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ORIGINAL ARTICLE

Prevalence and genotypic characterization of human parvovirus B19 in hemophilia patients

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ABSTRACT

Background and Objectives: Parvovirus B19 (B19V) is usually transmitted through respiratory tract, but can also be received through blood transfusion. This study evaluated the seroprevalence, DNA existence, and circulating genotypes of B19V in hemophilia patients.
Materials and Methods: Serum samples of cases and controls were analyzed for B19V using ELISA and real-time PCR. Finally, obtained sequences were used for genotyping.
Results: Among cases, 3% were anti-B19V IgM positive and 47% were anti-B19V IgG positive and B19V DNA was detected in 16% of them. However, among controls, 38% were anti-B19V IgG positive (P>0.05) and 5% were B19V DNA positive (P= 0.019). Also ~13% of cases were positive and all of controls were negative for IgG avidity test (P= 0.029). Viral load in case group was higher than control group (P = 0.037).
Conclusion: Since hemophilia patients receive large amounts of blood factors, prevalence of B19V in these patients might be higher than normal subjects.

Keywords: Human parvovirus B19; Hemophilia; Seroprevalence; Blood transfusion; Blood disease; Iran

INTRODUCTION

Parvovirus B19 (B19V), a non-enveloped, single-stranded DNA virus with 25 nm diameter, is the only human pathogen of the family *parvoviridae*, genus *erythrovirus.* Respiratory system is the main route of transmission for parvovirus B19, while vertical transmission and blood products transfusion are other transmission routes (1). There are three clinical conditions proved to increase the susceptibility to severe consequences of B19V infection: (i) blood disorders, (ii) immunodeficiency, and (iii) pregnancy (2, 3).

As a hereditary bleeding disease, hemophilia patients need to constantly receive alternative products of factor VIII or IX (4). Despite the efficiency of coagulation factors for the reduction of morbidity and enhancement of survival among patients, treatment with factors derived from plasma is shown to increase the likelihood of B19V infection (5, 6). It has been demonstrated that the production of anti-B19V IgM antibody may be accompanied by the elevation in serum parameters that are typically observed in rheumatoid arthritis. Up to 17% of patients infected with B19V suffer from a chronic form of arthritis

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with clinical symptoms similar to rheumatoid arthritis (7, 8).

Regarding the complications of infection with B19V in hemophilia patients and the lack of sufficient data about this virus, this study was focused on exploring the seroprevalence and molecular characterization of B19V in these patients, thus paving the way for further epidemiologic studies.

MATERIALS AND METHODS

Patients and serological assay. This research was a case-control study conducted in Mashhad, Khorasan, Iran, from September 2018 to October 2019. A total of 100 serum samples of patients with Hemophilia and 100 serum samples from healthy subjects collected. Serum levels of anti-B19V IgM and IgG antibodies were measured using ELISA kit (Euroimmune AG, Lübeck, Germany). Moreover, IgG avidity test was performed on IgG positive samples using commercial ELISA kit (SERION ELISA AVIDITY, Germany). The study was approved by the Tehran University of Medical Sciences Ethics Committee (ethic code number: IR.TUMS.SPH.REC.1396.3267).

DNA extraction and PCR assays. The Real-Time PCR was carried out according to previous study (9). For genotyping B19V, positive samples for the consensus NS1-VP1u real-time PCR were sequenced. To acquire the whole sequence, the first PCR was carried out for 35 cycles of 30 sec at 95°C, 30 sec at 55°C, 1 min and 30 sec at 68°C, with, PVB-1 (5-CACTATGAAAACTGGGCAATAAAC-3), and B19SR (5-CCAGGCTTGTGTAAGTCTTC-3) primers. After that, the first-round product was amplified by PVB-3 and B19SR primers under similar reaction conditions used for the first round. Then, sequencing was performed using an ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA) and BigDye terminator cycle sequencing kit (Applied Biosystems) in accordance with the manufacturer's instructions. The B19V sequences were aligned using BioEdit, ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and a phylogenetic tree was made with Mega 7.0 and Kimura 2 parameter neighbor-joining method with 1,000 bootstrap (version 7, http://www.megasoftware.net). The reference sequences of genotype 1a were obtained from GenBank. The GenBank accession numbers for nucleotide sequences of the NS1-VP1u region of B19V reported in this study are ON334154 to ON334165.

