Original Article

Access this article online

Quick Response Code:

Website: https://journals.lww.com/ijhm

DOI: 10.4103/ijh.ijh_19_25

Serological and molecular detection of parvovirus B19 in hereditary bleeding disorder patients

Huda Ibraheem Abd Al-Lateef, Shahla'a Fadhil Sabir, Mushtaq Mufleh Khazeem, Nidal Karim Maroof Al-Rahal, Marwa L. Jabur[1]

Abstract:

BACKGROUND: Hemophilia and other hereditary bleeding disorders patients who received blood and blood product transfusions are particularly vulnerable to viral infection including risk of transmission of parvovirus B19, which remains a critical issue.

OBJECTIVES: The aim of this study was to screen Parvovirus B19 infection in hereditary bleeding disorders patients.

PATIENTS, MATERIALS, AND METHODS: Sixty-one patients diagnosed with hemophilia and other hereditary bleeding disorders were recruited in this study. Out of 61 patients, 73.77% had hemophilia A, 13.11% had hemophilia B, 6.56% had von Willebrand disease, and 6.56% had congenital factor VII deficiency. Patients' clinical data were collected and evaluated during their follow-up visits. The presence of inhibitors in patients with hemophilia A was also recorded. Detection of parvovirus B19 DNA was performed using nested polymerase chain reaction protocol, and immunoglobulin G (IgG) detection was done using the enzyme-linked immunosorbent assay technique. Statistical analysis was performed using GraphPad Prism software (v7.0).

RESULTS: Despite the lack of significance, more than half (55.74%) of different types of hereditary bleeding disorders (34 patients) were seropositive for Parvo B19 IgG antibodies, while all patients had negative parvovirus B19 DNA. Additionally, 15 (33.33%) patients with hemophilia A had inhibitors to factor VIII, without increased association among those patients with positive IgG antibodies ($P > 0.9999$). Furthermore, the prevalence of hepatitis C virus and hepatitis B virus among all studied patients was 33 (54.1%) and 3 (4.92%), respectively, without signification association with a positive serological test of Parvo B19 virus ($P = 0.0621$ and $P = 0.6959$, respectively).

CONCLUSIONS: This study revealed that most patients had past exposure to Parvovirus B19, as indicated by IgG seropositivity, while the absence of detectable viral DNA suggests no current infections which underscore the likelihood of past transmission via blood or blood derived products. Therefore Regular monitoring of viral infections in hereditary bleeding disorder patients are crucial for improving infection control measures.

Keywords:

Hemophilia, hereditary bleeding disorder, immunoglobulin G, inhibitors, parvovirus B19

The National Center of Hematology, Mustansiriyah University, [1]Department of Clinical Laboratory Science, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq

Address for correspondence:
Dr. Shahla'a Fadhil Sabir, The National Center of Hematology, Mustansiriyah University, Baghdad, Iraq.
E-mail: shahlaa.fadhil@uomustansiriyah.edu.iq

Submission: 03-03-2025
Revised: 11-04-2025
Accepted: 12-04-2025
Published: 29-05-2025

Introduction

Inherited bleeding disorders represent a broad range of conditions that influence platelets, clotting factors, or the blood vessel wall, with clinical presentation varying based on the specific underlying cause.[1] A deficiency in coagulation factor VIII or IX results in hemophilia A and hemophilia B consequently, an inherited bleeding disorder linked to the X chromosome.[2] Around 80%–85% of the hemophilia population

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Al-Lateef HI, Sabir SF, Khazeem MM, Al-Rahal NK, Jabur ML. Serological and molecular detection of parvovirus B19 in hereditary bleeding disorder patients. Iraqi J Hematol 2025;14:117-22.

is hemophilia A, which represent more common hemophilia than hemophilia B.[3] About 70% of those with hemophilia have a severe form according to the serum level of the coagulation factor.[4]

