Frequency and Genotype of Human Parvovirus B19 among Iranian Hemodialysis and Peritoneal Dialysis Patients

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Keywords

Hemodialysis patients · Peritoneal dialysis patients · Human parvovirus B19 · Serology · Genotype · Anemia

Abstract

Objectives: The aim of this study was to evaluate the frequency and genotype of human parvovirus B19 and its relation with anemia among Iranian patients under dialysis. **Methods:** Fifty hemodialysis (HD) and 33 peritoneal dialysis (PD) patients were enrolled. B19 IgG and IgM antibodies were assessed by ELISA, and the presence of B19 DNA was evaluated by nested PCR. PCR products were sequenced directly and phylogenetic analysis was performed. **Results:** In the HD group, the prevalence of B19 antibodies was 54% for IgG and 4% for IgM. B19 DNA was detected in 10% of the cases, and 10% showed B19 IgG and viremia simultaneously. In the PD group, the prevalence of B19 IgG and IgM was 57.6 and 0% respectively, whereas B19 DNA was found in 12.1% of the group. A total of 9.1% showed B19 IgG and viremia

concurrently. There was no significant difference regarding anemia and B19 infection in either group. All B19 isolates were clustered in genotype 1A. **Conclusion:** Our findings indicate that B19 infection plays no role in leading chronic anemia in dialysis patients. However, persistent B19 viremia and the circulation of the same strains in dialysis patients may indicate a potential risk for the contamination of dialysis equipment and nosocomial spread of B19 infection within dialysis units.

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Introduction

Parvovirus B19 (B19) is a small nonenveloped single-stranded DNA virus belonging to the Parvoviridae family and Parvovirinae subfamily [1]. Respiratory spread appears to be the most common route of B19 transmission and the virus is easily transmitted with close contact but can also be acquired through blood and blood prod-

ucts. The vertical route of transmission from mother to fetus and nosocomial transmission of the virus have also been documented [2, 3].

In immunocompetent subjects, acute B19 infection is usually mild and associated with transient anemia, arthritis, and rash. It was shown that after acute B19 infection, B19 DNA remains in bone marrow, kidney, liver, myocardium, and other organs [4–6]. In contrast, in immunosuppressed cases, acute B19 infection may be associated with severe symptoms. Generally, solid organs or stem cell recipients are at increased risk for viral reactivation that may originate from a persisting previous viral infection or from transmitted pathogens through the transplantation [7]. Due to notable tropism of B19 to human bone marrow and erythroid precursor cells for replication, anemia is considered the main complication of B19 infection [8]. B19 infection can also cause aplastic crisis in patients with chronic hemolytic anemia.

Patients with renal failure on dialysis have disruptions in their immune system due to the immunosuppressive effects of uremia, deficient erythropoietin production, and significantly decreased erythrocyte survival [9, 10]. Therefore, dialysis patients reveal increased susceptibility to acute and chronic anemia after B19 infection. Moreover, B19 infection can induce aplastic crisis in dialysis patients similar to hemolytic anemia cases [11]. On the other hand, some authors believe that erythropoietin administration during B19 infection can facilitate viral replication [12]. Nosocomial spread of infection in the dialysis unit is also a potential threat, although this is not well explained in the literature [3]. Finally, persistent B19 infection in patients on chronic dialysis could be clinically symptomatic after renal transplantation and the consumption of immunosuppressants [13]. Therefore, it seems that B19 infection in dialysis patients can be noticeable. Nowadays, persistent B19 infection has a special significance and is of great interest in different settings, including dialysis patients [14–18].

Three distinct genotypes of B19 have been described: genotype 1, with subtypes 1A and 1B, genotype 2, and genotype 3, with subtypes 3A and 3B [19, 20]. Available data show some geographical restrictions for B19 genotypes. Genotype 1 is the most common B19 genotype detected worldwide, while genotype 2 has been reported from western countries like Germany [21], Finland [22], the USA and Brazil [23, 24]. Genotype 3 has been restricted to samples from French and Brazilian patients and Ghanaian blood donors [19, 24, 25].

However, there are insufficient data regarding the distribution and molecular epidemiology of B19 in dialysis patients, while this population may be vulnerable to anemia due to B19 infection. We therefore aimed to evaluate the frequency of B19 in Iranian hemodialysis (HD) and peritoneal dialysis (PD) patients, and to explore their molecular characterization as well as its relation to anemia.

Patients and Methods

Study Population

In this cross-sectional study, 83 dialyzed patients (50 on HD and 33 on PD) who were referred to the main dialysis units of Tehran, Iran, were consecutively enrolled in the study from September 2014 to November 2014. A questionnaire was provided to collect epidemiological and clinical data such as age, gender, length of time on dialysis, and previous medical history. The study protocol was approved by the ethical committee of the Iranian Society for Support of Patients with Infectious Diseases and informed consent was obtained from all patients. Anemia in dialysis patients has been defined by the World Health Organization [26] as a hemoglobin concentration <13.0 g/dL for adult males and postmenopausal women, and <12.0 g/dL for premenopausal women.

