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Detection of Parvovirus B19 Infection in Thalasemic Patients in Isfahan Province, Iran

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Abstract

Background: Parvovirus B19, a member of the Erythrovirus genus of *Parvoviridae* family, causes various clinical illnesses including infectious erythema, arthropathy, hydrops fetalis or congenital anemia, and transient aplastic crises. The B19 virus can be transmitted through respiratory secretions, blood products, and blood transfusion.

Objectives: The aim of this study was to detect the B19 virus in thalassemia patients in Isfahan, Iran.

Patients and Methods: The prevalence of parvovirus B19 infection was compared between thalassemia major patients and healthy subjects. Plasma samples were collected from 30 thalassemia patients from Isfahan, Iran. Thirty patients without any blood complications were considered as the control group. After DNA extraction from the plasma samples, polymerase chain reaction was performed for parvovirus B19 detection.

Results: The parvovirus B19-specific nucleotide sequence was detected in 6 patients (20%). None of the samples obtained from the 30 control subjects tested positive for B19.

Conclusions: In this study B19-Parvovirus infection were detected in patients with hematologic disorders in comparison with control subjects. Screening of patients with a high risk of parvovirus B19 infection can considerably reduce the incidence and prevalence of B19 infection.

Keywords: Parvovirus B19, Human, Thalassemia, Polymerase Chain Reaction, Iran

1. Background

Human parvovirus B19 was discovered by Yvonne Cossart in 1975 and was classified as a member of the *Parvoviridae* family in 1985. The B19 is a small, simple virus with a linear single-stranded DNA and is the only member of the *Parvoviridae* family, which is pathogenic to humans (1-5). This virus is resistant to most physicochemical factors and is mainly transmitted through respiratory secretions; however, it can be transmitted through blood and blood products (6, 7). During the production of blood products, the common deactivation stages of the virus cannot prevent parvovirus B19 transmission and the blood recipients may get infected. Thalassemia patients who often receive blood products are at risk of virus B19 infection. In addition, these patients are at risk of acquiring serious diseases transmitted through blood and blood products (7-9).

In immunologically healthy hosts, B19 infection can be benign or can occur without any signs or may cause erythema infectious in children and acute arthropathy in adults. In immunosuppressed patients, B19 infection may persist and lead to pure red cell aplasia, chronic anemia, and, less frequently, thrombocytopenia, pancytopenia, and neutropenia (10-12). During pregnancy, the virus can be transferred to the uterus, resulting in hydrops fetalis and fetal death (13). Patients with blood disorders are at risk of sever clinical diseases, especially those with chronic hemolytic anemia such as thalassemia, sickle cell anemia, and hereditary spherocytosis. In these patients, the rate of erythroid progenitor cell formation increases to compensate for the lysis of red blood cells. Parvovirus B19 infection can suppress erythropoiesis and induce acute erythroblastopenia, which is often referred to as transient aplastic crisis. This temporary halt in the production of red blood cells occurs only in patients with chronic hemolytic anemia due to the short lifespan of red blood cells. The risk of B19 transmission increases in these patients (5, 14, 15).

Us et al. reported the incidence of common B19 virus infection in leukemia, malignant lymphoma, chronic anemia, and thalassemia patients. Overall, their results showed that patients with malignant diseases, immu-

nosuppressive diseases, or hemolytic anemia are at high risk of B19 infection, and the relationship between these diseases and B19 infection has been confirmed (16). Most published studies in Iran have described the prevalence and detection of antibodies against parvovirus B19 by using serological methods such as enzyme-linked immunosorbent assay (ELISA). On the other hand, there is no information about the relationship between active B19 infection and thalassemia in Iran, and hence, it is very important to determine the rate of active B19 infection in apparently healthy individuals and thalassemia major patients.

2. Objectives

In this study, nested polymerase chain reaction (PCR) assay, which has high sensitivity, was used for the genomic detection of parvovirus B19 in thalassemia patients in the Isfahan province. Moreover, the relationships between possible risk factors such as age, gender, and history of blood transfusion and B19 infection were evaluated using statistical analysis. Any data on confirmation of B19 infection and other factors may be useful for the treatment, management, and prevention of B19 infection.