Statistical analysis was carried out using the GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA). The two-tailed Student's t test was used to compare the variables. P values of <0.05 were considered statistically significant.

RESULTS

The age range of participants was 9 to 65 years old with an average of 33.7 ± 12.65. In the control group, all 100 participants (100%) were male and in the case group, 13 (13%) were female and 87 (87%) were male. 3 out of the 100 samples in the case group (3%) were IgM positive and 97 (97.9%) were negative. Also, of 100 samples in the control group, 100 (100%) were negative for B19V-IgM antibody. Comparison of these two groups in terms of anti-B19V IgM was not statistically significant (P= 0.2462). Evaluation of the relative frequency of B19V-IgG antibody revealed that 47 (47%) and 38 (38%) were positive in case and control groups respectively (P= 0.2524). Results of real-time PCR showed that B19V genome was detected in 16 (16%) and 5 (5%) participants in case and control groups respectively (P=0.0192). IgG Avidity test was performed to determine recent B19 infection in all 85 B19V-IgG positive samples. Of the 47 patients in the case group, 6/47 (12.76%) were positive and 41/47 (87.24%) were negative. Also, all of the 38 control samples (100%) were negative. The prevalence of anti-B19V IgG avidity was higher in case group (P = 0.0289) (Table 1).

To find out the relation between B19V prevalence and age, patients were categorized into four age groups including <11, 11-29, 30-49, and >49 years old and frequency of B19V-IgM, IgG and IgG avidity antibodies were determined in each age group (Fig. 1A). Our results showed a higher level of B19 viral load in case group compared to control group. In the case group, 6 patients had $\geq 10^6$ copies/ml, 2 patients had 10^5-10^6 copies/ml, and 7 patients had 10^4-10^5 copies/ml of B19 DNA. However, viral load in the control group (5 patients) was between 10^4 and 10^5 copies/ml. Comparison of viral load in the case and control groups was statistically significant (p = 0.037) (Fig. 1B).

12 PCR positive samples were sequenced for detection of a 926 bp fragment of NS1-VP1u region

Table 1. Frequency of anti-B19V IgM, anti-B19V IgG and anti-B19V IgG avidity in case and control groups

	Anti-B19V IgM		Anti-B19V IgG		Anti-B19V IgG avidity		B19V DNA	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Case	3%	97%	47%	53%	12.76%	87.24%	16%	84%
Control	0%	100%	38%	62%	0%	100%	5%	95%
P value	0.2462		0.2524		0.0289		0.0192	

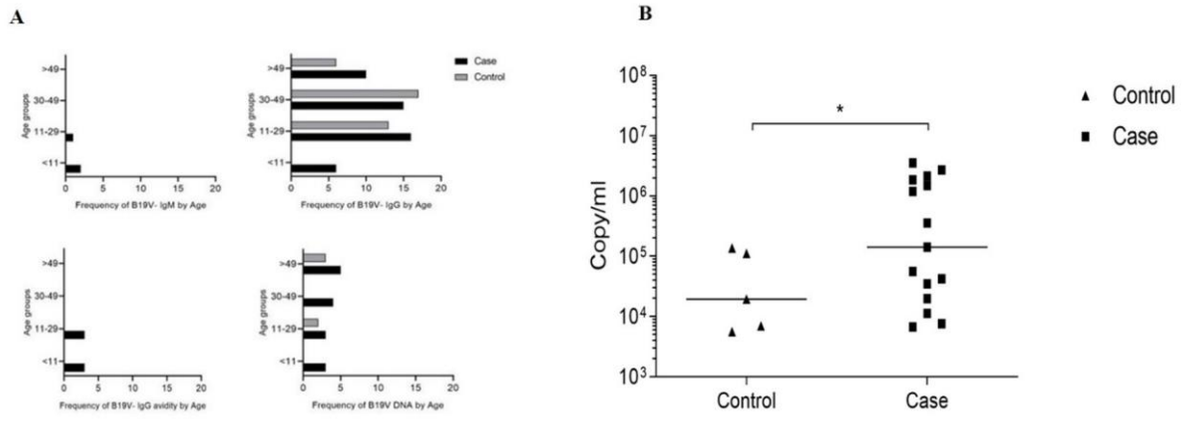

Fig. 1. (A): Overall frequency of B19V DNA and anti-B19V IgM, IgG, IgG avidity antibodies by age groups. (B): The mean viral load of B19V among case and control groups. * $P < 0.05$.

and genotyping was performed via a phylogenetic tree analysis with reference sequences obtained from GenBank. Sequences of our B19 isolates had 98-99% homology with genotype 1a sequences accessible in GenBank database and none of them clustered with other genotypes (Fig. 2).