Serological Assessment

All plasma samples were tested for B19 virus-specific antibodies using enzyme-linked immunosorbent assay (ELISA) kits for the detection of B19 IgG and IgM antibodies (EUROIMMUN, Lübeck, Germany). These assays were performed according to the protocols provided by the manufacturer. The serum hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc), and hepatitis C antibody (anti-HCV) levels were also detected by ELISA. The commercial enzyme immunoassay kits used were from Dia.Pro Diagnostic BioProbes (Milan, Italy). Human immunodeficiency virus (HIV) infection was diagnosed by ELISA (MP Biomedicals, Illkirch, France) with confirmatory Western blot analysis (Diaplus, San Francisco, CA, USA).

DNA Extraction and PCR

B19-DNA was extracted from 200 μ L of plasma using the RTP DNA/RNA Virus Mini Kit (Invitex, Berlin, Germany) according to manufacturer's instructions. The extracted DNA was stored at -20°C before PCR analysis.

In order to evaluate the suitability of extracted DNA, β -globin gene amplification was performed using PCO3 (5'-ACACAACGTGTGTTCACTAGC-3') and PCO4 (5'-CAACTTCATCCACGTTCACC-3') primers which amplify a 110-bp fragment. PCR was carried out in a 25- μ L amplification mixture containing 1 μ L of extracted DNA, 1.5 mM of MgCl_2 , 15 mM of Tris-HCl (pH 8.0), 0.2 mM of dNTP, 50 mM of KCl, 10 pmol of each primer, and 1.5 U of Taq polymerase (YTA PCR Master Mix). β -Globin-positive samples were subjected to nested PCR.

A fragment of the DNA sequence coding for the major capsid protein (VP2) of parvovirus B19 was amplified by nested PCR. The first round of PCR was carried out using the primer pair TJI

Table 1. ELISA and PCR results in HD and PD patients

Dialysis patients	B19 IgM, <i>n</i> (%)	B19 IgG, <i>n</i> (%)	B19 DNA, <i>n</i> (%)	B19 IgM + IgG, <i>n</i> (%)	B19 IgG + B19 DNA, <i>n</i> (%)
HD (<i>n</i> = 50)	2 (4)	27 (54)	5 (10)	1 (2)	5 (10)
PD (<i>n</i> = 33)	0 (0)	19 (57.6)	4 (12.1)	0 (0)	3 (9.1)

(5'-TTCTTTTCAGCTTTTAGG-3' [nucleotides 3775–3792]) and R4154 (5'-TATTCCTGTGACATATTT-3' [nucleotides 4171–4154]), which were described previously by Melon et al. [27]. The first round of PCR was performed with 10 µL of DNA extract added to an amplification mixture containing 2.5 µL of 10× PCR buffer, 1 µL of MgCl₂, 0.75 µL of dNTP mix, 0.4 µL of Taq polymerase, 1 µL of each primer in a total volume of 15 µL (8.35 µL of distilled water). The PCR profile was an initial 5 min denaturation at 94°C, followed by 35 cycles of amplification, including denaturation for 30 s at 94°C, annealing for 40 s at 45°C, and extension for 50 s at 72°C. Strand synthesis was completed at 72°C for 10 min and stored in 4°C for 5 min. A total of 2 µL of the first-round PCR products was then subjected for the second-round PCR under the same conditions but with the primers TJ2 (5'-TATAAGTTTCCTCCAGTGCC-3' [nucleotides 3818–3837]) and TJII (5'-TGAATTGCATGGTCTTCATG-3' [nucleotides 3975–3956]) in a total volume of 23 µL of amplification mixture (16.35 µL of distilled water). The expected size of the second PCR product was 158 bp. Each batch included negative controls containing water and B19 plasmids as positive controls. The PCR products were electrophoresed on a 1.5% agarose gel with the 100-bp DNA ladder (SinaClon, Tehran, Iran), stained with ethidium bromide, and visualized by ultraviolet transillumination.

DNA Sequencing, Genotyping and Phylogenetic Analysis

Nested PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and then sequenced directly for both directions at Pishgam Biotech Company (Tehran, Iran) using the Sanger method. The phylogenetic analysis was based on B19 VP2 sequences of the patients described in this study and reference sequences of various B19 subtypes downloaded from the GenBank database.

Nucleotide sequences were aligned using the CLUSTAL W program in CLC Main Workbench 6.5 software (CLC Bio, Qiagen). The genetic distance was calculated using the Kimura 2-parameter matrix. A phylogenetic tree was subsequently constructed by the neighbor-joining method and tested with the bootstrap resamplings (1,000 replicates) using the MEGA program, version 5. The B19 sequences obtained in this study were deposited in the GenBank under accession No. KU891254-62.

