy/reaction magnitude.

Our study did not demonstrate molecular positivity for the B19V DNA in the studied group (Table). However, the prevalence of B19V DNA may vary significantly depending on the region and evaluated target group. In this regard, in Brazil, other studies have demonstrated the presence of B19V DNA in 0% to 1% of blood

donors in the Southeast region⁽⁸⁻¹⁰⁾, and one of these studies with a number of donors similar to ours (91 individuals). However, the study conducted in the Southeast region includes both male and female donors, whereas our study has a relatively homogeneous group, i.e. women of childbearing age. In addition, the difference in geographical location between studies is also an important factor and should be considered. All previous studies were carried out in the state of São Paulo, a more populous region when compared to the Federal District. In addition, although the different genotypes (1, 2 and 3) circulate together, there is a difference in their relative frequencies⁽¹¹⁾. Therefore, there may be differences between the B19V genotypes circulating in each region, influencing the difference in prevalence between the studies.

In the world context, the B19V DNA prevalence in blood donors ranges between 0.006% and 0.88%⁽²⁾. Another possibility regarding the lack of detection of B19V DNA may be related to the absence of viral persistence in blood donors evaluated. On the other hand, even if viral DNA were detected, it would not be possible to state that there would be necessarily a risk of transfusion transmission. The persistence of B19V has been demonstrated in different tissues, such as skin, liver, heart, among others. After acute infection, genomes or fragments of viral DNA can also be released into plasma from these tissues and thus the presence of B19V DNA in plasma would not necessarily be associated with the presence of the infectious viral particle⁽¹²⁾. It is still possible that in previous studies the rates of persistent infection in blood donors (very low detectable DNA load) are higher when compared to our donor group. In this regard, the absence of viral DNA in blood donors evaluated in our study does not allow us to affirm that the risk of transmission of B19V, through blood transfusion at the evaluated blood bank, does not exist. Thus, for the evaluation of the true prevalence of B19V DNA in this blood bank, it is necessary to include a much higher number of samples similarly to all the studies carried out in Brazil and by international groups.

Subsequently, the seroprevalence of anti-B19V class G immunoglobulin (IgG) was evaluated in the same samples. The importance of this evaluation in women of childbearing age is justified by the fact that once non-immunized (anti-B19V

TABLE – Comparison of prevalence of anti-B19V IgG and B19V DNA in studies conducted in Brazil

Study	Region (State)	Population	n	anti-B19V IgG	B19V (DNA)
Slavov <i>et al.</i> (2012) ⁽⁸⁾	São Paulo	Blood donors (both sexes)	100	60%	1%
Slavov <i>et al.</i> (2012) ⁽⁹⁾	São Paulo	Blood donors (both sexes)	47	57.4%	0%
Slavov <i>et al.</i> (2014) ⁽¹³⁾	São Paulo	Blood donors (both sexes)	40	50%	0%
Slavov <i>et al.</i> (2016) ⁽¹⁰⁾	São Paulo	Blood donors (both sexes)	91	-	1%
Rios <i>et al.</i> (2016) ⁽¹⁴⁾	Goiás	Women of childbearing age	101	8.9%	-
Silva <i>et al.</i> (2018) (our study)	Federal District	Blood donors, women of childbearing age	89	60.7%	0%

IgG: class G immunoglobulin; DNA: deoxyribonucleic acid.

IgG negative), these women are susceptible to infection. In case the infection occurs during pregnancy, especially in the first trimester, fetal anemia, hydrops, fetal death and spontaneous abortion may occur⁽¹⁵⁾. Part of the serum collected was tested by the Elisa technique using the Biotrin Parvovirus B19 IgG kit (DiaSorin, Saluggia, VC, Italy) according to the manufacturer's recommendations. Our results demonstrated 60.7% seroprevalence of anti-B19V IgG in the studied group. The seroprevalence of anti-B19V IgG in healthy blood donors in different parts of the world may range from 9.78% and 79.1%, depending on the geographical region and the test used for diagnosis⁽²⁾. In Brazil, serum levels in donors vary between 50% and 60%^(8,9,13), similar to our results the results obtained by our study (Table). This demonstrates that although detection of viral DNA in different parts of Brazil varies, anti-B19V IgG seroprevalence are relatively close and considered high. On the other hand, another study carried out with 101 women of childbearing age (25 to 34 years old) observed anti-B19V IgG positivity in 8.9% of the tested individuals in the city of Goiânia, Goiás, Central-West Brazil⁽¹⁴⁾. Despite the similar number of individuals tested in the Goiânia study and in this study, there was a considerable difference in the anti-B19V IgG seroprevalence. This difference may occur due to different causes, such as different kits used for the detection of IgG [ELISA Ridascreen Parvovirus B19

IgG from Lab R-Biopharm (1998 to 1999) and ELISA Parvovirus B19 IgG of Biotrin (2015), respectively].

Our results did not show the presence of viral DNA in the samples evaluated. However, a high prevalence of anti-B19V IgG was observed in women of childbearing age, blood donors from the Blood Center of Brasília. These results demonstrate that despite the apparent low risk of virus transmission, this fact can not be completely ruled out due to the high levels of IgG seroprevalence, showing that there is B19V circulation in this group. Therefore, a study with a larger number of samples is necessary for the evaluation of the real transfusion risk of B19V in this region of Brazil. Nevertheless, the establishment of strategies that guarantee the supply of blood and blood products safely must be considered, such as the implementation of nucleic acid testing (NAT) for all B19V genotypes. It has also been observed that approximately 40% of women of childbearing age are not immune to B19V (anti-B19V IgG negative) and they can be infected during pregnancy, especially during epidemic periods⁽¹⁵⁾.

This is the first study that evaluates the prevalence of B19V in the Federal District and provides important data about the circulation of B19V and its seroprevalence (IgG) in the Center-West region of Brazil.

RESUMO

O parvovírus B19 (B19V) pode ser transmitido por via respiratória, verticalmente – da mãe para o feto – e via transfusão de sangue e transplante de órgãos. A infecção por transfusão de sangue ou hemoderivados ocorre devido à resistência do B19V aos métodos de inativação viral. Nosso estudo avaliou a presença do ácido desoxirribonucleico (DNA) B19V e a prevalência de imunoglobulina da classe G (IgG) anti-B19V em mulheres em idade fértil, doadoras de sangue do Distrito Federal, Brasil. Nossos resultados demonstraram a ausência de DNA de B19V nesses doadores. No entanto, foi observada a soroprevalência de IgG anti-B19V em 60,7% dessa população. Este estudo fornece dados importantes da circulação do B19V no Centro-Oeste do Brasil.

Unitermos: eritrovírus; parvovírus B19 humano; prevalência; estudos soropidemiológicos.

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