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ORIGINAL ARTICLE

Investigation of human parvovirus B19 prevalence in a large healthy umbilical cord blood donors

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ABSTRACT

Background and Objectives: Umbilical cord blood (UCB) was used to source hematopoietic stem cells in the past. Despite the apparent advantages of UCB transplantation, virus reactivation poses a considerable danger in allogeneic hematopoietic stem cell transplantation (HSCT). Human Parvovirus B19 is regarded as a potential threat to UCB contamination. This study aimed to evaluate the prevalence of parvovirus B19 in cord blood donors by Semi-Nested PCR. This study is the first large-scale report of the B19 DNA in cord blood donors in Iran.

Materials and Methods: A total of 691 umbilical cord blood were collected under standard procedure. Then, DNA from buffy coat and plasma were extracted, and semi-nested PCR was performed for all samples.

Results: Two out of 691 samples (0.29%) indicated viremia in plasma and buffy coat.

Conclusion: In this line, designing and validating a quantitative PCR assay for detection, quantification, and discrimination of Human B19 DNA genotypes of cord blood donors is necessary to enhance the safety of this source of stem cells.

Keywords: Parvoviridae; Human parvovirus B19; Umbilical cord blood; Hematopoietic stem cells; Nested polymerase chain reaction

INTRODUCTION

Umbilical Cord Blood (UCB) has been an alternative source of hematopoietic stem cells (HSCs) with lower graft-versus-host disease (1). UCB can be used to treat patients, especially children with hematological malignancies, and reconstitute the blood system (2-4). UCB is more profitable than other HSCs sources due to its instant availability and less requirement for HLA matching, with lower GVHD (5); additionally, because it is harvested after infant delivery makes no risk to either mother or child (1). Despite many advantages for UCB transplantation, neutropenia and delayed immune reconstitution increase concerns about viral infection and related mortality (6). Viral reactivations are significant concerns of allogeneic hematopoietic stem cell transplantation (HSCT) (7).

Several viral infections, including cytomegalovirus (CMV), hepatitis B virus (HBV), hepatitis C

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virus (HCV), and HHV 3, 6, 7, and 8 are thought to be possible dangers to UCB contamination. Another viral pathogen involved in viral infectious complications is human Parvovirus B19 (5). Human parvovirus B19 is a small, non-enveloped virus transmitted via the respiratory tract, blood products, transplacental, and organ transplantation. Parvovirus B19 particles were first discovered in 1975 by Cossart while he was evaluating assays for hepatitis B virus in serum (8, 9). B19 is an Erythrovirus, belongs to the *Parvoviridae* family of small DNA viruses. It is an icosahedral, non-enveloped virus by 18-26 nm diameter (10, 11) composed of a single-stranded DNA genome of around 5500 nucleotides in length (12). DNA genome can be integrated into the host's genome and causes clinical features, such as erythema infectious (fifth disease) in children (10, 13), acute arthritis, and prolonged anemia in immunocompromised patients (8). However, chronic infection by B19 can be seen in subjects with immune dysfunction, such as patients with human immunodeficiency or congenital immunodeficiency or recipients of organ transplants under immunosuppressive drugs (14). The virus infection may be severe during pregnancy because of affects, leading to fetal anemia, abortion, and hydrops fetalis (8, 15).

One of the parvovirus B19 transmission routes is hand to mouth contact, so the women of childbearing age, mothers of small children, and nursery school teachers are at a high risk of B19 infection (16, 17). Although the most current way of B19 transmission in respiratory secretion, transplacental transmission is particularly important (8, 18). Parvovirus B19 is a widespread human pathogen. It is heat-stable and resists treatment with solvent and detergents, making it challenging to inactivate or remove the virus from blood or plasma (10), and it can be transmitted through blood products infusion (11, 17). Although B19 has been detected in none erythroid tissue such as myocardial cells, it has a unique affinity for erythroid progenitor cells and arrests erythropoiesis (13). The presence of human B19 DNA in cord blood could be a risk signal for viral transmission from mother to fetus, as well as a danger to recipients of cord blood stem cells. This study aimed to investigate human Parvovirus B19 infection by semi nested PCR assay in umbilical cord blood samples. This study is the first large-scale report of the B19 DNA in cord blood donors in Iran.

MATERIALS AND METHODS

Study samples. Six hundred ninety-one pregnant women, with an average age of 26 years old, were selected as UCB donors at Akbarabadi hospital in Tehran and Shooshtari hospital in Shiraz, Iran, at the time of admission. Before obtaining UCB samples, maternal patients provided informed consent. Women with a known history of infectious diseases, hepatitis, diabetes mellitus, abortion, or genetic disease were excluded. A 10 ml blood sample was taken from cord blood in sterile EDTA-containing tubes for each participant.

DNA isolation. In order to separate human cord blood mononuclear cells (CBMNCs), cord blood (CB) samples were centrifuged at 3000rpm for 10 minutes. Buffy coat, as well as plasma, were separated in different sterile tubes. DNA was extracted from buffy coats and the plasma using the High Pure Viral Nucleic Acid kit (Roche, Germany), according to manufacturer's instructions, and stored at -70°C. 10 µL of cloned BMV as an internal control was added to each sample. A previously confirmed DNA sample screened by B19 qPCR was used as a positive control. The positive and negative control was also included in each run. A nanodrop spectrophotometer (Nano Drop 1000, Thermo Fisher Scientific, USA) evaluated the quality of genomic DNA.

Real-time PCR assay for internal control. BMV cloned fragment internal control was evaluated by the Real-Time PCR method. A mix containing 5µl quantifies probe PCR master mix (2×), forward and reverse primer (0.4 µM), and BMV probe (0.2 µM) were prepared. Amplification was processed using the following thermal profile; initial denaturation at 95°C for 3 min, followed by 45 cycles of 10 seconds at 95°C and 30 seconds at 60°C.