Due to the high risk of fatal bleeding in hemophilia before effective treatments emerged, its close link with transfusion medicine is understandable.[5] The life expectancy of persons with inherited bleeding disorders varies, depending on whether patients receive appropriate treatment. Notably, with proper care and treatment, patients with hemophilia now have a life expectancy close to that of the normal population or a little bit less.[6] However, management of inherited bleeding disorders with recombinant coagulation factor concentrates nowadays becomes more popular, but one of the most serious complications of treatment is the emergence of inhibitory antibodies against transfused clotting factors, with persistent concerns regarding the immunogenicity of different concentrates type.[7] Still giving blood products (frozen source plasma and cryoprecipitate) is useful when no recombinant products are available.[8] However, the administration of pooled plasma transfusion in the 1970s and 1980s resulted in many hemophilia patients contracting blood-borne infections such as HIV and hepatitis C.[9]

Infected blood donated by seemingly healthy and asymptomatic individuals can transmit a range of blood-borne pathogens through transfusion. The range and specifics of viral serological screening tests are used to screen individual donors, but the risk of false-negative results may still occur, resulting in the transmission of blood-borne pathogens.[10] The human parvovirus B19 is a nonenveloped human DNA virus that spreads from person to person, often through the respiratory system, and can also be transmitted via blood, with the risk of infected pregnant women potentially passing the virus to their babies.[11] The risk of iatrogenic transmission of parvovirus B19 via blood products is feasible because the primary infection often results in high viremia levels, with asymptomatic individuals often having over 10^{12} geq/mL in their blood during the early phase of acute infection.[12] Blood and plasma donations are frequent sources of Parvo B19 virus contamination, and the virus has been transmitted through medicinal products derived from plasma.[13] Therefore, this research assesses the prevalence of Parvo B19 virus infection among individuals with hemophilia and other hereditary bleeding disorders.

Patients and Methods

This cross-sectional prospective study was carried out from October 2020 to August 2022. Sixty-one patients diagnosed with different hereditary bleeding disorders were recruited in this study. All patients have been diagnosed and registered at the National Center of Hematology and were evaluated through their follow-up visits to the center. Patients' clinical data included age, sex, type of hereditary bleeding disorder, history of blood product transfusion, and any surgical intervention. The results of inhibitors testing (Bethesda units) were also recorded for patients with hemophilia A. Patients with any history of malignancy are excluded.

Hepatitis C virus (HCV) antibody and hepatitis B surface antigen (HBsAg) results were obtained from patients' records.

Ethics approval (Reference: nch-erc-20-23) was granted from the Scientific Ethical Committee of The National Center of Hematology/Mustansiriyah University, in accordance with the principles of the Declaration of Helsinki, and informed consent was obtained from the patients and/or one of the parents of pediatric patients.

Sample collection

Five milliliters of venous blood was withdrawn from each participant, 3 ml dispensed in a gel tube and centrifuged for 15 min at 3000 rpm. Then, separated sera were stored frozen at −20°C for the determination of parvovirus B19 immunoglobulin G (IgG). 2 ml was dispensed in an ethylenediaminetetraacetic acid (EDTA) tube for the total genomic DNA extraction.

Viral DNA extraction

Viral DNA was isolated from EDTA blood samples of patients using the WizPrep Viral DNA/RNA Mini Kit (V2) (WizBiosolution, Korea) in accordance with the manufacturer's guidelines. The extracted viral DNA samples were stored at −20°C until use.

Nested polymerase chain reaction for B19 DNA detection

Detection of parvovirus B19 DNA was performed using nested polymerase chain reaction (PCR) protocol as described previously by Zerbini *et al*.[14] with modification. Briefly, the protocol involves a two-round PCR reaction. The first round produces an 1112 bp product, and 2 µl

Table 1: Primers used in nested polymerase chain reaction

	Oligonucleotide sequences	Product size (bp)
First round primers	5'-CTTTAGGTATAGCCAACTGG-3' 5'-ACACTGAGTTTACTAGTGGC-3'	1112
Second round primers	5'-CAAAAGCATGTGGAGTGAGG-3' 5'-CCTTATAATGGTGCTCTGGG-3'	104

Table 2: The polymerase chain reaction protocol used for amplification

Round of nested PCR	Step	Temperature (°C)	Time (min)	Number of cycles
First round	Initial denaturation	95	5	1
	Denature	95	1	35
	Anneal	56	1.5	
	Extension	72	1	
	Final extension	72	5	1
Second round	Initial denaturation	95	5	1
	Denature	95	1	35
	Anneal	56	1.5	
	Extension	72	1	
	Final extension	72	5	1

PCR=Polymerase chain reaction

of this product is then used in the second round, which yields a 104 bp product. Primers used in the two rounds are shown in Table 1.

