



# Human parvovirus B19 and parvovirus 4 among Iranian patients with hemophilia

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## Background

Human parvovirus B19 (B19V) is one of the smallest DNA viruses and shows great resistance to most disinfectants. Therefore, it is one of the common contaminant pathogens present in blood and plasma products. Parvovirus 4 (PARV4) is a newly identified parvovirus, which is also prevalent in parenteral transmission. In this study, we aimed to evaluate the prevalence of B19V and PARV4 DNA among patients with hemophilia in Birjand County in eastern Iran.

## Methods

This was a cross-sectional epidemiological study comprising nearly all people with hemophilia in this region. Whole blood samples were taken after patient registration and sent for plasma isolation. After nucleic acid extraction, B19V was detected with real-time polymerase chain reaction, PARV4 DNA was then detected using sensitive semi-nested PCR.

## Results

In total, there were 86 patients with hemophilia, with mean age  $28.5 \pm 1.5$  years. Of these, 90.7% were men and 9.3% women; 84.9% had hemophilia A and 7.0% had hemophilia B. We found 11 patients (12.8%) were positive for B19V DNA and 8 were positive (9.3%) for PARV4 DNA. The prevalence of B19V was higher in middle-aged groups rather than younger people, whereas PARV4 infection was more common in younger patients ( $P < 0.05$ ).

## Conclusion

There was a high prevalence of B19V and PARV4 infection in this high-risk group of patients with hemophilia. Due to the clinical significance of the B19 virus, imposing more precautionary measures for serum and blood products is recommended.

**Key Words** Parvovirus B19, PARV4, Hemophilia, Prevalence, Iran

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## INTRODUCTION

Human parvovirus B19 (B19V), the smallest human DNA virus, belongs to the *Erythroparvovirus* genus within the Parvoviridae family [1]. B19V is a non-enveloped single-stranded DNA virus with three distinct genotypes that are distributed differently around the world [2]. As a causative agent of globally prevalent infection, B19V is mainly transmitted via the respiratory route; it can also be trans-

mitted through blood products and vertical transmission [3, 4]. The most important clinical diseases related to B19V infection are erythema infectiosum (fifth disease), hydrops fetalis, transient aplastic crisis, arthropathy, and congenital aplasia [5].

Hemophilia is a hereditary bleeding complication with spontaneous bleeding into joints that leads to arthritis and that requires continuous injection of factor VIII or IX alternative products [6]. Although the use of coagulation factors can lead to improved morbidity and survival in patients

with hemophilia, there is still a risk of B19V infection by continuous administration of plasma-derived factors [7]. Common inactivation procedures remove enveloped viruses but do not appear to effect B19V owing to its characteristics of greater resistance to physicochemical agents and having such a small non-enveloped capsid [8].

Due to the high prevalence of B19V in the general population [9] and the large amount of blood donations that are used in the production of plasma-derived factors, there is a risk of contamination in these products [10]. Given that development of IgM anti-B19V may be associated with arthritis in young people and adults [11], joint illness and arthritis could be important B19V-related clinical issues in hemophilia [12]. In addition, synovial bleeding and joint trauma that occur among people with hemophilia could lead to transmission of circulatory viruses to the synovium and establish a persistent synovial infection with B19V [13].

PARV4 is another member of the Parvoviridae family, which has been discovered recently [14]. PARV4 has 30% sequence diversity with other viruses of its family and does not have a close relationship with other family members; that may, in fact, put PARV4 in a new group within the Parvoviridae family [15]. To date, this new virus has been detected in pooled plasma, blood products, autopsy samples, the bone marrow, and the liver [16]. High prevalence of PARV4 has been reported in high-risk groups such as patients positive for human immunodeficiency virus, hepatitis B virus, and hepatitis C virus (HCV) [17]. Nevertheless, clinical outcomes of PARV4 infection in the above mentioned coinfections remain unclear [18]. PARV4 infection has been reported to be associated with encephalitis [19], however, and may cause manifestations such as pharyngitis, arthralgias, and vomiting [14].

Considering the risk of B19V as well as PARV4 infections for patients with hemophilia, and the absence of clear data regarding these viruses in this population, this study aimed to determine the prevalence of B19V and PARV4 viral DNA in this high-risk group for further application in epidemiologic studies and to assist with effective health-related decision-making. To the best of our knowledge, this is the first report on the B19V and PARV4 viremia among patients with hemophilia in Iran.

