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Human parvovirus B19 among hemophilia A patients in Basrah, Southern Iraq

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Abstract:

BACKGROUND: Hemophilia A patients, especially if there is shortage in recombinant Factor VIII concentrate, may require occasional blood and/blood products transfusion, rendering them more susceptible to acquire infections including *Parvo B19* virus (B19V).

OBJECTIVES: To assess the presence of B19V viral DNA among hemophilia A patients and look for its possible association with disease-related variables.

SUBJECTS AND METHODS: This case–control study was carried out from October 2019 to August 2020. A total of 95 male patients with Hemophilia A and 95 healthy subjects matched for age and gender were enrolled in the study. The identification of B19V DNA was achieved using the real-time polymerase chain reaction (PCR). Hepatitis C virus (HCV) antibodies and hepatitis B surface antigen (HBsAg.) were tested by ELISA method.

RESULTS: The frequency of B19V among hemophilia A patients was 13.7% compared to 6.3% among healthy subjects. None of the control group has been tested positive for HCV antibodies or HBsAg. While among patients, the frequencies of hepatitis C and B were 8.4% and 2.1%, respectively. Patients with hemophilia A and B19V have significantly higher rate of arthropathy at the time of blood sampling and blood and/blood products transfusion, especially fresh-frozen plasma (FFP) and cryoprecipitate compared to those who did not receive such blood products (92.3% vs. 50%), $P < 0.05$.

CONCLUSIONS: Parvovirus B19 was detected in a significant proportion of hemophilia patients especially those with a history of FFP and cryoprecipitate transfusion. The use of PCR technique is essential to detect viruses in donor's blood to avoid infection among this high-risk group.

Keywords:

Hemophilia A, Iraq, Parvo B19

Introduction

Hemophilia A is the most common severe congenital bleeding disorder which results from the deficiency in the clotting protein Factor VIII (FVIII). FVIII deficiency is an X-linked recessive disorder occurring in 1 in every 5000 male births without an ethnic predominance.[1]

Patients with hemophilia, especially if there is a lack or shortage of recombinant FVIII concentrate, may require occasional blood and/blood products transfusion (plasma and cryoprecipitate) to compensate for missing blood or stop of bleeding. The main problems in hemophilic patients that are related to the blood and blood product transfusion are infections especially by viruses. The most common viruses are hepatitis C and B viruses (HCV), HIV and *Parvovirus B19* (B19V).[2]

Human B19V, is the smallest human DNA virus, related to the Erythroparvovirus genus inside the Parvoviridae family.[3] This virus is transmitted mainly by the respiratory route and has the ability to be transmitted

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in different methods like mother to fetus and by blood or blood products. The majority of blood banks lack the screening test for B19V, and common inactivation strategies eliminate enveloped viruses but do not have an impact on B19V owing to its characteristics of grander resistance to external chemical and physical agents in addition to having such a little nonenveloped capsid,[4] making these patients at high risk of acquiring the infection which may aggravate the sign and symptoms of joint involvement (arthropathy).[2] The occurrence of B19 infection in patients with hemophilia may lead to production of autoantibody responses that are related to joint lesions. It was reported that B19 DNA can persist in the synovial membranes of patients with hemophilic arthritis significantly more frequently in comparison to control individuals with arthrosis or joint trauma leading to persistent B19V,[5] therefore, the occurrence of B19 infection in patients with hemophilia may lead to production of autoantibody responses that are related to joint lesions.[4]

Previous studies in Basrah reported a high frequency of B19V infection; 76.9% in patients with arthropathy,[6] 47.4% in pediatric patients with acute lymphoblastic leukemia[7] and 89.4% in patients with sickle cell disease and thalassemia[8] compared to 20% in healthy individuals.[7]

The frequency of seropositivity among hemophiliac children was found to be significantly higher than normal.[9] When B19V infection is suspected, screening of patients with polymerase chain reaction (PCR) rather than with immunoglobulin G and M (IgG and IgM) antibody-based serology is recommended for the diagnosis of the infection.[10]

As there are no clear data concerning the risk of B19V infections among patients with hemophilia A in Basrah, we have conducted this study to assess the presence of B19V viral DNA in this high-risk group in Basrah province and look for its possible association with selected disease-related variables.

