



Association between Parvovirus B19 and anemia in HIV-infected patients

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Abstract

Background: Human parvovirus B19 (B19V) can cause anemia in some patients, including those with compromised immunity system. There are a few studies on molecular epidemiology of B19V and its association with anemia in Iran. Therefore, the aim of this study was to determine the B19V DNA, IgM, IgG, genotyping, and viral load in HIV patients in different groups of pregnant women, general population, injection drug users (IDU), and Elite controllers. Also, the possible association of B19V with anemia was studied.

Methods: In this case-control study, B19V DNA, anti-B19V IgM, anti-B19V IgG, viral load, and hemoglobin level were assessed in 113 HIV positive patients and 72 healthy controls. Also, CD4+ T cell counts and HIV load were measured in the patients' group. All statistical analyses were done using STATA 14.2 software (Stata Corporation, College Station, Texas, USA). P value < 0.05 was considered statistically significant.

Results: Among HIV patients, 19 (16.8%) cases had B19V DNA, 3 (2.7%) had B19V IgM, and 7 (6.2%) had B19V IgG. In control group, the prevalence of B19V DNA, IgM, and IgG was 6 (8.33%), 7(9.7%), and 19 (26.4%), respectively. In subpopulations based on transmission routes, general population had the highest B19V IgG and DNA positivity prevalence and viral load level. There was no significant association between B19V antibodies and DNA with anemia.

Conclusion: The results demonstrated that B19V infection cannot be considered as a high-risk factor for anemia in adult HIV patients. However, further studies are needed to determine the exact role of B19V infection in HIV patients.

Keywords: Anemia, Parvovirus B19, Human immunodeficiency virus, Iran

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Introduction

Human parvovirus B19 (B19V) is a small non enveloped, ssDNA (single-stranded) virus which belongs to

Parvoviridae family (1, 2). The transition routes for this virus include the respiratory route, mother to fetus, blood

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↑What is “already known” in this topic:

Parvovirus B19 infection is involved in several clinical manifestations such as erythema infectiosum, chronic arthritis, hydrops fetalis, and transient aplastic crisis in patients with sickle cell anemia and pure red cell aplasia in immunocompromised hosts. This virus can lead to self-limited anemia in healthy individuals; however, it can develop into chronic anemia in immunocompromised cases.

→What this article adds:

The present study evaluated the association between anemia and B19V in HIV patients. Also, the threat of B19V is limited in the HIV patients receiving ART, whereas persistent B19 viremia in the untreated patients may lead to anemia.

transfusion, and blood products (3, 4). B19V infection can cause various clinical manifestations such as erythema infectiosum, chronic arthritis, hydrops fetalis, transient aplastic crisis in patients with sickle cell anemia, and pure red cell aplasia in immunocompromised hosts (5, 6). The main site of B19V replication is inside the erythroid progenitor cells, bone marrow (7). Although it leaves no clinical impact on healthy individuals, it could cause anemia in immunocompromised hosts (3, 8). In the course of B19V infection, a brief period of viremia occurs which is mostly self-limited. This low-level viremia may persist in healthy individuals for about 12 months. However, in immunocompromised patients, it could lead to viral persistence and chronic bone marrow suppression and finally anemia due to the absence of an adequate antibody response (9, 10). This anemia occurs because of the inadequate immune response in immunosuppressed individuals who fail to control the primary infection of the virus (11). B19V infection and the consequent anemia are considered serious issues in HIV patients. A proportion of patients are cured by blood transfusions and high-dose intravenous immunoglobulin (12-15).

This study aimed to investigate the presence of B19V specific markers and its genotype patterns in different groups of HIV positive patients, including pregnant women, elite controllers, injection drug users (IDUs), and general population with different counts of CD4⁺ T cells. Also, in this study, it was aimed to determine the possible association between B19V and anemia in these patients.

Methods

Study population

A total of 113 HIV-infected patients and 72 control (healthy) persons participated in this case-control study between January 2016 and December 2017 in Tehran and Kermanshah.