Statistical Analysis

Statistical analyses were conducted using SPSS statistics software (version 16, Chicago, IL, USA). The χ^2 test or Fisher exact test was used to compare variables. Data are presented as the mean $\pm$ SD or, when indicated, as an absolute number and percentage. *p* values <0.05 were considered statistically significant.

Results

A total of 50 patients on HD (mean age 53.18 ± 16.44 years) and 33 patients on PD (mean age 53.66 ± 13.02 years) were enrolled in the study. The overall prevalence of B19-specific IgG in patients under dialysis was 55.4% (46 of 83) and of B19 IgM was 2.4% (2 of 83). B19 DNA was detected in 10.8% (9 of 83) of cases. One sample was positive for both antibodies, while 8 cases (9.64%) revealed B19 IgG and viremia concurrently. The mean Hb in HD and PD patients was 10.51 ± 1.57 and 11.23 ± 2.1 g/dL, respectively. Anemia was found in 86.74% of dialysis patients. Erythropoietin (EPO) was received by 60.2% cases. All HD patients were negative for HBsAg, anti-HBc, and anti-HIV. One HD patient had anti-HCV.

In the HD group, 38% were male and 62% were female. The mean duration of dialysis was 56.58 ± 52.46 months and the dialysis interval was 3 times a week. The prevalence of B19-specific immunoglobulin was 54% (27 of 50) for IgG and 4% (2 of 50) for IgM. The prevalence of B19 viremia was 10% (5 out of 50) in HD subjects. One sample was found to be positive for both antibodies, while 5 cases (10%) showed B19 IgG and viremia simultaneously. Anemia was present in 92% of the HD cases. In the present study, we did not observe a significant relation between the presence of B19 IgG, IgM and viremia and age, sex, mean duration of dialysis, and anemia.

In the PD group, 54.5% were male and 45.5% were female, and the mean duration of dialysis was 38.94 ± 35.21 months. The prevalence of B19 IgG and IgM was 57.6% (19 of 33) and 0% respectively, whereas B19 DNA was found in 12.1% (4 of 33) of this group. Three cases (9.1%) showed B19 IgG and viremia concurrently. Anemia was present in 78.8% of the PD patients. We did not find any significant relation between the presence of B19 IgG, IgM and viremia and age, sex, mean duration of dialysis, and anemia.

No significant difference between the PD and HD groups was found regarding B19 IgG, IgM, and viremia. Table 1 summarizes the ELISA and PCR results in the 2 groups.

Fig. 1. Phylogenetic tree from the B19 VP2 region of 9 human parvovirus B19 strains isolated from Iranian HD and PD patients. Reference sequences for 1A, 1B, 2, 3A, and 3B subtypes from the GenBank database were also included. The numbers next to the nodes of the tree represent bootstrap values (1,000 replicates). The Iranian sequences determined in this study are indicated by the IR prefix. IR-1 to 4 relate to PD patients and IR-5 to 9 were isolated from the HD group.

Phylogenetic analysis revealed that all B19 isolates in the PD and HD groups clustered to genotype 1, and were classified as subtype B19-1A (Fig. 1). We also identified the nucleotide and amino acid divergence in our isolates. Comparing the nucleotide sequences revealed the overall variation to be 0.10879 (range 0.00–0.19990). The mean intragenotypic nucleotide distance within Iranian isolates and other 1A isolates was 0.01167 (range 0.000–0.03267). The mean variation between Iranian strains was 0.00848 and the maximum distance was 0.01924.

By comparison of the amino acid sequences, the overall variation was 0.30818 (range 0.00–0.5877). The mean intragenotypic amino acid distance within Iranian isolates and other 1A isolates was 0.03154 (range 0.000–0.08894). The mean variation between Iranian strains was 0.02405 and the maximum distance was 0.06596.

According to Figure 1 and the genetic distance analysis on isolates detected from the PD center, IR-1 and IR-4 were the same, while IR-2 and IR-3 had a similar genetic distance and fell in another branch of the phylogenetic tree. This suggests that 2 different B19 isolates are circulating in our PD center. Regarding isolates recovered from 1 HD center, IR-8 and IR-9 clustered in 1 branch of the phylogenetic tree, which may indicate that these isolates are similar, while IR-7 from the same dialysis center revealed some genetic distance (0.02151) but was close to IR-8 and IR-9. Moreover, IR-5 and IR-6, which were obtained from another HD center, showed minimal genetic distance, demonstrating that they are only close to each other.