3. Patients and Methods

In this cross-sectional study, 30 patients were randomly selected from inpatients a hospitalized in the thalassemia and hemophilia wards in Seyed-Al-Shohada Hospital in Isfahan. These patients were clinically diagnosed as thalassemia patients. Moreover, 30 apparently healthy individuals without a history of thalassemia and in the same age range as the patients were selected as the controls. The indexes of blood factors of the B19-infected and uninfected patients were recorded and statistically analyzed. Whole blood samples were collected in vacuum tubes containing ethylenediaminetetraacetic acid and plasma samples were prepared by centrifugation at 1500 rpm for 5 min and were aliquoted (500 μL) in microtubes and stored at -20°C until analysis. Demographic date on gender, age, duration of disease, laboratory findings regarding the number and characteristics of red and white blood cells, and history of blood transfusion were recorded in data forms. Statistical analysis of the data were performed using SPSS software (ver. 15). The chi-square test at a confidence level of 95% ($P < 0.05$) and student t-test were used.

Extraction of viral DNA from the plasma samples from the patients was performed using the GF-1 viral nucleic acid extraction kit (Vivantis, USA), according to the manufacturer instructions and the extracted DNA was stored at -70°C. The nested PCR method described by Zerbini et al. (1996) and Wen et al. (2011) was used for detecting specific viral DNA sequences (17, 18). This method is >10,000 times more sensitive than the standard PCR method. To control contamination and verify the absence of inhibitors of PCR, distilled water was used as the negative control and a positive serum sample containing the B19 genome was used as the positive control. The materials required in this method are as follows: 25 μL of reaction mixture containing 2.5 μL of 10× PCR buffer, 1 μL of 10 mM dNTP Mixture, 1.5 μL of 50 mM $MgCl_2$, 0.5 μL of *Taq* DNA polymerase (5 U/μL), 1 μL of each forward and reverse primer (10 pmol/μL), 14.5 μL of deionized water (TAG Copenhagen, Denmark), and 3 μL of extracted DNA sample. 1A and 2S primers were used at the first stage, and 3A and 4S primers were used at the second stage (Table 1). The annealing temperature and Mg^{2+} concentration were optimized by gradient PCR. Thirty-five cycles were used in both the stages of PCR with the following temperature conditions: 95°C for 1 minute, 55°C for 1.5 minutes, and 72°C for 1 minute, followed by final extension at 72°C for 7 minutes. At the second stage of nested-PCR, 2 μL of the recent reaction mixture was added to 23 μL of the PCR mixture with 3A and 4S primers.

Finally, the PCR products were analyzed by gel electrophoresis in 2% agarose gel and were evaluated on the basis of the position of the DNA marker bands on the gel, the amount of product in the first stage was 1112 bp and that in the second stage was 104 bp.

4. Results

This study included 60 subjects comprising 30 thalassemia patients and 30 healthy individuals. The thalassemia subjects comprised 15 (50%) female and 15 (50%) male patients, whereas the control group comprised 12 (40%) female and 18 (60%) male patients. The B19 infection rate in the thalassemia patients was 20%. None of the healthy subjects tested positive for B19. The Chi-square test showed a significant difference between the thalassemia and control groups (Table 2).

Parvovirus B19-infected and uninfected patients in the thalassemia group were analyzed in terms of gender, age, and history of blood transfusion by using the chi-square test. Statistical analysis of the possible risk factors for B19 infection showed no significant differences in gender, age, and history of blood transfusion between the B19-infected and uninfected patients (Table 3).

The hematological parameters in the participants are presented in Table 4. The Student *t*-test was used to assess the differences in the quantitative variables including the values of the hematological parameters investigated between the B19-infected and uninfected patients. Differences in the descriptive indicators of the hematological parameters between the B19-infected and uninfected thalassemia patients are shown in Table 5.