Inclusion criteria were defined as cases referred to Iranian Research Center for HIV/AIDS, accessibility to fresh samples, and patients being from Kermanshah and Tehran. Exclusion criteria included any medical history of radiotherapy and/or chemotherapy, or systemic inflammatory disease such as rheumatoid arthritis and biologic anticancer therapies. Patients were classified into 4 subgroups, including pregnant women, general population, IDU, and elite controller. Elite controllers were defined as a group of HIV-positive individuals who, without any antiretroviral therapy (ART), had plasma viral loads below the detection limit along with a CD4 cell count similar to the healthy controls. Patients were further classified into 3 subgroups according to their CD4⁺ T cells/mm³ as CD4⁺ T <200, CD4⁺ T = 200–500, and CD4⁺ T >500. This study has been approved by the local institutional ethical committee under the Ethics Code of 14121472, and all procedures were in accordance with the Helsinki Declarations. Those participants who met the case and control definitions were asked to fill a written informed consent.

Serology

All plasma samples were screened for anti-B19V-IgM and anti-B19V-IgG using an enzyme-linked

immunosorbent assay (ELISA) Kit (Abcam, MA, USA), according to the manufacturer's standard protocol.

Nucleic acid extraction

DNA extraction from 200 mL of plasma was performed by QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendation. The extracted DNA was stored at –70°C until further analysis.

B19V detection, genotyping, and viral load

For amplification of target DNA, the following items were used: 1 µL of DNA, 2X Master mix (sinacolon, Iran), 1 µM of each primers (8) (Metabion, Germany), 0.8 mM deoxynucleotide mix, 2.5 mM MgCl₂, 0.02 U µl⁻¹ Taq polymerase (Invitrogen, CA, USA), 5% DMSO and DDS up to 25 µL. Amplification was performed in a thermocycler (EppendorfMastercycler®, Massachusetts, USA) for 40 cycles with the following temperatures: 95°C for 1 minute (denaturation), 55°C for 45 seconds (annealing), 72°C for 1 minute (extension), and 72°C for 10 minutes (final extension). Agarose gel electrophoresis (1.5%) of PCR products was performed using 1mM Tris-Borate-EDTA (TBE) buffer and the 100-bp DNA ladder (Sinaclon, Iran) at 90 V for 50 minutes. Finally, the desired DNA bands were observed using green viewer dye (Sinaclon, Iran).

Genotyping was conducted based on the protocol described by Azadmanesh et al (12). QIAquick PCR purification Kit (Qiagen, Hilden, Germany) was used for purification of nested PCR products from samples. Then, the positive samples for the NS1/VP1 region of B19V genome were sent for sequencing performed by Bioneer (Korea). The B19V DNA positive samples were analyzed using real time-PCR technique to determine B19 viral load, whose assessment has already been described by Koppelman et al (16). Accordingly, the set of primers which target a certain region in NS1 (nucleotides 2082–2099 and 2254–2274 of the genome with GenBank accession number AF162273) was used. Both protocols used the same set of primers targeting the same region of NS1 and were described previously by Koppelman et al.

CD4+ T cell count and HIV viral load

The CD4 count was performed using a flow cytometer FACSCalibur (Becton Dickinson Biosciences, USA). To determine HIV viral load, Cobas TaqMan (COBAS TaqMan; Roche Molecular Systems, Branchburg, NJ) was applied.

Anemia determination

According to the WHO standards, a hemoglobin value of below 13 g dL⁻¹ for men, below 12 g dL⁻¹ for women, below 11 g dL⁻¹ for pregnant women, and below 12 g dL⁻¹ for children were considered as severe anemia (17). CELLDYN RUBY Hematology Analyzer (Abbott Diagnostics) was used to determine hemoglobin level.

Statistical analysis

Continuous data were represented as mean ± standard

deviation (SD) or median \pm interquartile range (IQR). The statistically significant differences between experimental groups were assessed by one-way ANOVA or Kruskal–Wallis H test. Categorical data were displayed as N (%) and Chi-square or Fisher's exact tests were used for comparison. Also, the odds ratio (95% CI) as the effect size of the association between the markers of B19V and HIV was estimated using logistic regression model. All statistical analyses were done using STATA 14.2 software (Stata Corporation, College Station, Texas, USA). Statistical significance was set at p value less than 0.05.

Results

In the present study, 113 HIV-positive patients and 72 adults with no immunologically compromised cases were enrolled. In HIV group, 72 (63.7%) of patients were male, with the mean \pm SD age of 36.221 \pm 9.933 years, and this proportion for healthy controls was 49 (68.1%), with the mean \pm SD age of 38.44 \pm 10.351 years.