Amplification was performed using the Thermal Cycler C1000 (Bio-Rad, USA). The PCR protocol used for amplification is summarized in Table 2.

Five microliters of second-round PCR amplification was analyzed using 2% agarose gel electrophoresis. Visualization of bands was performed using RedSafe DNA stain (Nippon Genetics, Germany).

Parvovirus B19 immunoglobulin G detection

Enzyme-linked immunosorbent assay technique was used to detect parvovirus B19 IgG using (Demeditec Diagnostics, Germany) kit according to instructions of the company for the qualitative determination of IgG-class antibodies to parvovirus B19 in humans.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (version 7.0; GraphPad Software, San Diego, CA, USA). Mean, standard deviation, and other descriptive statistics were calculated using column analysis. For categorical data, contingency table analysis was performed using Chi-square or Fisher's exact tests. For significance threshold, $P < 0.05$ was considered statistically significant.

Results

Sixty-one patients were recruited in this study, and the mean age was 27 ± 12.06 years. The male: female (M:F) ratio was 9.1:1, comprising 90.2% of males and 9.8% of females.

Among the 61 patients, the distribution of bleeding disorders was as follows: 73.77% had hemophilia A, 13.11% had hemophilia B, 6.56% had von Willebrand disease, and 6.56% had congenital factor VII deficiency.

Table 3: Demographic, clinical parameters, and virological characteristics of patients

Characteristics	Patients (*n*=61), *n* (%)
Age, mean±SD	27±12.06
Sex	
Male	55 (90.2)
Female	6 (9.8)
Male:female ratio	9.1:1
Hemophilia type	
A	45 (73.77)
B	8 (13.11)
VWD	4 (6.56)
Congenital (factor VII deficiency)	4 (6.56)
Hemophilia type A patients treated with VIII	34 (75.55)
Formation of inhibitors in type A hemophilia patients	
Yes	15 (33.33)
No	30 (66.67)
Patients with blood transfusion	
Yes	39 (63.93)
No	22 (36.07)
Patients with surgery	
Yes	16 (26.23)
No	45 (73.77)
Viral screen (IgG)	
Parvovirus B19 (IgG)	
Yes	34 (55.74)
No	27 (44.26)
HCV	
Yes	33 (54.1)
No	28 (45.9)
HBsAg	
Yes	3 (4.92)
No	58 (95.08)
Parvovirus B19 DNA	
Negative	61 (100)

HCV=Hepatitis C virus, HBsAg=Hepatitis B surface antigen, SD=Standard deviation, VWD=Von Willebrand disease

Fifteen (33.33%) of studied hemophilia A patients had inhibitors to factor VIII.

Thirty-nine patients (63.93%) had received blood product transfusions at least once during their lives. Additionally, 16 (26.23%) patients had a past history of surgical intervention. The positive viral screen for the included patients was as follows: 34 (55.74%) parvovirus B19 (IgG), 33 (54.1%) HCV-Ab, and 3 (4.92%) HBsAg. Parvovirus B19 DNA screen was negative for all patients included. The patient's characteristics and parameters are shown in Table 3.

Table 4 shows that there was no significant difference regarding parvovirus B19 virus status among the studied parameters. Despite the lack of significance, more than half of the different types of patients were seropositive for parvovirus B19 IgG.

