Iranian Journal of Virology 2011;5(2): 9-12
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Original Article

Detection of Human Parvovirus B19 Markers in Blood Samples of Donors

Mahmoodian-Shooshtari M[1*], Sharifi Z[1]

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.

Abstract

Background and Aims: Human parvovirus B19, a member of the parvoviridae family, with single-stranded DNA is a very minute non-enveloped virus. B 19 virus is mostly transmitted via the respiratory tract but some studies have been reported which B19 virus can be transmitted through blood and/or blood products. The aim of this study was to evaluate the prevalence of B19 among blood donors in Tehran.

Methods: In this cross-sectional study, the collection of samples was performed in Tehran blood transfusion center for a period of 6 months, from March 2005 through August 2006.
Sera of 1640 blood donors who were negative for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBs Ag) and hepatitis C virus (HCV) were tested for immunoglobulin G (IgG) and immunoglobulin M (IgM) anti-B19 using Enzyme Linked Immunosorbent Assay (ELISA). Then, all of the sera were tested for presence of B19 DNA through semi-nested Polymerase Chain Reaction (PCR).

Results: Out of 1640 blood donors, 8 (0.5%) subjects had IgM antibody thereby being reported positive; 676 subjects (41.2%,) confidential intervals (CIs 95%= 42.7-50) were positive for anti-B19 IgG. B19 DNA was not found in any of the subjects (0%).

Conclusion: The result of this study showed that none of the blood donors had detectable parvovirus B19 DNA. This means that there was a very low risk of transmission of parvovirus B19 through blood or blood derived products. It is recommended that more blood samples to be studied specially in high risk groups.

Keywords: Parvovirus B19; Blood donors; Prevalence; Antibodies

Introduction

Human parvovirus B19 is a member of the parvoviridae family with single-stranded DNA. B19 is the smallest and non-enveloped virus (1). Human parvovirus B19 causes a number of clinical illnesses including infectious erythema (fifth disease), hydropes fetalis, transient aplastic crises, arthropathy and congenital aplasia (2).

B19 virus is mostly transmitted via the respiratory tract but some studies have been reported which B19 virus can be transmitted through blood and/or blood products (3).

In the production of blood products, current virus-inactivating steps seem to be ineffective to prevent transmission of parvovirus B19 (4). In particular, hemophilia patients receiving blood products on a regular basis are at risk of acquiring B19 infection (4, 5).

B19 as global infection is common in childhood, continues at a low rate throughout the life, and most people are sero-positive.

***Corresponding author:** Mahmoud Mahmoodian Shooshtari, PhD. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.
Tel: (+98) 21 88 6015 01-3, Fax: (+98) 21 88 60 15 55
Email: m.shooshtari@ibto.ir

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[DOI: 10.21859/isv.5.2.9]

Previous studies have shown that 40-60% of the world's adult population have antibodies specific for B19 and immunity to B19 infection depending upon previous exposure and the presence of neutralizing anti-B19 IgG antibodies, mainly directed to VP1 capsid proteins of the virus (6). IgM antibody against B19 lasts for a few months and IgG antibody persists for many years or lifelong.

It is still not clear whether screening for parvovirus measurment ofB19 IgG antibodies should be introduced for routine blood donors (7). There are some reports from different countries regarding the seroprevalence of B19 infection ranging from as low as 16.2% in Singapore, 32.8% to as high 80% in Japan, 75% in Belgium, 60% in England, 49% in America, 40-46.8% in Germany, and 64% in North Africa (8-12). However, in our country, a few seroepidemiological studies in adults or donor population are available. Therefore, the present study was designed to find the seroepidemiology of B19 in healthy voluntary blood donor's population in Tehran.

Methods

This cross-sectional study was performed by sample collection in Tehran blood transfusion center for a period of 6 months, from March 2005 to Aug 2006.

Sera of 1640 blood donors aged 17 to 65 years who were negative for HIV antibody (anti-HIV), Hepatitis B surface antigen (HBs Ag) and third generation HCV antibody (anti-HCV) were selected randomly.

Two milliliter of blood was taken from each blood donor and then centrifuged at 3500 g at room temperature. Sera were separated and transferred into fresh tubes. Serum samples were kept at-70 °C until uesd. IgM and IgG anti-B19 was determined using a commercially available ELISA (Enzyme Linked Immunosorbent Assay) (IBL, Hamburg, Germany©). The ELISA method were performed according to manufacturer instructions.

B19 DNA extraction

Serum samples were frozen at-70 °C before nucleic acid extraction procedure. B19 DNA was extracted from serum by "High Pure Nucleic acid Kit" (Roche Diagnostics High Pure Viral nucleic acid, Germany) according to manufacturer instructions and DNA was stored at-70°C.

Semi – nested PCR

25 µL reaction mixture containing 2µL of the DNA sample, 2.5 µl 10× PCR buffer with 1.5 mM $MgCl_2$, 0.5 µl 10 mM dNTPs, 0.5 µl of outer primer 10 pmol/µl, 0.3 µl Taq DNA polymerase (5 U/µl), and 15.5 µl nuclease free water was amplified in a thermal cycler. the cycling profile for the first round PCR consisted of 94 °C for 5 min, 30 cycles of 94 °C for 60 s, 58 °C for 30 s, and 72 °C for 60 s, followed by a final extension of 72 °C for 10 min and a final hold program of 4 °C.