The mean CD4 count was 402.69 \pm 341.451 cells/mm³ in the patients and 1172.32 \pm 279.116 cells/mm³ in the healthy controls (p<0.001). The mean of the B19 viral load in HIV patients was significantly higher than that of the healthy group (p=0.002). B19V DNA was detected in 19 (16.8%) patients. B19V IgG and IgM were detected in the blood of 7 (6.2%) and 3 (2.7%) patients, respectively. One patient was positive for both IgG and IgM antibodies. Also, one patient was positive for B19V DNA and IgM simultaneously. The results of statistical analysis did not show any significant differences between the case and

control groups in B19V IgM, while the prevalence of B19V IgG in controls was significantly higher than in the cases (26.4% vs 6.2%, p<0.001, OR: 95% CI=0.184: 0.073-0.466). All B19V samples were confirmed as Genotype I, subtype B19-1A, using a phylogenetic analysis. More details are demonstrated in Table 1.

Considering the patients being classified according to their CD4⁺ cell count, there were no significant differences in the distribution of B19V, antibodies, and DNA in the 3 subgroups. However, patients with CD4 cell counts > 500 mm⁻³ had a significantly higher prevalence of IgG and IgM. On the other hand, patients with CD4 cell counts 200-500 mm⁻³ had a significantly higher prevalence of B19V DNA. Among these patients, the average B19 viral load was higher in the subgroup with the lower CD4 count (p= 0.038). The mean of the B19 viral load was the highest, 882.2 \pm 129.06, in patients with CD4 count < 200. More details on the prevalence of B19V DNA, antibodies, and viral load in the CD4 count subgrouping of patients are presented in Table 2.

Patients were classified into 4 subgroups: 12.43% pregnant women, 23.8% general population, 18.4% IDUs, and 6.5% elite controllers. The mean of the B19 viral load was 334.9 \pm 105.2 copies/mL (lowest) in elite controllers and 764.8 \pm 129.41 copies/mL (highest) in pregnant women (p=0.036). The elite controller samples had a significantly higher prevalence of IgM and IgG (p=0.007 and p=0.036, respectively). More details are illustrated in Table 3.

The prevalence and level of B19V DNA were higher in anemia patients compared to the non anemia group (Table 4). Moreover, the prevalence of anti-B19 IgM antibodies

Table 1. Comparison of participants' characteristics between 113 HIV+ patients and 72 healthy controls.

Characteristics	HIV+ patients	Healthy Control	p	OR (95% CI)	
Age (Year)*	36.221 \pm 9.933	38.44 \pm 10.351	0.146	NA	
CD4 count*	402.69 \pm 341.451	1172.32 \pm 279.116	<0.001	NA	
B19 Viral load*(copies/ml)	1302.4 \pm 186.33	227.6 \pm 109.72	0.002	NA	
Sex ⁺	Male	72 (63.7%)	49 (68.1%)	0.454	0.824 (0.441-1.542)
	Female	41 (36.3%)	23 (31.9%)	0.057	0.912 (0.427-1.842)
B19V DNA (Genotype I)	Presence	19 (16.8%)	6 (8.3%)	0.045	2.223 (0.843-5.867)
	Absence	94 (83.2%)	66 (91.7%)	0.064	NA
Anemia ⁺	Presence	16 (14.2%)	5 (7%)	0.139	0.837 (0.77-6.32)
	Absence	97 (85.8%)	67 (93%)	0.072	NA
IgM ⁺	Positive	3 (2.7%)	7 (9.7%)	0.052	0.253 (0.063-1.01)
	Negative	110 (97.3%)	65 (90.3%)	0.058	
IgG ⁺	Positive	7 (6.2%)	19 (26.4%)	<0.001	0.184 (0.073-0.466)
	Negative	106 (93.8%)	53 (73.6%)	<0.001	
Sub-population ⁺	Healthy	-	72 (100%)	NA	NA
	Pregnant Women	23 (12.43%)	-		
	IV Drug User	34 (18.4%)	-		
	Elite controller	12 (6.5%)	-		
	General population	44 (23.8%)	-		
HIV load	No Data ⁺	6 (5.3%)	-	NA	NA
	Undetectable ⁺	57 (50.4%)	-		
	Detectable ⁺	31.42 \times 10 ⁴ \pm 8.08 \times 10 ⁴	-		

NA; Not applicable; * indicated as Mean \pm Standard Deviation; + indicated as N (%).