Table 4: Association of human parvovirus B19 infection status profile and demographic, clinical parameters, and virology screen characteristics for patients

Characteristics	B19 screen profile		*P*
	IgG (–), *n* (%)	IgG (+), *n* (%)	
Total patients (*n*=61)	27 (44.3)	34 (55.7)	-
Sex			
Male (*n*=55)	24 (43.64)	31 (56.36)	0.7657
Female (*n*=6)	3 (50)	3 (50)	
HCV			
+ (*n*=33)	11 (33.33)	22 (66.67)	0.0621
– (*n*=28)	16 (57.14)	12 (42.86)	
HBsAg			
+ (*n*=3)	1 (33.33)	2 (66.67)	0.6959
– (*n*=58)	26 (44.83)	32 (55.17)	
History of blood transfusion			
Yes (*n*=39)	26 (66.67)	13 (33.33)	0.8110
No (*n*=22)	14 (63.64)	8 (36.36)	
History of previous surgery			
Yes (*n*=16)	5 (31.25)	11 (68.75)	0.2224
No (*n*=45)	22 (48.89)	23 (51.11)	
Type of hereditary bleeding disorders			
A (*n*=45)	21 (46.67)	24 (53.33)	0.1746
B (*n*=8)	1 (12.5)	7 (87.5)	
VWD (*n*=4)	2 (50)	2 (50)	
Congenital (*n*=4)	3 (75)	1 (25)	
Formation of inhibitors in type A hemophilia patients			
Yes (*n*=15)	7 (46.67)	8 (53.33)	>0.9999
No (*n*=30)	14 (46.67)	16 (53.33)	

Statistical analysis was performed using Chi-square test. B19=Human parvovirus B19, HCV=Hepatitis C virus, HBsAg=Hepatitis B surface antigen, Ig=Immunoglobulin, VWD=Von Willebrand disease

Table 5: Association between hemophilia A patients with inhibitors and hepatitis C virus

Characteristics	HCV		*P*
	IgG (–), *n* (%)	IgG (+), *n* (%)	
Formation of inhibitors in type A hemophilia patients			
Yes (*n*=15)	6 (40)	9 (60)	>0.9999
No (*n*=30)	12 (40)	18 (60)	

HCV=Hepatitis C virus, Ig=Immunoglobulin, *n*=number

Table 6: Association between hemophilia A patients with inhibitors and hepatitis B virus

Characteristics	HBsAg		*P*
	IgG (–), *n* (%)	IgG (+), *n* (%)	
Formation of inhibitors in type A hemophilia patients			
Yes (*n*=15)	14 (93.33)	1 (6.67)	0.6090
No (*n*=30)	29 (96.67)	1 (3.33)	

HBsAg=Hepatitis B surface antigen, Ig=Immunoglobulin, *n*=number

Table 7: Association between hemophilia A patients with inhibitors and patients who received blood product transfusions

Characteristics	Blood product transfusion		*P*
	No, *n* (%)	Yes, *n* (%)	
Formation of inhibitors in type A hemophilia patients			
Yes (*n*=15)	5 (33.33)	10 (66.67)	>0.9999
No (*n*=30)	10 (33.33)	20 (66.67)	

Table 8: Association between hemophilia A patients with inhibitors and patients who had surgery

Characteristics	History of surgery		*P*
	No, *n* (%)	Yes, *n* (%)	
Formation of inhibitors in type A hemophilia patients, *n* (%)			
Yes (*n*=15)	11 (73.33)	4 (26.67)	0.6121
No (*n*=30)	24 (80)	6 (20)	

Regarding the risk of inhibitor formation in type A hemophilia patients, data demonstrated no significant differences among patients with a history of HCV, HBsAg positivity, blood product transfusion, and previous surgical intervention, as shown in Tables 5-8.

Discussion

Parvovirus B19 is widespread globally and has infected more than half of the adult population.[15] The infection is responsible for a broad range of hematological pathogenetic mechanisms.

Our study demonstrates that more than one-half of patients with different hereditary bleeding disorders had evidence of previous human B19 parvovirus infection, as proven by seropositive IgG antibodies. Notably, none of the patients had an active infection as confirmed by 100% negative PCR results for parvovirus B19. This suggests prior exposure rather than ongoing infection, highlighting that patients with a hereditary bleeding disorder may become at risk for parvovirus B19 infection due to their frequent need for medical care, including blood product transfusions and surgical intervention throughout their lives. Therefore, this represents a suitable disease context in which to study Parvovirus B19 infection across different patient profiles.