## MATERIALS AND METHODS

### Patients and samples

This work was a cross-sectional study conducted in coordination with the Hemophilia Society of Southern Khorasan province in the city of Birjand, Iran, from September 2015 to October 2016. We enrolled 86 patients who had hemophilia and were receiving plasma-derived coagulation factors as participants in this study. The study procedures were explained to all patients and after signing a written consent form, participants completed a questionnaire. Serum samples were then taken and stored at -70°C in the Birjand University of Medical Sciences central lab. Specimens were subsequently

subjected to DNA extraction using a Viral Nucleic Acid Extraction Kit (Yekta Tajhiz Azma, Iran). The extracted DNA was transported to the virology laboratory of Iran University of Medical Sciences for further molecular analysis. Demographic and clinical data of patients were kindly provided by the Hemophilia Society; the confidentiality of patient information was strictly maintained. The study was approved by the Iran University of Medical Sciences Ethics Committee (ethic code number: IR.IUMS.Res. 1395.94-03-30-26536).

### Real-time PCR analysis for B19V

To detect B19V DNA in the serum of patients with hemophilia, a sensitive real-time PCR test was used to amplify a 154-base pair (bp) fragment of the NS1 gene. The primer and hydrolysis probe were as follows: B1F (5'-CCACTA-TGAAAACCTGGGCAATA-3'), B1R (5'-GCTGCTTTCAGT-AGTTCTTCA-3') and probe 5'-FAM-AATGCAGATGCCCTCCACCCAG-TAMRA-3' [20]. The reaction mixture contained 7.5 µL of 2X qPCR master mix for probe (Yekta Tajhiz Azma, Iran), 0.5 µM each of forward and reverse primers, 0.2 µM probe, and 3 µL of template and nuclease-free water in a reaction volume of 15 µL. The amplification cycle was conducted using a QIAGEN real-time PCR cycler (Rotor-Gene Q 2plex Platform, QIAGEN GmbH, Germany) with a two-step method, including a 95°C hold for 10 min and then 35 cycles of 95°C for 20 sec and 62°C for 30 sec.

### PCR test for PARV4

A semi-nested PCR test was performed to identify the ORF2 of PARV4 DNA. The primers were as follows: Seq1 (5'-CCGGAACCTTCAAGTCAAGCCA-3') and Seq2 (5'-CCGCTCAAGGTCTGGTTCAACAA-3') in the first step, producing a 750-bp product, followed by Seq1 and Seq3 (5'-CAAGGTGGACTCCGACATCTGG-3'), producing a 490-bp product [21]. The PCR reaction contained 12.5 µL of PCR 2X master mix (Yekta Tajhiz Azma, Iran), 0.5 µM each of Seq1 and Seq2 primers, and 25 ng of template to a total volume of 25 µL using sterile nuclease-free water. The PCR program began with a predenaturation step at 94°C for 7 min and then 32 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min, and a final extension step of 10 min to guarantee production of full-length strands. A total of 3 µL of the first-step PCR product was used as a template for the semi-nested run using Seq1 and Seq3 primers under the same reactions and cycling conditions as the first stage, apart from annealing temperature, which was changed to 60°C.

### Statistical analysis

All demographic, clinical and experimental data were analyzed using Statistical Package for the Social Sciences SPSS version 16 (SPSS Inc., Chicago, IL, USA). The frequency of qualitative variables and differences between groups were compared using Fisher's exact test and independent *t*-tests, respectively. *P*-values less than 0.05 were considered statistically significant.

**RESULTS**

**Baseline population data**

Serum samples were collected from September 2015 to October 2016 from the included 86 patients with hemophilia. The clinical and demographic data of participants are given in Table 1. In summary, the mean age of patients was 28.5±1.5 years, with a range of 5–48 years; of these, 90.7% of patients were men and 9.3% women. A total 40% of participants were under 20 years of age and were mainly students. Hemophilia A was the most prevalent (84.9%) disease among our patients; hemophilia B (7%) and other forms of bleeding disorders were rare. Anti-HCV was detected in 10.5% of