Bold P-values represent as statistically significant

Table 2. Markers of B19V infection in HIV+ patients based on the CD4 count.

B19V Characteristics	<200	200-500	>500	p
DNA ⁺	8/49 (16.32%)	9/39 (23.7%)	2/25 (8%)	0.302
IgM ⁺	0/49 (0.0%)	1/39 (2.6%)	2/25 (8%)	0.099
IgG ⁺	3/49 (6.1%)	1/39 (2.6%)	3/25 (12%)	0.323
B19 Viral load*(copies/ml)	882.2 \pm 129.06	469.3 \pm 187.11	237.1 \pm 126.50	0.038

+ indicated as n/N (%); * indicated as Mean \pm Standard Deviation.

Bold P-values represent as statistically significant

Table 3. Markers of B19V infection in HIV+ patients based on the HIV+ sub-populations

B19V Characteristics	Pregnant Women	IDU	Elite Controller	General population	p
DNA ⁺	5/23 (21.7%)	5/34 (14.7%)	1/12 (8.33%)	8/44 (18.18%)	0.780
IgM ⁺	1/23 (4.4%)	0/34 (0.0%)	2/12 (16.7%)	0/44 (0.0%)	0.007
IgG ⁺	1/23 (4.4%)	1/34 (2.9%)	3/12 (25%)	2/44 (4.5%)	0.036
B19 Viral load [*] (copies/ml)	764.8 ± 129.41	672.4 ± 111.78	334.9 ± 105.2	441.0 ± 109.6	0.036

+ Indicated as n/N (%); * indicated as Mean ± Standard Deviation.
Bold P-values represent as statistically significant

Table 4. Markers of B19V infection in HIV+ patients with Anemia.

B19V Characteristics	Anemia group	Non-anemia group	p
DNA ⁺	17/101 (16.83%)	2/12 (16.66%)	0.675
IgM ⁺	1/16 (6.25%)	2/97 (2.06%)	0.370
IgG ⁺	0/16 (0.0%)	7/97 (7.2%)	0.591
B19 Viral load [*] (copies/ml)	911.3 ± 187.52	302.4 ± 186.33	0.002

+ Indicated as n/N (%); * indicated as Mean ± Standard Deviation. Bold P-values represent as statistically significant

was higher in anemia patients than that of non-anemia group; nonetheless, the difference was not statistically significant ($p=0.370$).

Discussion

In the present study, the molecular and serological characterization of B19V was investigated in 4 groups of HIV patients in Tehran and Kermanshah, Iran. To our knowledge, this was the first study reporting the prevalence and genotyping of B19V in HIV patients and assessing the association between B19 infection and anemia within the mentioned subgroups. No previous study has been done with such categories and on elite controllers.

In this study, the prevalence of IgG, IgM, and B19V DNA in HIV-infected patients was 6.2%, 2.7%, and 16.8%, respectively. Studies that have been conducted in Iran included (a) Azadmanesh et al who reported 11.1%, 1%, and 13.1% for the prevalence of the IgG, IgM, and B19V DNA, respectively and (b) Abdollahi et al who stated the prevalence of B19V IgM and IgG as 81.1% and 0%, respectively (12, 18). In another study, the prevalence of IgM, IgG, and B19V DNA was reported to be 5.1%, 67.3%, and 15.3%, respectively (19). This inconsistency of the B19V prevalence might have been due to the following reasons: the degree of immunodeficiency, sample size, and laboratory techniques. Also, it was demonstrated that the prevalence of B19V DNA was higher in cases than in controls (16.8% vs 8.33%, $p<0.045$), while the prevalence of B19V IgG was significantly lower in cases than in controls (6.2% vs 26.4%, $p<0.001$).