Subjects and Methods

This case–control study was carried out from October 2019 to the end of August 2020. A total of 95 male patients, aged 2 months to 63 years, with Hemophilia A were recruited in the study. All patients have been diagnosed and registered at Basrah Center for Hereditary Blood Diseases and were evaluated through their follow-up visits to the center.

All patients with hemophilia A who have visited the Center for Hereditary Blood Diseases during the study period were included. While patients with acquired hemophilia and patients with other congenital bleeding disorders were excluded from the study.

Patients clinical data included age at diagnosis, family history of similar condition, bleeding episodes, recombinant FVIII concentrate treatment modalities (on demand or prophylaxis), and history of blood transfusion and fresh-frozen plasma (FFP) or cryoprecipitate. The disease severity on registry (depending on FVIII level) and the results of inhibitors testing (Bethesda units) were also recorded.

The severity of hemophilia was classified depending on the plasma levels of FVIII activity: Severe if <1%, moderate if between 1% and 5% and mild if >5 and <40% of normal.[11]

A total of (95) healthy participants matched for age and gender were also enrolled in the study as a control group. These participants were free of chronic diseases such as thalassemia, sickle cell anemia, and other diseases requiring the transfusion of blood and blood components (e.g. FFP, cryoprecipitate).

An informed consent was taken from the patients and/one of the parents of pediatric patients.

Two milliliters of venous blood was withdrawn from each participant into EDTA tube for the total genomic DNA extraction, by using Wizard Genomic DNA Purification Kit, promega/USA, and this was done according to instructions of the company.

To identify DNA of *Parvovirus B19* in the blood of patients with hemophilia and the control group, a sensitive real-time PCR test was used to amplify a 154-base pair (bp) fragment of the NS1 gene with cyber green master mix.

Primers for parvovirus B19:
- B1F CCACTATGAAAACTGGGCAATA 60 C
- B1R GCTGCTTTCACTGAGTTCTTCA 60 C

The procedure of quantitative PCR (q-PCR) to detect of Parvovirus B19 and the amplification cycle has followed the cycles described in Table 1.

HCV antibodies and hepatitis B surface antigen (HBsAg) tests were done by ELISA method using kits of Fortress diagnostics company (United Kingdom, Antrim) with washer and reader of human company (Germany, Wiesbaden).

Statistical analysis

Data analysis of all subjects was performed using the Statistical Package for the Social Sciences software (SPSS) version (20), (IBM Corp., Armonk, N.Y., USA). The

comparison between groups and qualitative variables was done by the Chi-squared test while quantitative and continuous variables were tested using Student's *t*-test, Mann–Whitney test, or Fisher exact test.

Statistical tests with probability values <0.05 were considered statistically significant.

Results

Demographic distribution of patients and controls

All subjects were males, with most hemophilia A patients were ≤10 years of age. There was no statistically significant difference in the age and residence of and patients the control group, $P > 0.05$, Table 2.

Selected hemophilia patients characteristics

The mean age at the time of diagnosis for all People with hemophilia (PWH) was 4.62 ± 9.21 years, while the median age was 1.

Patients with hemophilia A were divided into three groups according to the severity of hemophilia, the determination of severity mild 37 (39%), moderate 33 (34.7%), and severe 25 (26.3%).

Hemarthrosis was the most common presentation which was reported in 80 (84.2%), followed by mucous membrane bleeding with or without cutaneous bleeding in 76 (80%), muscle bleeding in 60 (63.2%), and bleeding after circumcision in 20 (21.1%). Inhibitors were detected in 13 (13.7%) of PWH.

Parvovirus B19

The frequency of B19V among hemophilia A patients was 13.7% compared to 6.3% among healthy subjects [Table 3]. None of the control group has been tested positive for hepatitis C antibodies or HBsAg. While among PWH, the frequencies of hepatitis C and B were 8.4% and 2.1%, respectively. One PWH with B19V has also hepatitis B.