DISCUSSION

Our study evaluated the prevalence and genotypic characterization of B19V in hemophilia patients compared to healthy blood donors. Our results documented that 16 (16%) patients were positive for the presence of virus genome, while 3 (3%) and 47 (47%) were positive for anti-B19V-IgM and -IgG antibodies, respectively. In the control group, 5 (5%) healthy subjects were positive for the presence of virus genome, while 0 and 38 (38%) were positive for anti-B19V-IgM and -IgG antibodies, respectively. The low prevalence of B19 IgM antibody in comparison to high prevalence of viral DNA in hemophilia patients could be due to the disease stage, because at the early stage of disease anti-B19V IgM antibody has not been produced or the IgM level is not detectable.

Although in many cases B19 infections are transmitted through the respiratory tract, the prevalence rate of B19V is completely different from country to country. The prevalence of B19 IgG in healthy controls in our study was lower than countries such as Belgium, Italy, Netherlands and Spain (8, 10, 11). This can be due to other factors including differences in genetics, geographic area, socioeconomic status (SES), age, and also the laboratory techniques applied for virus detection (12). For example, Abarca et al. (13), documented that the B19V seroprevalence in people with low SES is significantly higher than those with high SES, regardless of their ages. Moreover, surveys on patients with sickle cell disease (SCD) and betathalassemia have determined several variables like different epidemiological features, SES, overpopulation, immunological and hematological status and continual blood transfusions as factors affecting the B19V seroprevalence (14). However the most common finding among different studies is the increase of prevalence rate of B19V infection with age (15, 16). Our results indicated that serum anti-B19V IgG levels were increased in association with increased age. Our previous study also revealed that level of anti-B19V IgG increased with age in