Discussion

This is the first report to investigate the serological and molecular characterization of parvovirus B19 in patients on HD and PD in Iran. The prevalence of B19-specific immunoglobulin was 54% for IgG and 4% for IgM, and B19 viremia was found in 10% of HD subjects, while 10% of HD cases showed B19 IgG and viremia simultaneously. In the PD group, the prevalence of B19 IgG and IgM was 57.6 and 0% respectively, whereas B19 DNA was found in 12.1%. B19 IgG and viremia was concurrently shown in 9.1% of PD cases. Moreover, in the present study we did not observe a significant relation between the presence of B19 IgG, IgM, and viremia and anemia in both groups.

Anemia is common in patients with chronic kidney disease under dialysis [28] but the morbidity and mortality mainly depend on the etiology of anemia and the stage

of renal disease [29]. The significance of B19 infection in patients with chronic renal failure remains to be clarified. Patients undergoing dialysis may have increased susceptibility to acute and chronic anemia after B19 infection. The immunosuppression state in dialysis patients due to underlying diseases and immunosuppressive treatment may prevent an effective antiviral immune response relating to persistent viral infection and chronic suppression of erythropoiesis with the development of chronic anemia or B19-related recurrent anemia [30, 31]. Potentially life-threatening transient aplastic crisis due to B19 infection is also a major concern in some dialysis cases [11]. Finally, persistent B19 infection in patients on chronic dialysis can become clinically manifested after renal transplantation and the administration of immunosuppressive medications [13].

Previous investigations have described the B19 distribution in different settings and shown a variable epidemiological pattern among populations [32–35]. However, most surveys mainly focused on the prevalence of B19 in kidney transplant subjects, and few studies have been carried out on the B19 frequency in dialysis patients. In a study by Małyszko et al. [32], B19 IgM and IgG antibodies were detected in 16 and 49% of dialyzed patients, respectively. There were no significant differences among dialyzed patients with and without acute or chronic B19 infection regarding Hb, erythrocyte count, and erythropoietin concentration.

In a study on 62 hemodialyzed patients, Guiserix et al. [36] reported that B19 infections play no role in leading chronic anemia in these subjects. Moreover, there was no correlation between time on dialysis and IgG antibodies.

Alves et al. [29] conducted a survey on 120 dialyzed patients (106 on HD and 14 on PD) and showed that the overall B19 seroprevalence was 67.5%. There was no significant correlation between B19 infection and anemia. They suggested that B19 infection creates little problem in dialyzed patients.

We previously carried out a study on HIV patients and a control group [35]. The prevalence of B19 IgG, IgM, and DNA in the control group was 25, 1.6, and 9.4%, respectively, while in the HIV group the prevalence of IgG, IgM, and B19 DNA was 11.1, 1, and 13.1%, respectively. B19 IgG and viremia was simultaneously shown in 3% of HIV cases. In comparison with the present study, we reported a higher rate of IgG, IgM, and B19 DNA, and concurrent presence of IgG and viremia. Therefore, the prevalence of parvovirus B19 is very variable and the degree of immunodeficiency can explain the differences in the IgG seroprevalence and viremia found in different studies. It

seems that dialysis patients have no problem in producing B19 antibodies in accordance with our results, while some studies have reported low rates of B19 IgG among HIV patients due to underlying immunological problems [34, 35]. However, persistent B19 infection with coexisting IgG and viremia was found commonly in our HD cases, possibly due to a qualitative defect of antibodies for neutralizing the virus [37, 38]. The clinical significance of B19 infection in patients undergoing dialysis is not clear, but persistent B19 viremia should be considered a potential risk through the contamination of dialysis equipment and subsequent threat to dialysis patients during transplantation procedures.

In agreement with other reports from most parts of the world, including Iran, genotype 1A was the only detectable genotype in this study group. In our survey, a low degree of nucleotide diversity among B19 strains was detected, which concurs with other reports (<2%) [39]. However, in comparison to our previous study conducted in HIV patients and normal controls, we report a higher degree of nucleotide diversity in samples isolated from dialysis patients. Moreover, we have identified the same strains isolated from 1 dialysis center, revealing the possibility of nosocomial spread of B19 infection among dialysis patients. There are limited studies on this issue, al-

though Ozeki et al. [3] described 1 HD case with transient aplastic crisis due to nosocomial B19 infection.

In conclusion, this study investigated the serological and molecular characterization and importance of parvovirus B19 infection in patients under dialysis in Iran. Our findings indicate that B19 infection plays no role in leading chronic anemia in dialysis patients. However, persistent B19 viremia and the circulation of the same strains in dialysis patients may demonstrate a potential risk for the contamination of dialysis equipment and nosocomial spread of B19 infection within dialysis units. Further studies with larger samples are required to clarify the nosocomial spread of B19 infection in dialysis units as a potential threat, which could be clinically symptomatic after renal transplantation.

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Disclosure Statement

No conflicts of interest exist for any of the authors.

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