The outer primers were NS1, NS2 (5'–ATT GCATAACAGACTTTGAGC-3 and 5'-CAGACTTTGAGCAGGTTATG-3').

For semi – nested PCR amplification, 23 µl PCR master mix containing the inner primers Vp1 and NS2 (5'-AGCATCAGGAGCTAT ACTTCC-3' and 5'-CAGACTTTGAGCAGG TTATG-3') and 2 µl of the first round PCR products was used for the second round PCR under the same condition.

PCR products were analyzed by gel electrophoresis on 1.5% agarose gel in TAE buffer. A single 717 bp band was showed after the second PCR.

Results

The specific antibody was tested in all 1640 blood samples and only 8 (0.5%) were positive for anti B19 IgM, while 676 (41.2%) were positive for anti B19 IgG (Table 1).

Representative semi- nested PCR and gel electrophoresis results of amplified B19 genome are shown in Fig 1, that all samples were negative for B19 DNA.

Discussion

Previous studies have shown that B19 virus can be transmitted through blood and/or blood products (13). Zakrzeswka et al, (13) showed 9 of 25 clotting products to be B19 DNA positive by PCR. They found B19 DNA in

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[DOI: 10.21859/isv.5.2.9]

Table 1. (a) Anti B19 Ig G positivity and (b) anti B19 IgM positivity in blood donors.

(a)

Age	Total no. of donors	Positive for B19 IgG (%)	Negative for B19 IgG (%)
17-65	1640	676 (41.2)	964 (53.7)

(b)

Age	Total no. of donors	Positive for B19 IgM (%)	Negative for B19 IgM(%)
17-65	1640	8 (0.5)	1632 (99.5)

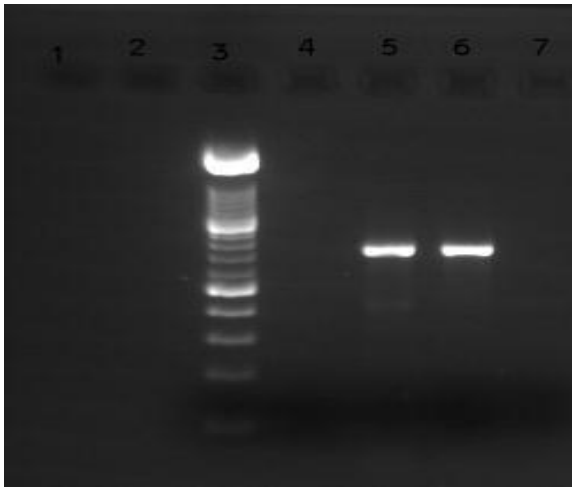


Fig. 1. Semi nested PCR product on 1.5% LE agarose gel electrophoresis results of amplified B19 genome. Columns 1 and 2 (negative controls), Column 3 (100 bp ladder molecular size marker), Columns 5 and 6 (717 bp bond amplified in positive samples), Columns 4 and 7 (donor serums).

low-purity non-inactivated blood product as well as in solvent detergent, steam-and dry-heat-treated products and also in monoclonally purified clotting factor concentrates. The significant difference in prevalence of B19 IgG between hemophiliacs and healthy persons demonstrates that there is a high risk of transmission of parvovirus B19 through plasma-derived clotting products (4).

In most of the countries, B19 virus infection occurs during childhood and by the age of 15 years, approximately 50% of children have anti B19 antibody. B19 prevalence may further increase during lifetime and reach values higher than 85% in elderly.

The prevalence in Japan was found to be 55% by ELISA using monoclonal antibodies (14). Our finding show that the seroprevalence of anti B19 IgG (41.2%, CI 95% = 42.7-50) is lower than the prevalence reported from developed countries such as Belgium (75%), England (60 %), Germany (48.8%), and America (49%). The explanation to this finding could be due to the fact is that in our study most of the blood donors were young. The mean age of the donors was 40 years and ranged from 17 to 65 years.

Mc Ornish *et al*, (16) studied the prevalence of parvovirus B19 viremia in blood donors. They found that 1:3300 donors were B19 DNA PCR positive, whereas during the seasonal outbreaks, 1:260 was viremic. The presence of parvovirus B19 DNA in 2,440 donated bloods from the United Kingdom and sub-Saharan Africa (Ghana, Malawi, and South Africa) was screened (16). Sensitive qualitative and real-time quantitative PCR assays revealed a higher prevalence of persistent infection with the simultaneous presence of immunoglobulin G (IgG) and viral DNA (0.55 to 1.3%) than previously reported (16).

In contrast with some other countries like Vietnam with high prevalence of Parvovirus B19 DNA (21.4%) (17), in our finding, we did not detect any positive sample for parvovirus B19 DNA in all samples. This means that there is a very low risk of transmission of parvovirus B19 through blood or blood derived products.

High prevalence of IgG in the blood products matching with the lack of any positive cases of B19 DNA indicates the low risk of transfusion of parvovirus B19. It is recommended that

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more blood samples to be studied specially in high risk groups.

Acknowledgment

This study was supported by the Blood Transfusion Research Center. We are grateful to High Institute for Research and Education in Transfusion Medicine for initiating and encouraging this program of research.

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[DOI: 10.21859/isv.5.2.9]