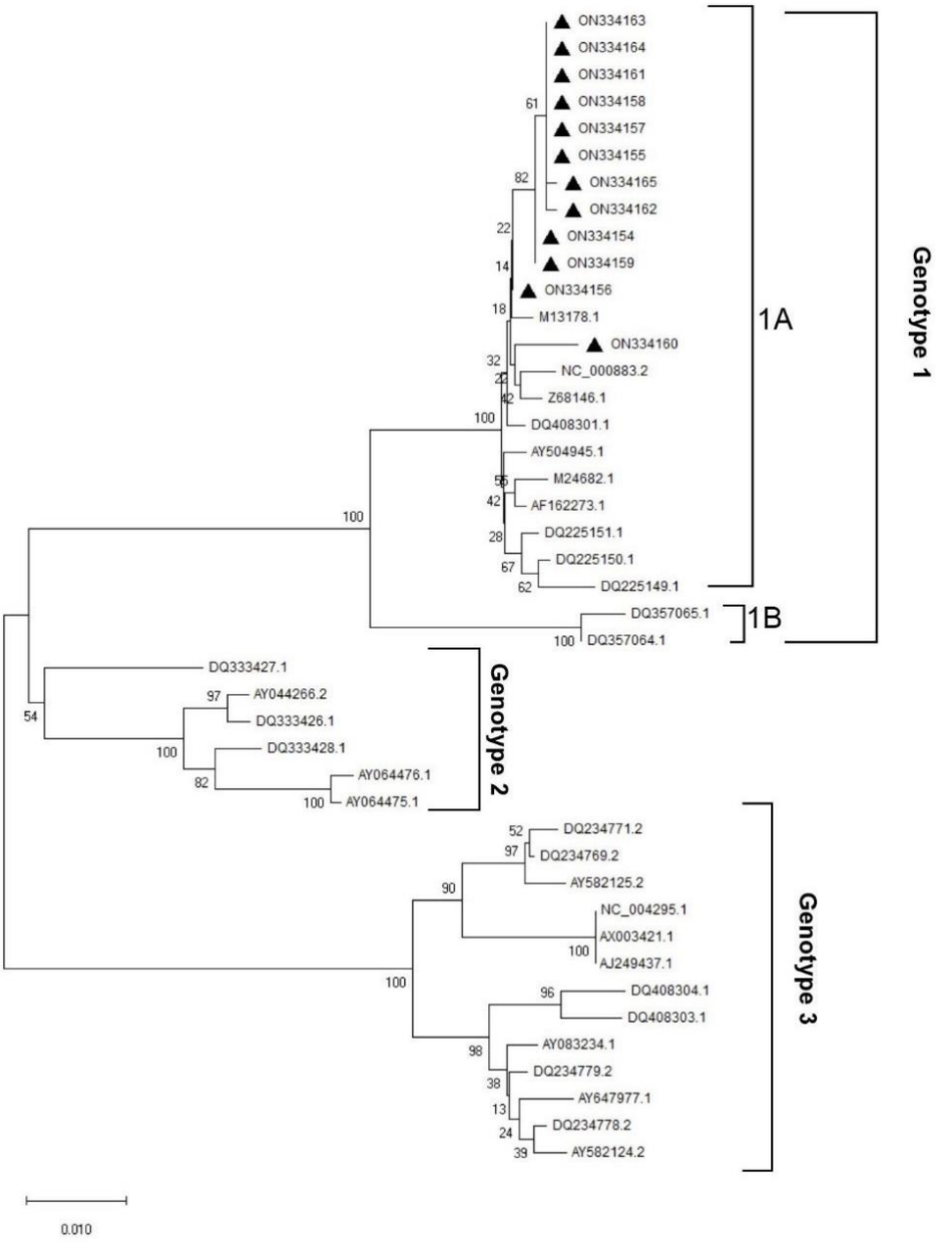

Fig. 2. Genetic relation between B19 viruses isolated from Iranian hemophilia patients compared to GenBank reference sequences. Mega 6.0 software and Kimura 2 parameter maximum likelihood method were used to draw a phylogeny tree based on NS1-VP1u area (926 bp). Genotype 1a was dominant in Iranian isolated strains (Black triangle).

measles- and rubella-like illness (9). Similarly, it has been demonstrated that the incidence of anti-B19V IgG/IgM in SCD and betathalassemia patients can increase by age (14). On the other hand, some studies have reported that the prevalence rate of B19V infection is increased with blood products transfusion (17). Also results of a study on Iranian blood donors represented an almost considerable B19V prevalence and viremia (18), which indicates high probability of transmission of B19V infection through blood transfusion. Different studies show that the prevalence of anti-B19V IgG in hemophilia patients is significantly higher than the healthy controls (19). Higher prevalence of anti-B19V IgG in hemophilia patients than healthy controls indicates a high risk of receiving B19V infection through plasma- and blood derived

products. Our study also showed a higher anti-B19V IgG and IgG avidity in hemophilia patients than controls however, only IgG avidity results were statistically significant. These results indeed, imply on higher abundance of recent B19V infections caused by frequent plasma and blood transfusions in hemophilia patients.

The geographical distribution of B19V genotypes is different from region to region. Our evaluation according to NS1-VP1u region of B19V DNA showed that the only circulating genotype in Mashhad city is the genotype 1a which was similar to another study in Iran (9). Actually, in Iran and many other countries only genotype 1 has been reported for B19V (20). Such strong dominance of one circulating genotype can facilitate monitoring B19V distribution pattern and also controlling the infection especially through new vaccine candidates.

CONCLUSION

We reported a high prevalence rate of B19V active infection (16%) in our hemophilia patients, as was showed by previous investigations. Despite clearance protocols used to clear blood and blood products from virus infections, there is still a high prevalence of B19V infections in hemophilia patients. Due to the severe complications caused by B19V infection, B19V screening methods and virus inactivation procedures for blood products are very essential.