Figure 1: A shows the results of one of our PWH positive for B19V, while Figure 1. B shows the melting curve analysis of B19V detection, the temperature of cycle was 60°C–94 C° for 1 min, the degree of plot specificity was displayed in one peak at 80°C, the intercalating dye was SYBR green.

The study also revealed that PWH and B19V have significantly higher rate of arthropathy at the time of blood sampling and blood and/blood products transfusion, $P < 0.05$ [Table 4]. *Parvo B19* infection was significantly higher among PWH who have received FFP and cryoprecipitate compared to those who did not receive such blood products, $P < 0.05$ [Table 5].

Table 1: Procedure of q-polymerase chain reaction to detect of parvovirus B19

Step	Temperature (°C)	Time	Cycle
Initial denaturation	95	5 min	1
Denature	95	20 s	40
Anneal	60	45 s	
Extension	72	1 min	
Melting curve analysis	60-95	5 s/step	1

Table 2: Selected sociodemographic characteristics of hemophilia patients and control group

Variable	PWH total 95, *n* (%)	Control group total 95, *n* (%)	*P*
Age (years)			
≤10	39 (41.1)	37 (39)	0.968*
11-20	24 (25.3)	21 (21.1)	
21-30	19 (20)	20 (21)	
31-40	10 (10.5)	13 (13.7)	
41-50	1 (1.1)	1 (1.05)	
>50	2 (2.1)	3 (3.15)	
Mean age±SD (years)	16.5±12.6	17.4±14.2	0.647**
Residence			
Center	50 (52.6)	54 (56.8)	0.560***
Periphery	45 (47.4)	41 (43.2)	

*Fisher's exact test, **Mann-Whitney test, ***Chi-squared test. SD=Standard deviation, PWH=Patients with haemophilia

Table 3: The frequency of parvovirus B19, hepatitis B and hepatitis C virus infections among patients with haemophilia and control group

Type of infection	Control (*n*=95), *n* (%)	PWH (*n*=95), *n* (%)	*P**
Parvo B19			
Yes	6 (6.3)	13 (13.7)	0.090
No	89 (93.7)	82 (86.3)	
HCV			
Yes	0	8 (8.4)	0.007
No	95 (100)	87 (91.6)	
HbsAg			
Yes	0	2 (2.1)	0.497
No	95 (100)	93 (97.9)	

*Fishers exact test. HCV=Hepatitis C virus, PWH=Patients with haemophilia, HbsAg=Hepatitis B surface antigen, Parvo B19=Parvovirus B19

Discussion

Hemophilia A is the second most common type of all hereditary blood disorders after VWD, and the most common type of hemophilia in the world.[12]

Ninety-five patients with hemophilia A were included in this study out of the total 200 patients with hemophilia A that were registered in the Center for hereditary Blood Diseases in Basra.

Patients with hemophilia A were divided into three groups according to plasma pro-coagulant levels: Mild (39%), moderate (34.7%), and severe (26.3%). The

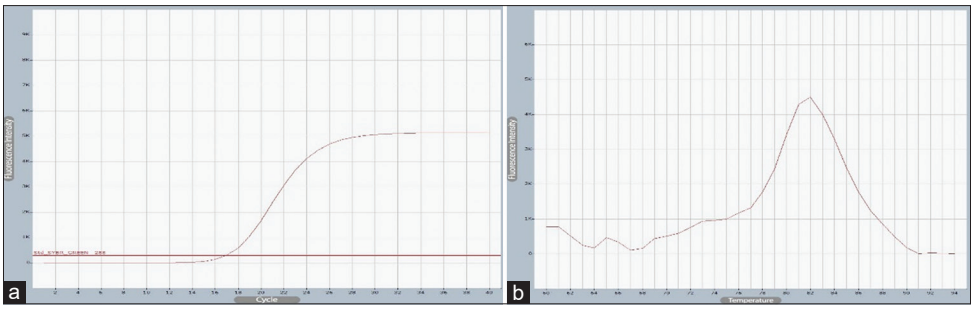

Figure 1: (a) Amplification curves of positive *Parvovirus B19* results by real-time polymerase chain reaction, (b) Melting curve of positive *Parvovirus B19* results by real-time polymerase chain reaction