**Table 1.** Demographic, clinical, and virological characteristics of patients with hemophilia.

	N (%)	Anti-HCV N (%)	B19 N (%)	PARV4 N (%)	P
Gender					0.237
Male	78 (90.7)	9 (11.5)	10 (12.8)	7 (9.0)	
Female	8 (9.3)	0	1 (12.5)	1 (12.5)	
Hemophilia type					0.064
A	73 (84.9)	9	10 (13.7)	6 (8.2)	
B	6 (7.0)	0	1 (16.7)	2 (33.3)	
Other	7 (8.1)	0	0	0	
Severity <sup>a)</sup>					0.05
Severe	38 (44.2)	4	3 (7.9)	3 (7.9)	
Moderate	34 (39.5)	3	7 (20.6)	4 (11.8)	
Mild	14 (16.3)	2	1 (7.1)	1 (7.1)	
Age (yr) <sup>b)</sup>					0.02
≤10	13 (15.1)	0	1 (7.7)	2 (15.4)	
11–20	23 (26.7)	0	2 (8.7)	3 (13.0)	
21–30	17 (19.8)	3 (17.6)	3 (17.6)	2 (11.8)	
31–40	13 (15.1)	3 (23.1)	4 (23.1)	1 (7.7)	
41–50	13 (15.1)	1 (7.7)	1 (7.7)	0	
>50	7 (8.1)	2 (28.6)	0	0	
Total	86 (100.0)	9 (10.5)	11 (12.8)	8 (9.3)	

<sup>a)</sup>There was a significant difference in B19V and PARV4 according to severity of hemophilia, between the moderate and mild groups.

<sup>b)</sup>There was a significantly greater prevalence of B19V among participants aged 20–40 years, but a significant PARV4 prevalence in those aged <20 years, compared with other age groups.

Abbreviations: B19V, human parvovirus B19; HCV, hepatitis C virus; N, number; PARV4, human parvovirus 4.

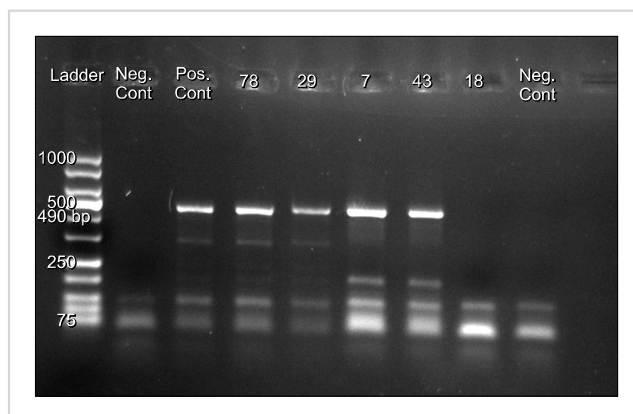
patients.

**Detection of B19V DNA**

The results of real-time PCR for B19V revealed that 11 patients (12.8%) were positive for B19V DNA. The mean age of positive patients was 28.8±2.4 years with an age range of 9–45 (Table 2). The prevalence of B19V active infection was found mostly among participants aged 31–40 years (23.1%) and 21–30 years (17.6%) (Table 1). Differences between the age groups of 20–40 years and less than 20 years were significant ( $P < 0.05$ ). Based on serologic data, 9 patients (10.5%) were seropositive for HCV, of which 2 (22.22%) were infected with B19V; this was much higher than seronegative patients (11.7%). Based on the severity of hemophilia, the prevalence of B19V was more predominant in moderate forms (20.6%) than in mild and severe forms of hemophilia (7.14% and 7.9%, respectively), with a significant difference ( $P \leq 0.05$ ). With respect to the type of hemophilia, most patients had hemophilia A (84.9%), including 13.7% with B19V infection.

**Detection of PARV4 DNA**

According to the results of PCR (Fig. 1), 8 patients (9.3%) were positive for PARV4 DNA. Patients who were positive for PARV4 had a mean age 24.5±1.3 years, with a range of 5–40 years. We observed a unique age range pattern compared with B19V: PARV4 infection was more prevalent in young people and declined with age. Just two patients—a



**Fig. 1.** Result of PCR for PARV4 produced fragments of 490 base pairs.

**Table 2.** Characteristics of patients positive for B19V and PARV4.

	B19 positive	PARV4 positive	Total
N (%)	11/86 (12.8)	8/86 (9.3)	17/86 (19.8)
Age (yr, mean±SD)	28.8±2.4	24.5±1.3	26.7±1.8
Male/female	10/1	7/1	15/2
N of anti-HCV + (%)	2/11 (18.2)	1/8 (12.5)	3/17 (17.6)
B19V & PARV4 coinfection, N (%)	2/11 (18.2)	2/8 (25.0)	2/17 (11.8)

Abbreviations: B19V, human parvovirus B19; HCV, hepatitis C virus; N, number; PARV4, human parvovirus 4.