Table 1. Primer Sequences and PCR Product Fragment Sizes for B19

Primer/Gene Location	Base Sequence '3 → '5	PCR Fragment Size, bp
Upstream primer		1112
1A 2095 - 2924	CTTTAGGTATAGCCAACTGG	
2S 4016 - 3997	ACACTGAGTTTACTAGTGGC	
Downstream primer		104
3A 3187 - 3206	CAAAAGCATGTGGAGTGAGG	
4S 3290 - 3271	CCTTATAATGGTGCTCTGGG	

Table 2. Parvovirus B19 Infection Frequency Distribution Between the Thalassemia and Control Groups

Patient Groups	Number of Sampled Patients, %	Number of B19-Positive Patients, %	P Value
Thalassemia	30 (50)	6 (20)	0.01
Control	30 (50)	0 (0)	0.01
Total	60 (100)	6 (10)	-

Table 3. Demographic Data of Thalassemia Patients[a]

Patient Characteristics	Demographic Data	Negative Result of Nested-PCR	Positive Result of Nested-PCR	P Value
Age distribution, y				0.401
1 - 20	8 (26.7)	7 (29.2)	1 (16.7)	
20 - 40	12 (40)	9 (37.5)	3 (50)	
40 - > 60	10 (33.3)	8 (33.3)	2 (33.3)	
Gender				0.326
Female	15 (50)	11 (45.8)	4 (66.7)	
Male	15 (50)	13 (54.2)	2 (33.3)	
History of blood transfusion				0.702
Yes	25 (83.3)	20 (83.3)	5 (83.3)	
No	5 (16.4)	4 (16.7)	1 (16.7)	
Total	30	24	6	

[a]Values are presented as No (%).

Table 4. Descriptive Indicators of Hematological Parameters in the Study Subjects[a]

Indicators	Thalassemia Group (N = 30)	Control Group (N = 30)	df	t	P Value
WBC, $\times 10^3/\mu L$)	4.6 ± 7.9	1.12 ± 6.9	58	1.1	0.26
RBC, $\times 10^6/\mu L$	0.59 ± 3.5	0.54 ± 5.2	58	11.4	0.001
HGB, g/dL	1.9 ± 9.4	1.7 ± 14.4	58	10.59	0.005
HCT, %	4.3 ± 29	4.1 ± 43.1	58	12.7	0.003
PLT, $\times 10^3/\mu L$	153 ± 403	63 ± 301	58	3.38	0.001
M.C.V, fl	10 ± 82	4.18 ± 89	58	3.45	0.002
M.C.H, pg	3.3 ± 26.8	1.5 ± 30	58	4.7	0.001

[a]Values are presented as mean ± SD.

Table 5. Differences in the Descriptive Indicators of Hematological Parameters Between B19-Infected and Uninfected Thalassemia Patients[a]

Indicators	Positive Nested-PCR	Negative Nested-PCR	df	t	P Value
WBC, $\times 10^3/\mu L$	2.8 ± 5.1	4.8 ± 8.6	58	1.7	0.09
RBC, $\times 10^6/\mu L$	0.84 ± 3.6	0.53 ± 3.5	58	0.31	0.75
HGB, g/dL	1.9 ± 9.3	2.02 ± 9.4	58	0.01	0.98
HCT, %	4.6 ± 27.7	4.3 ± 29.3	58	0.82	0.41
PLT, $\times 10^3/\mu L$	100 ± 359	163 ± 414	58	0.78	0.44
M.C.V, fl	8.3 ± 77.2	10.9 ± 83.2	58	1.26	0.21
M.C.H, pg	2.9 ± 25.4	3.3 ± 27.1	58	1.15	0.25

[a]Values are presented as mean ± SD.

5. Discussion

The present study included 30 thalassemia major patients from Isfahan province. PCR analysis showed that parvovirus B19-specific nucleotide sequences were present in 20% of the thalassemia patients, which is consistent with the results of previous studies conducted in other countries. Although the immunoglobulin G (IgG) concentration was high in the serological tests, the immunoglobulin M (IgM) and DNA concentrations were reported to be in the same range. In a study by Siritantikorn et al. in Thailand, some evidence of active B19 infection was found in 13% of the thalassemia patients. Moreover, the prevalence of anti-parvovirus B19 IgG was 38% and that of anti-parvovirus B19 IgM was only 4% in the patients. The prevalence of viral DNA and anti-B19 IgG was higher in thalassemia patients with no history of blood transfusion than in those who received blood products, which can be owing to the low prevalence of B19 infection in blood donors in Thailand (6).