Table 4: Selected demographic and clinical characteristics of patients with haemophilia in relation to parvovirus B19 infection

Variable	PWH (*n*=95)		*P*
	Parvovirus B-19 negative (*n*=82), *n* (%)	Parvovirus B-19 positive (*n*=13), *n* (%)	
Age (years)			
≤10	33 (40.2)	6 (46.2)	0.691*
11–20	21 (25.6)	3 (23.1)	
21–30	15 (8.3)	4 (30.8)	
31–40	10 (12.2)	0	
41–50	1 (1.2)	0	
≥51	2 (2.4)	0	
Residence			
Center	44 (53.7)	6 (46.2)	0.615**
Periphery	38 (46.3)	7 (53.8)	
Severity			
Mild	34 (41.5)	3 (23.1)	0.193*
Moderate	29 (35.4)	4 (38.8)	
Severe	19 (23.1)	6 (46.2)	
Arthropathy at time of phlebotomy			
No	60 (73.2)	4 (30.8)	0.002*
Yes	22 (26.8)	9 (69.2)	
Inhibitors			
No	70 (85.4)	12 (92.3)	0.499*
Yes	12 (14.6)	1 (7.7)	
Blood and blood products transfusion			
No	41 (50.0)	1 (7.7)	0.004*
Yes	41 (50.0)	12 (92.3)	

*Fishers exact test, **Chi-square test were used. PWH B19V positive=Hemophilia patients infected with parvovirus B19, PWH B19V negative=Hemophilia patients without parvovirus B19, PWH=Patients with haemophilia

World Federation of Hemophilia reported that 22.32% of PWH A, in upper middle income countries (for which Iraq belongs), have mild disease, 22.66% with moderate disease, 43.67% have severe hemophilia, and 11.25% with unknown severity.[13]

The blood-borne diseases are among the most problems for patients who are in need for blood or blood product transfusions. Hepatitis C, hepatitis B, and Parvovirus B19 and HIV are of the most common pathogens.[14]

The use of blood products (FFP and cryoprecipitate) has rapidly declined following the availability of FVIII concentrates; however, PWH may require blood and/blood products transfusion to compensate for the lost blood and missed FVIII if there is shortage in recombinant FVIII.[15]

In this study, significant differences were reported in the frequency of B19 parvovirus between PWH (13.7%) and control group (6.3%), as detected by q-PCR technique.

Around (46.2%) of positive PWH were under 10 years old, this is the period of maximum activity of children who are exposed to different environmental accidents during playing and in the school which brought them more vulnerable to injuries and subsequent bleeding with the increased chance for blood or blood product transfusion that enhance the probability of acquiring the virus.

The prevalence of Parvovirus B 19 in PWH in this study was (13.7%). It was not affected by residence or severity of hemophilia, although it was higher among severe cases, $P > 0.05$.

The frequency of Parvovirus B19 among PWH in the current study is comparable to that reported by Javanmard *et al*., in Southern Khorasan, Iran (12.8%), but differs regarding the frequency of HCV seropositivity that was reported in (10.5%),[4] while 8.7% of PWH in Basrah were tested positive for HCV.

In Brazil (35.7%) of PWH have Parvovirus B19 DNA and (10.7%) have anti-HCV IgG.[16] While in Japan, Parvovirus B19 DNA was found in (7.5%) from 40 patients but IgG was found in all patients (100%).[17] B19V is a virus capable of infections presenting with different courses, so that the acute-phase infection can be followed by a delayed clearance, active chronic infections, or silent persistence in tissues, depending on the interplay with host factors and the efficacy of the immune system response. Therefore, an accurate laboratory diagnosis of B19V infection will necessary rely on a multiparametric approach, combining