Recent CDC reports identified an increased test positive in clinical samples and pooled plasma which reflects a rise in parvovirus B19 activity throughout the United States. The prevalence of recent infections increased from less than 3% during 2022-2024 to 10% by June 2024, with the most significant rise observed among children aged 5-9 years, where infection rates surrged from 15% to 40%. Samples with elevated parvovirus B19 DNA levels among plasma donors markedly increased from 1.5% in December 2023 to 19.9% in June 2024.[16]

Several studies from Iran, South Africa, and Brazil reported that the prevalence of parvovirus B19 DNA in hemophilia patients was 12.8%, 8%, and 35.7%, respectively.[17-19] Our study showed negative B19 DNA results, indicating the absence of an active infection. However, the seroprevalence of anti-parvovirus IgG in hemophilia patients was 58%, and this is comparable to the findings of Slavov *et al*., indicating that half of the patients had anti-IgG prevalence.[19] Other studies from Japan and Iran showed that the seroprevalence of B19 IgG was 100% and 47%, respectively, while the B19 DNA was 7.5% and 16%, respectively, among hemophilia patients.[20,21] Furthermore, a study from Basrah (Southern Iraq) revealed that the frequency of the virus DNA was 13.7% out of 95 hemophilia A patients.[22]

Parvo B19 virus is transmitted through the respiratory route, but it can also spread through blood, and/or pooled blood products, and possibly even through tattooing procedures.[23] Approximately 64% and 26% of patients showed a history of blood product transfusions and/or past surgical intervention. Consequently, this may be attributed to the limited availability of recombinant factor therapy for hereditary bleeding disorders, which potentially increases the risk of transfusion-related viral infection.[24,25] However, our study found no significant difference in seropositive parvovirus infection among patients. Lin *et al.*'s study showed that seroprevalence was higher in patients receiving blood products and women who had undergone abortions compared to controls; however, no such increase was observed in individuals with tattoos.[26] Another study comparing between individuals who have and have not received blood transfusions has found a notably higher seropositivity rate among those who received transfusions.[19]

Comparative analysis of patients with and without a history of blood product transfusions has shown a slightly higher, though not statistically significant, rate of seropositivity for viral infections including B19 parvovirus, hepatitis B virus (HBV), and HCV among patients with a history of surgical procedure and blood product intake. Our observation showed that more than 66% of patients with HCV and HBV positive had seropositive IgG B19 parvovirus.

Among patients with hemophilia type A, 33.33% had factor inhibitors. Impressively 53% of those with seropositive parvovirus B19 had inhibitors. However, no statistically significant increase in the risk of inhibitors was observed among hemophilia A patients with seropositive parvovirus B19 compared to those with seronegative Parvo B19 virus.

Conclusions

This study revealed that patients with hereditary bleeding disorders had past exposure to Parvovirus B19,