In another study by Kishore et al. in India, the prevalence of anti-B19 IgM was 41.1% in the thalassemia major patients and 6.2% in the control patients without any blood disorders. Moreover, the prevalence of anti-B19 IgG was 81% in the study patients and 21% in the control patients (9). In a study by Regaya in Tunisia, the prevalence of anti-B19 IgG was 56.5% in patients with chronic blood disorders, while viral DNA was reported in only 8.7% of the patients, which is lower than that reported in our study (5). In another study in Turkey, the prevalence of viral DNA and IgM antibodies was 29.1% (16). Furthermore, in a study by Kishore et al., the prevalence of anti-B19 IgM, which represents new or acute infection, was 17.1% in children with hematologic malignancies (19). In a study by Gupta et al. B19 DNA was reported in 27.3% of 66 patients with aplastic anemia, as compared to 2.2% of the control patients. Furthermore, the prevalence of anti-B19 IgM was 25.8% in the study patients and 2.2% in the control patients (20).

In the present study, statistical analysis of possible risk factors for B19 infection showed no significant differences in gender, age, history of blood transfusion, and hematological parameters between the B19-infected and uninfected patients. However, the white blood cell count, hemoglobin level, hematocrit level, platelet count, mean corpuscular volume, and mean corpuscular hemoglobin level were decreased in thalassemia patients with parvovirus B19 DNA.

Many studies reported that the prevalence of parvovirus B19 infection increased with age among populations (6). Moreover, an increase in the prevalence of parvovirus B19 infection associated with blood transfusion and blood products has been reported in Hong Kong and Taiwan (21, 22).

Some previous studies conducted in various countries showed that the prevalence of B19 infection in the investigated individuals was very high, which may be owing to those studies were conducted in groups at high risk of B19 infection such as patients suffered from hematologic disorders, immunodeficiency or leukemia. This may be owing to repeated blood transfusions in patients with hematologic disorders or immunodeficiency and lymphoid tumor lines in the patients with leukemia.

B19 infection is common, but most individuals are not actively infected, as observed in previous studies conducted in Iran and other countries, in which the prevalence of anti-B19 IgG and viral DNA was reported to be 92% and 0%, respectively, in apparently healthy individuals. Similarly, in the present study, none of the control patients tested positive for B19 infection. B19-DNA was detected in 3 out of 64 healthy controls in Vietnam. The prevalence of B19-infection was significantly higher in HBV-infected patients than healthy control group (24.1% vs 4.7%). (21.4%) (23). Generally, parvovirus B19 is transmitted through respiratory secretions, but this virus can also be transmitted through blood transfusions. Patients who have chronic anemia and hematologic malignancies such as leukemia, thalassemia, and hemophilia and require continuous blood infusions may receive units of red blood cells containing parvovirus B19. The probability of acquiring transfusion-transmitted diseases is associated with exposure to infected blood units, which depends on the prevalence of asymptomatic infected blood donors in the general population. The prevalence of B19 in the blood of healthy donors has been reported to be in the range of 1 in 20,000 to 1 in 500,000 donors (9, 24).

Data on the relationship between B19 infection and blood disorders have been reported previously. Hence, it is needed that, the cases of chronic anemia and hematologic malignancies in which parvovirus B19 is involved to be distinguished in order to replace a suitable treatment for the patients. In cases of transient aplastic crisis caused by parvovirus B19, erythrocyte transfusions are usually used for treatment, and intravenous immunoglobulin infusions containing antibodies neutralizing the human parvovirus are used to treat or mitigate persistent signs of B19 infection in immunocompromised or anemic patients (1, 25).

It is recommended that all patients with hematological disorders should consider undergoing PCR screening for the presence of antibodies against the B19 virus and B19-specific nucleotide sequences for better diagnosis and treatment. Currently, there exist no guidelines or protocols for B19 screening during blood donations. However, it is recommended that further studies be performed to confirm the findings of this study and other hypotheses (the B19 virus shows great tropism for erythroid progenitor cells and causes an increase in these cells in thalassemia patients, predisposing them to B19 replication).

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Footnotes

Authors' Contributions: Study concept and design: Mohammad Reza Mahzounieh. Data acquisition: Miss Nikoozad and Mohammad Reza Ghorani. Data analysis and interpretation: Mohammad Reza Mahzounieh. Manuscript drafting: Razieh Nikoozad. Study supervision: Mohammad Reza Mahzounieh.

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