CORRESPONDENCE

Prevalence and Viral Load of Human Parvovirus B19 (B19V) Among Blood Donors in South-East Brazil

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Abstract The infection of human parvovirus B19 (B19V) is a common event in the general population, including volunteer blood donors. In some cases it can be asymptomatic and can remain persistent for a long period of time. The objective of this study was to examine the B19V DNA prevalence and viral load in first-time volunteer blood donors. Blood samples were collected from 91 primary blood donors at the Regional Blood Center of Ribeirão Preto, Southeast Brazil. Viral detection and quantitation was performed by an in-house TaqMan[®] real-time PCR with high sensitivity. B19V DNA was detected in one male blood donor (1.0 %) and was characterized by a very low viral load (537.36 copies/mL). Our studies demonstrate that B19V DNA at low titer may be present in apparently healthy individuals. Sensitive molecular diagnostic tools can be applied for the screening of fresh blood derived products in order to prevent transfusion-transmitted B19V infection.

Keywords *Human parvovirus B19* · B19V · Viral quantitation · Blood donors

Human parvovirus B19 (B19V), a small icosahedral virus (19–22 nm), is the prototype member of the *Erythrovirus* genus (Parvoviridae family). B19V infection is a common finding in the general population and demonstrates marked seasonality. Clinical manifestations of B19V infection in children and adults may include erythema infectiosum (fifth disease) or arthropathy, nevertheless, great proportion of the infected individuals remain asymptomatic [1]. Therefore, in the general population B19V infections among blood donors can be considered a normal event and many of them have asymptomatic course [2, 3]. A peculiar characteristic of B19V infection is that not always the virus is cleared and in many cases it can establish persistency in different body tissues [4]. Persistent B19V infection has been observed in immunologically normal individuals [5] including qualified blood donors [6]. The presence of persistent B19V infection in blood donors raises serious questions regarding transfusion safety of the obtained hemoderivatives, especially plasma products and packed erythrocytes.

Moreover, B19V is highly resilient to almost all virus inactivation procedures [7], causes infections with a high viral load and these properties predispose to easy viral contamination of the blood products. The relevance of the low B19V load in asymptomatic donors and for the respective recipients of blood is still unclear. Therefore, the objective of this study was to evaluate B19V DNA prevalence and viral load (VL) during 1 month blood collection in a non-metropolitan blood transfusion center in Southeast Brazil (Ribeirão Preto city, Western part of the São Paulo State).

Ninety one peripheral blood samples were collected from 91 first time volunteer blood donors (mean age 35.7 years, 18–65 years, 55 % males). All of them were approved on the preliminary interview for blood donation

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and they did not demonstrate signs or overt infectious disease. The donors were negative for the routinely tested parenterally transmitted viral diseases (anti-HIV-1/2 IgG/IgM, anti-HCV IgG, HBsAg, anti-HBc IgG, and anti-HTLV-1/2 IgG, all negative), syphilis (Fluorescent Treponemal Antibody Absorption Test, FTA-ABS) and American trypanosomiasis or Chagas disease (anti-*Trypanosoma cruzi* IgG negative). The volunteer blood donors were attended at the Regional Blood Center of Ribeirão Preto, city of Ribeirão Preto, São Paulo State, Brazil and all of them signed a written informed consent. Viral DNA was extracted from 140 µl of plasma using the QIAamp Viral RNA Mini Kit (QIAGEN, São Paulo, Brazil), extracting simultaneously viral RNA and DNA, following the manufacturer's instructions. B19V detection and VL quantitation was performed using a previously described TaqMan® real-time PCR with sensitivity 10 B19V DNA copies/reaction and a wide order of magnitude [2]. In brief, the 25 µl volume reactions were run in duplicates using standard reaction conditions as implied by 7500 Real-Time PCR system (Life Technologies, São Paulo, Brazil). The samples with positive amplification were submitted to DNA sequencing of the B19V polymorphic region (VP1u-NS1 gene junction) using an in-house optimized nested-PCR with first round primers: forward JUN1 (5'GGACCAGTT CAGGAGAATCAT3') and reverse JUN2 (5'CCAGCTT GTAGCTCATTGC3') and second round primer pair: JUNNF (5'GACCAGTTTTCGTGAACTGTTAGT3') (forward) and JUNNR (5'CAGGCTTGTGTAAGTCTTCACT AG3') (reverse). The reaction conditions were the same for the first and the second reaction, except the cycle number (initial denaturation at 95 °C for 5 min and 35 cycles of initial denaturation at 95 °C/0:30 s, annealing at 55 °C/0:30 s and final elongation at 72 °C/1 min). The reaction was terminated with a final extension of 72 °C for 10 min. The second reaction included 40 cycles of PCR amplification. The DNA amplification products were run in 2 % agarose gel stained by GelRed (Biotinum, Hayward, CA, USA). Measures to prevent PCR contamination were strictly adhered to.