as indicated by IgG seropositivity, while the absence of detectable viral DNA suggests no current infections which underscore the likelihood of past transmission via blood or blood derived products. Highlighting the value of regular screening and continued monitoring to help protect these patients and ensure the safety of the blood products they rely on.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Alli N, Vaughan J, Louw S, Schapkaitz E, Mahlangu J. Inherited bleeding disorders. S Afr Med J 2018;108:9-15.
2. Castaman G, Matino D. Hemophilia A and B: Molecular and clinical similarities and differences. Haematologica 2019;104:1702-9.
3. Hedner U, Ginsburg D, Lusher JM, High KA. Congenital Hemorrhagic Disorders: New Insights into the Pathophysiology and Treatment of Hemophilia. Hematology Am Soc Hematol Educ Program 2000. p. 241–65. [doi: 10.1182/asheducation.V2000.1.241.241].
4. Josephson N. The hemophilias and their clinical management. Hematology Am Soc Hematol Educ Program 2013;2013:261-7.
5. Schramm W. The history of haemophilia – A short review. Thromb Res 2014;134 Suppl 1:S4-9.
6. Poon MC, Card R. Hemophilia management in transfusion medicine. Transfus Apher Sci 2012;46:299-307.
7. Ljung R, Auerswald G, Benson G, Dolan G, Duffy A, Hermans C, *et al*. Inhibitors in haemophilia A and B: Management of bleeds, inhibitor eradication and strategies for difficult-to-treat patients. Eur J Haematol 2019;102:111-22.
8. Giangrande PL. Blood products for hemophilia: Past, present and future. BioDrugs 2004;18:225-34.
9. Key NS, Negrier C. Coagulation factor concentrates: Past, present, and future. Lancet 2007;370:439-48.
10. Allain JP, Stramer SL, Carneiro-Proietti AB, Martins ML, Lopes da Silva SN, Ribeiro M, *et al*. Transfusion-transmitted infectious diseases. Biologicals 2009;37:71-7.
11. Ragni MV, Koch WC, Jordan JA. Parvovirus B19 infection in patients with hemophilia. Transfusion 1996;36:238-41.
12. Siegl G, Cassinotti P. Presence and significance of parvovirus B19 in blood and blood products. Biologicals 1998;26:89-94.
13. Marano G, Vaglio S, Pupella S, Facco G, Calizzani G, Candura F, *et al*. Human Parvovirus B19 and blood product safety: A tale of twenty years of improvements. Blood Transfus 2015;13:184-96.
14. Zerbini M, Musiani M, Gentilomi G, Venturoli S, Gallinella G, Morandi R. Comparative evaluation of virological and serological methods in prenatal diagnosis of parvovirus B19 fetal hydrops. J Clin Microbiol 1996;34:603-8.
15. Berns KI, Parrish CR. Parvoviridae. In: Knipe DM, Howley PM, Cohen JI, Griffin DE, Lamb RA, Martin MA, *et al*., editors. Fields Virology. 6th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2015. p. 1768-91.
16. Centers for Disease Control and Prevention. Increase in Human Parvovirus B19 Activity in The United States; 2024. Available from: https://www.cdc.gov/han/2024/han00514.html?utm_source=chatgpt.com. [Last accessed on 2025 Mar 03].
17. Javanmard D, Ziaee M, Ghaffari H, Namaei MH, Tavakoli A, Mollaei H, *et al*. Human parvovirus B19 and parvovirus 4 among Iranian patients with hemophilia. Blood Res 2017;52:311-5.
18. Rubinstein R, Karabus CD, Smuts H, Kolia F, Van Rensburg EJ. Prevalence of human parvovirus B19 and TT virus in a group of young haemophiliacs in South Africa. Haemophilia 2000;6:93-7.
19. Slavov SN, Kashima S, Rocha-Junior MC, Oliveira LC, Silva-Pinto AC, Yamamoto AY, *et al*. Frequent human parvovirus B19 DNA occurrence and high seroprevalence in haemophilic patients from a non-metropolitan blood centre, Brazil. Transfus Med 2014;24:130-2.
20. Nishida Y, Arai M, Yamamoto Y, Fukutake K. Serological and virological markers of human parvovirus B19 infection in patients with haemophilia. Haemophilia 1997;3:137-42.
21. Keshavarz M, Janati-Namin N, Arjeini Y, Mokhtari-Azad T, Rezaei F. Prevalence and genotypic characterization of human parvovirus B19 in hemophilia patients. Iran J Microbiol 2022;14:568-73.
22. Al-Khegane MA, Ibrahim WN, Hassan MK. Human parvovirus B19 among hemophilia A patients in Basrah, Southern Iraq. Iraqi J Hematol 2021;10:112-7.
23. Shneerson JM, Mortimer PP, Vandervelde EM. Febrile illness due to a parvovirus. Br Med J 1980;280:1580.
24. Kleinman SH, Glynn SA, Lee TH, Tobler LH, Schlumpf KS, Todd DS, *et al*. A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion. Blood 2009;114:3677-83.
25. Yu MY, Alter HJ, Virata-Theimer ML, Geng Y, Ma L, Schechterly CA, *et al*. Parvovirus B19 infection transmitted by transfusion of red blood cells confirmed by molecular analysis of linked donor and recipient samples. Transfusion 2010;50:1712-21.
26. Lin KH, You SL, Chen CJ, Wang CF, Yang CS, Yamazaki S. Seroepidemiology of human parvovirus B19 in Taiwan. J Med Virol 1999;57:169-73.