32-year-old woman and a 19-year-old man—were coinfecting with B19V and PARV4. The prevalence of PARV4 was higher among people with moderate hemophilia (11.8%). Although there were fewer patients with hemophilia B (7%) than type A (84.9%), interestingly, the positive rate for PARV4 was more noteworthy in type B (33.33%) than type A (8.2%); however, the difference was not statistically significant ( $P > 0.05$ ). In patients who were seropositive for HCV, the rate of PARV4 infection was 1/9 (11.11%), and it was 7/77 (9.1%) in seronegative patients.

## DISCUSSION

Hemophilia is a condition that requires continuous administration of coagulation factors to prevent spontaneous bleeding, and this process makes people with the disease vulnerable to blood-borne pathogens [22]. Parvoviruses have a small capsid without a lipid envelope layer; as a result, they are resistant to several viral clearance procedures used to process plasma pools [23]. PARV4 is a parvovirus that has been recently identified, which has been reported in various high-risk groups as well as in the general population [24].

The result of the present study showed 12.8% prevalence of B19V DNA and 9.3% of PARV4 DNA. A similar work in South Africa revealed an 8% prevalence of B19V infection among 26 patients with hemophilia [8], which is a lower rate than that of the current study; however, the sample size in the South African study was very small. Slavov in Brazil demonstrated that active infection of B19V was as high as 35.7% among 28 patients [25]. This difference in reported rates of B19V viremia may be partly related to sample size and also to other risk factors in some populations, such as coinfection with HCV, which was 10.7% in the study by Slavov. In the current study, the seroprevalence of HCV was 10.5%, and the rate of B19V infection among these HCV-seropositive patients was 22.22%. This finding indicates shared routes of transmission for HCV and B19V, as well as shared risk factors.

It has been shown that the seroprevalence of B19 among hemophilia patients in different countries is very high. A surveillance study among 1,043 young patients with hemophilia established that they were 1.7 times more prone to have antibodies against B19 compared with people who were unexposed to blood or blood products [12]. Another work demonstrated an odd ratio of 7.6 among those who received plasma-derived factors [6]. In a population with hemophilia in Japan, the seroprevalence of B19 IgG was 100% and B19 DNA was found in 7.5% of patients [26].

It has been reported that the seroprevalence of B19 IgG was 74% among 180 patients with hemophilia in the Shiraz district of Iran [27]. Although these high seroprevalence rates represent previous infections, there was also a moderately high rate of active infection in previous studies, as in the results of our work, indicating that receipt of coagulation factors remains a risk factor for B19V.

The novel PARV4 is a prevalent blood-borne pathogen,

as earlier studies have demonstrated. In the current work, there was a 9.3% prevalence of PARV4 DNA. In a serologic screening study for PARV4, 55% of patients with hemophilia were seropositive [28]; another work revealed 44% prevalence with an incidence rate of 1.7% per year [29]. This high rate of seroprevalence is proportional to the moderately high rate of active infection obtained in the current study.

In this study, the rate of active infection with B19V was significantly higher in middle aged patients (20–40 yr); this may be related to greater exposure to environmental factors as well as receiving more coagulation factors than older patients. Interestingly, the figure for PARV4 was higher in younger age groups (under 20 yr), possibly because of recently contaminated resources.

Another finding of this study showed that the rate of these viruses in patients with severe hemophilia was greater than in those with milder forms of the disease, although the rate was higher for moderate hemophilia. This finding supports the hypothesis of virus transmission through regular receipt of plasma-derived factors. It should be noted that the results of the current study are subject to some limitations, such as small sample size and absence of a control group.

In conclusion, we found a moderately high prevalence of active infections of PARV4 and B19 viruses (9.3% and 12.8%, respectively) among people with hemophilia, as in previous studies that have also pointed out high seroprevalence of these pathogens. Despite nucleic acid testing and clearance procedures for blood products, there is still a high prevalence of B19V infections among patients with hemophilia. It has not been determined that these sorts of infections occur solely through receiving plasma-derived factors; therefore, further investigation is needed. The occurrence of B19 infection in patients with hemophilia may lead to production of autoantibody responses that are related to joint lesions. Thus, there is a need for more preventive measures in B19 virus screening methods and virus inactivation procedures for pooled plasma.

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## Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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