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REFERENCES

1. Reinheimer C, Allwinn R, Doerr HW, Wittek M. Seroepidemiology of parvovirus B19 in the Frankfurt am Main area, Germany: evaluation of risk factors. *Infection* 2010; 38: 381-385.
2. Dobec M, Juchler A, Flaviano A, Kaeppeli F. Prolonged parvovirus b19 viremia in spite of neutralizing antibodies after erythema infectiosum in pregnancy. *Gynecol Obstet Invest* 2007; 63: 53-54.
3. Agi E, Hojjatipour S, Namvar A, Bolhassani A. Impact of blood transfusion on the prevalence of HHpgV-1, HPgV-1, and B19V among Iranian HCV-infected patients with Hemophilia. *J Pediatr Hematol Oncol* 2020; 42(4): e213-e218.
4. Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, Kitchen S, Llinas A, et al. Guidelines for the management of hemophilia. *Haemophilia* 2013; 19(1): e1-47.
5. Nikoozad R, Mahzounieh MR, Ghorani MR. Detection of parvovirus B19 infection in thalasemic patients in Isfahan province, Iran. *Jundishapur J Microbiol* 2015; 8(11): e26590.
6. Wu Cg, Mason B, Jong J, Erdman D, McKernan L, Oakley M, et al. Parvovirus B19 transmission by a high-purity factor VIII concentrate. *Transfusion* 2005; 45: 1003-1010.
7. Colmegna I, Alberts-Grill N. Parvovirus B19: its role in chronic arthritis. *Rheum Dis Clin North Am* 2009; 35: 95-110.
8. Lunardi C, Tinazzi E, Bason C, Dolcino M, Corrocher R, Puccetti A. Human parvovirus B19 infection and autoimmunity. *Autoimmun Rev* 2008; 8:116-120.
9. Rezaei F, Sarshari B, Ghavami N, Meysami P, Shadab A, Salimi H, et al. Prevalence and genotypic characterization of Human Parvovirus B19 in children with measles-and rubella-like illness in Iran. *J Med Virol* 2016; 88: 947-953.
10. Stelma FF, Smismans A, Goossens VJ, Bruggeman CA, Hoebe CJPA. Occupational risk of human Cytomegalovirus and Parvovirus B19 infection in female day care personnel in the Netherlands; a study based on seroprevalence. *Eur J Clin Microbiol Infect Dis* 2009; 28: 393-397.
11. Suárez González A, Otero Guerra L, De La Guerra GV, La Iglesia Martínez Pd PD, Solís Sánchez G, Rodríguez Fernández A. Varicella and parvovirus B19 immunity among pregnant women in Gijon, Spain. *Med Clin (Barc)* 2002; 119: 171-173.
12. He M, Zhu J, Yin H, Ke L, Gao L, Pan Z, et al. Human immunodeficiency virus/human parvovirus B19 co-infection in blood donors and AIDS patients in Sichuan, China. *Blood Transfus* 2012; 10: 502-514.
13. Abarca K, Cohen BJ, Vial PA. Seroprevalence of parvovirus B19 in urban Chilean children and young adults, 1990 and 1996. *Epidemiol Infect* 2002; 128: 59-62.
14. Soltani S, Zakeri A, Tabibzadeh A, Zandi M, Ershadi E, Rezayat SA, et al. A literature review on the parvovirus B19 infection in sickle cell anemia and β-thalassemia

patients. *Trop Med Health* 2020; 48: 96.
15. Siritantikorn S, Kaewrawang S, Siritanaratkul N, Theamboonlers A, Poovorawan Y, Kantakamalakul W, et al. The prevalence and persistence of human parvovirus B19 infection in thalassemic patients. *Asian Pac J Allergy Immunol* 2007; 25: 169-174.
16. Drago F, Ciccarese G, Broccolo F, Javor S, Parodi A. Atypical exanthems associated with parvovirus B19 (B19V) infection in children and adults. *J Med Virol* 2015; 87: 1981-1984.
17. Slavov SN, Kashima S, Pinto ACS, Covas DT. Human parvovirus B19: general considerations and impact on patients with sickle-cell disease and thalassemia and on blood transfusions. *FEMS Immunol Med Microbiol* 2011; 62: 247-262.
18. Zadsar M, Aghakhani A, Banifazl M, Kazemimanesh M, Tabatabaei Yazdi SM, Mamishi S, et al. Seroprevalence, molecular epidemiology and quantitation of parvovirus B19 DNA levels in Iranian blood donors. *J Med Virol* 2018; 90: 1318-1322.
19. Azzi A, Manaresi E, Zakrzewska K, De Santis R, Musiani M, Zerbini M. Antibody response to B19 parvovirus VP1 and VP2 linear epitopes in patients with haemophilic arthritis. *J Med Virol* 2004; 72: 679-682.
20. Lavrent'eva IN, Antipova AI, Semenov AV, Bichurina MA. Genotyping of parvovirus B19 isolates circulating in Northwestern Federal District of Russia. *Zh Mikrobiol Epidemiol Immunobiol* 2013; (6):36-43.