Consistent with the findings of the present study, various studies have documented low frequency of anti-B19V IgG antibodies among HIV-infected patients. This may be due to the impairment of the immune system and its inability to produce neutralizing antibody against B19V, which finally leads to chronic infection (20, 21). In this study and Gyllensten's group research, the prevalence of IgM in patients with CD4 level < 200 was 0%, whereas in another investigation by Pedranti et al, this value has been reported up to 10.8 % for these patients (19, 22). An important limitation of epidemiology studies such as this study is the sampling period. When sampling is done immediately after the outbreak, the frequency is higher than interepidemic periods (1). Also, here, it was demonstrated that 15 cases had B19 viral load, while IgG and IgM were negative; and 4 cases were positive for B19 IgG and had

viremia simultaneously. This situation could be due to an early acute or persistent phase of B19V infection. When the B19V infection gets chronic, it could lead to anemia in these patients. This result is inconsistent with the investigations conducted by He et al and Azadmanesh et al (12, 21). In this study, all B19V-positive samples were confirmed as genotype I. The genotype of the B19V in other studies vary in comparison to studies by Azadmanesh (Iran, genotype I), Grabarczyk (Poland, genotype I, II), and Plentz (Germany, genotype I, II, III) (12, 23, 24). This variability could be explained as B19V genotype differs according to the geographical area. In HIV patients, the B19 viral load was significantly higher compared to the control group ($p=0.002$) (Table 1). Also, B19 viral load in patients with CD4⁺ cells less than 200 was higher compared to other categories, demonstrating that B19V can replicate at a high level because of the lack of a functional immune system in these immunologically compromised patients (Table 2). However, in another investigation, the B19 viral load has been stated equal in all HIV-positive patients (19). This study also evaluated B19V characteristics in subgrouping of HIV patients, including pregnant women, general population, IV drug users, and elite controllers. This subgrouping was done considering the fact that the differences in the immunological and physiological status of these groups could be of great help in analysis. For example, elite controllers were considered the individuals with viral replication of undetectable levels without any ART and also with a CD4 level similar to healthy individuals (25). Here, it was found that the lowest mean of the B19 viral load was 334.9 ± 105.2 copies/mL observed in elite controller group ($p=0.036$) (Table 3). Also, it was shown that the elite controller samples had a significantly higher prevalence of IgM and IgG ($p=0.007$ and $p=0.036$, respectively), indicating an effective immune system functioning in these patients. On the other hand, in IDUs, the prevalence of IgG was at the lowest rate, which could be a sign of the compromised condition of the immune system.

As previously mentioned, anemia is one of the major complications following B19V infection. On the other hand, there are several significant abnormalities in HIV patients among which anemia is highly important. This abnormality can use as a predictive marker for disease progression and mortality. Different factors can affect hemoglobin level, including immunological and hemato-

logical conditions, opportunistic infections, and antiretroviral drugs and antimicrobials agents. Zidovudine (AZT) is an antiretroviral drug which can lead to anemia (26). In this study, AZT was the most commonly antiretroviral drug used by patients. Therefore, this study focused on these aspects in search for cases of anemia. Another factor affecting anemia was the B19V infection. Due to the inability of immune system to produce neutralizing antibodies against B19V, this infection could turn into a persistent infection or recurrent viremia which would further associate with chronic anemia (27). Abkowitz et al detected 17% B19V DNA in 30 HIV patients with hematocrits (HCT) of ≤ 24 and 31% of the patients with hematocrits less than 20. Therefore, they proposed that this virus probably causes severe anemia in HIV patients (28). In another similar investigation, B19V DNA has been found in 3 of 98 HIV patients with anemia. However, they found no significant association between anemia and B19V antibodies and DNA (19). Similar to this study, Pedranti et al indicated that there was no association between B19V DNA, IgM, and IgG with anemia. In our subgroup, anemia was observed at its highest rate of 6 cases (37.5%) and lowest of 0 (0%) cases in pregnant women and elite controllers, respectively. This high rate of anemia in pregnant women could be due to B19V infection resulting in weakened immune system finally failing to yield an adequate response to this infection (29).

Conclusion

The molecular and serological characterizations of B19V was investigated in HIV patients and healthy populations in Iran. Also, the association between anemia and B19V in HIV patients was evaluated. The results shrewdly indicated that the threat of B19V is limited in HIV patients receiving ART, whereas persistent B19 viremia in the untreated patients could lead to anemia. More detailed studies should be conducted to elucidate the role of B19V in HIV patients.

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Conflict of Interests

The authors declare that they have no competing interests.

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