Table 5: Blood or blood product transfusion according to B19 virus infection

Variable	PWH B19 negative (*n*=82), *n* (%)	PWH B19 positive (*n*=13), *n* (%)	Total	*P**
Blood and blood products transfusion				
No	41 (50)	1 (7.7)	42 (44.2)	0.004
Yes	41 (50)	12 (92.3)	53 (55.8)	
Blood				
No	73 (89.0)	12 (92.3)	85 (90.5)	0.614
Yes	9 (11)	1 (7.7)	10 (10.5)	
FFP				
No	52 (63.4)	2 (15.4)	54 (56.85)	0.001
Yes	30 (36.6)	11 (84.6)	41 (42.1)	
Cryoprecipitate				
No	60 (73.2)	5 (38.4)	65 (68.4)	0.013
Yes	22 (26.8)	8 (61.6)	30 (31.6)	

*Fishers exact test. PWH B19V positive=Hemophilia patients infected with parvovirus B19, PWH B-19V negative=Hemophilia patients without Parvovirus B19, PWH=Patients with haemophilia, FFP=Fresh frozen plasma

as much as possible both molecular detection of viral components and immunological detection of virus-specific antibodies.[18] Nowadays, real-time PCR techniques must be considered the standard analytical method for the molecular detection of B19V.[19]

Higher frequency of Parvovirus B19 between PWH appears in the study of Mousavi *et al*., in Afghanistan. Out of 80 hemophilia patients; (43.7%) have detectable parvovirus B19 DNA, (91.25%) with anti-Parvovirus b19 IgG, (2.5%) with HBs Ag and 7 (8.7%) with anti-HCV.[20] The frequencies of HBs Ag and HCV are similar to that found in the current study.

Parvo B19 infection was significantly higher among PWH who have received FFP and cryoprecipitate compared to those who did not receive such blood products, $P < 0.05$.

There are many obstacles in managing PWH in developing countries, one of these obstacles is the shortage in factor concentrates supply. In such countries, physicians rely on blood and blood products (fresh whole blood, FFP, and cryoprecipitate) in treating PWH, therefore such PWH may acquire many viral infections even when the blood and blood products are screened for certain viruses like hepatitis B and C,[21] and this may explain the variation in the frequency of B19V in different studies.

In this study B19V among PWH according to the severity of hemophilia was (23.1%), (30.8%) and (46.2%) for mild, moderate and severe hemophilia, respectively.

Regarding the association of B19V with inhibitors, the study did not reveal significant association. This result is comparable to the results of Slavov *et al*., in which the distribution of parvovirus B19 according to severity was (20%) in patients with mild hemophilia, (20%) with moderate hemophilia, and (60%) with severe hemophilia, but differs in the percentage of patients who have B19V with inhibitors (30%).[16]

However, the findings of the current study differ from that reported by Mousavi *et al*., who have found in their study that the distribution of parvovirus B19 among PWH was (71.4%) for severe hemophilia and (28.6%) for mild and moderate together.[20]In another Iranian study that was done by Javanmard *et al*., B19V was detected in (27.3%) patients with severe hemophilia, (63.6%) moderate hemophilia, and (9%) with mild hemophilia.[4]

The differences in the frequency of B19V among PWH in different studies may be related to several factors like the number of samples, season at which the blood samples withdrawn, because B19V peak distribution is in late winter and spring,[22] exposure to blood products and the severity of hemophilia patients, as severe hemophilia patients need more of blood or blood product and that increases the chance of infection with B19V.[23]

Conclusion

From this study it can be concluded that the frequency of Parvovirus B19 is significantly higher among PWH and arthropathy at time of phlebotomy and in those with a history of blood and blood products transfusions (FFP and cryoprecipitate) than in Parvovirus B19-ve patients.

The use of PCR technique is essential to detect viruses in the blood of donors to avoid infection of patients with hemophilia. Adequate supply of recombinant FVIII concentrates will minimize the need for blood products and therefore the risk of different transfusion-transmitted viral infections in PWH. Sensitive nucleic acid testing methods such as real-time PCR is recommended to screen blood and blood products for parvovirus B19 combined with IgM ELISA for correct diagnosis. More direct approaches for making blood products B19-virus safe, such as inactivation of microorganisms by advance technique (in plasma products) to reduce the quantity of viral particles in final products are also recommended.

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Conflicts of Interest

There are no conflicts of interest.

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