Positive results for B19V DNA were obtained from one volunteer blood donor (1.0 %; n = 1/91). He was a young male of 24 years of age and was clinically asymptomatic. He started his blood donation participation in 2011 and since then has been donating blood and platelets for eight times. At the time of the donation he was negative for HBV, HIV, HCV, HTLV, syphilis and American trypanosomiasis serological markers. Immunohematologically, the individual was blood group O positive and he never presented detection of irregular antibodies. He also had no alterations in the hematological parameters (blood picture with normal counts). B19V infection was tested in a technical duplicate and was characterized by a very low

DNA VL (537.36 viral copies/mL, Ct = 34.16). Due to the low VL, identification of the viral genotype by sequencing of a partial fragment of the VP1u-NS1 genomic region was not possible, although weak B19V amplification was observed (data not shown).

In this study, B19V DNA at a very low VL was detected in 1 % of the first time blood donors attended at the above mentioned Blood Transfusion Center in the Western Part of the São Paulo State, Brazil. This result is not divergent from our previous observations, where B19V DNA was detected at the same percentage in an almost the same number of tested blood donors [2]. This prevalence is also similar to the data obtained in the USA (0.88 %) [8]. Nevertheless, the observed prevalence was higher compared to countries like Belgium (0.2 %) [9] and China (0.58 %) [10] and lower compared to Dutch blood donors (2.5 %) [11]. Such a difference is probably due to the different sample volume used in the B19V testing, demographic characteristics of the populations or regional specifications of the B19V circulation (genotypes) in a given geographic region.

The detection of a very low B19V load in a clinically asymptomatic individual probably means a persistent course of the infection. In a prospective study, following 79 B19V-infected blood donors, in most of the cases persistent B19V DNA was detected at a very low VL (between 75 and 999 IU/mL), which is similar to our results. However, the mean duration of B19V viremia in the infected donors was 21.5 months (approximately 2 years), which demonstrates prolonged viral carriage [12]. Persistent low B19V DNA in blood donors has also been demonstrated by other authors [6, 13]. The VL detection variability in the different studies (including our findings), probably depends on the different sensitivity of the used diagnostic method. It seems that low levels of plasma B19V DNA did not affect clinically the infected blood donors [3, 12]. This was observed also in our study, where the donor with low B19V VL was clinically asymptomatic and with normal hematological parameters. Moreover, he has been regularly donating blood during the period of 2011–2014 (eight times) and has never been declined from blood donation due to clinical manifestation.

The presence of very low persistent VL in the blood donations seems not to affect the process of plasma manufacturing as normally, the presence of anti-B19V IgG seems to neutralize the virus. That is why, a cut off value for the acceptable B19V load for blood manufacturing has been established and it cannot surpass 10⁴ IU/mL [14]. In our case the VL was much below the established limit, and we believe that its influence in contamination of blood products is insignificant. However, depending on the clinical condition of the patients (or their anti-B19V IgG status) treated with hemoderivatives, B19V infection can have

serious consequences. This is especially true for patients with altered rates of the red cell substitution like sickle cell disease and beta-thalassemia, where the B19V infection can be fatal and evolve to extensive bone marrow necrosis (bone marrow fat embolism) [15]. Therefore, we believe that careful monitoring of the B19V DNA VL in the obtained blood products is plausible in order to avoid serious B19V complications in predisposed patients receiving hemotherapy. The presented report also adds to the global distribution of B19V infection among blood donors and the incidence of the asymptomatic infections.

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