Semi nested PCR assay. A semi-nested PCR revealed the presence of parvovirus B19 DNA in the first stage, which targeted 725bp DNA, and then amplified 717bp DNA in the second step (Table 1).

PCR amplification was performed in a 25 µL reaction consisting of 2.5 µL of 10× buffer with 1.5 mM $MgCl_2$, 0.5 µL of each primer with10 pmol/µL, 0.25 µL Taq DNA Polymerase (5U/µL), and 18.25 µL nuclease-free water. The thermocycler profile started with denaturation in 94°C for 5 minutes, followed by

Table 1. Sequences of Parvovirus B19 primers

Primer	Sequences
NS1 (forward-stage1)	5′-ATTGCATACAGACTTTGAGC-3′
NS1 (reverse-stage1 & 2)	5′-TCAGAGCTTTCACCACCAC-3′
NS1 (forward-stage 2)	5′-CAGACTTTGAGCAGGTTATG-3′

30 cycles at 94°C for 45 seconds, 60°C for 45 seconds, 72°C for 45 seconds. The final extension was set at 72°C for 10 minutes.

The gel electrophoresis was then done using a 2 percent agarose gel in TAE 1× buffer. After the second round of semi nested-PCR, a single 717 bp band was detected using the specific ladder.

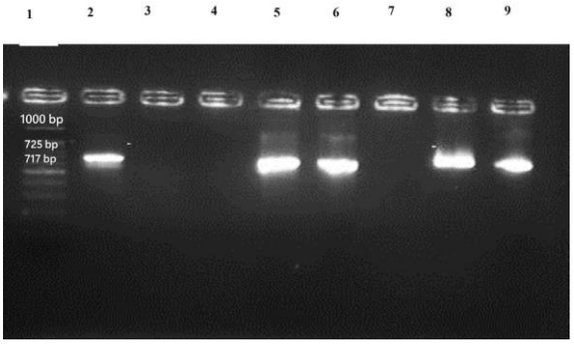

Fig. 1. Agarose gel electrophoresis of human parvovirus B19 Semi-nested Polymerase Chain Reaction of 2 positive samples, stained with ethidium bromide. Lane 1; denotes molecular weight (ladder); Lane 2, Positive control, lanes 3, 4 negative samples, lane 5, 6 positive samples (buffy coat), lane 7, negative control, lanes 8, 9 positive samples in the first step of nested PCR.

RESULTS

This study involved 691 cord blood samples from pregnant women by the average age of 26. Of those, 574 individuals had a natural delivery and 117 individuals delivered by surgery. Semi-nested PCR identified B19 DNA in 2 (0.29%) of 691 buffy coats and umbilical cord blood samples (Fig. 1). It means that these results for buffy coat coincide with those of each UCB plasma.

DISCUSSION

In recent years, the use of UCB as an alternative source of HSC transplantation for the treatment of hematopoietic disorders has increased (19). Viral infection and reactivations are major complications after allogeneic hematopoietic stem cell transplantation (HSCT) and are suggested to be associated with acute graft-versus-host diseases (aGVHD) and allograft rejection. The compromised immune system in allogeneic HSCT recipients, because of both the administration of serotherapy and immunosuppressive drugs to prevent or treat GVHD and rejection of the donor graft after HSCT make the patient more susceptible and vulnerable for viral reactivation (7, 20-22). There have been few reports on the prevalence of parvovirus B19 in umbilical cord blood reported from Iran (23). In this study, 691 UCB samples were screened by semi-nested PCR for detection of parvovirus B19 DNA. As other reports proved, PCR is the most sensitive method for parvovirus B19 detection (24, 25) and can detect 10-100 genome copies of viral particles (2). However, other methods such as direct hybridization or immunohistochemically assays cannot detect low-level viral loads below 10^4-10^5 copies/mL (9, 25). Parvovirus B19 is a pervasive human pathogen, and B19 antibody is prevalent in the general population, viremia or the presence of viral DNA is rare (9, 18, 26). The frequency of B19 viremia in voluntary blood donors has been estimated at rates of 1:167 to 1:35000, which equals to the prevalence of 0.006 to 1.3% (27), while one study discovered B19 DNA in bone marrow (B.M.) samples around 9% in healthy donors (28). Another study showed no evidence of B19 DNA in 13 samples of B.M. (26), although Tolfvenstam et al. found it 2% by PCR (29). The ability of parvoviruses to integrate into the host genome and induce chronic infections is well established. According to recent studies, B19 DNA can be detected in blood samples using ultrasensitive techniques up to 6 months after acute phase viremia. As shown above, healthy blood donors can exhibit b19 viremia in their serum. As a result, the presence of B19 DNA found by PCR in pregnant women may not be linked to a recent infection (30). The variation of B19 frequency depends on the immune status of the host, epidemic periods, and sensitivity of the methodology used (29). Parvovirus B19 could be able to associate in early spontaneous abortions along with human papillomavirus (HPV),

herpes simplex virus (HSV), and cytomegalovirus (CMV) (31, 32). These viruses were reported to have an asymptomatic infection with various clinical manifestations in patients (33-35). So there needs to be more investigation of B19 in UCB due to abortion capabilities.

Rollin et al. studied possible B19 transmission in patients with osteoarthritis following transplantation using mesenchymal stem cells (MSCs) from the B.M. Using a Real-Time PCR assay, they discovered one B19 DNA positive out of 18 (5%) (36). In Italy, fifty-eight fetal cord blood was screened by PCR-ELISA and In Situ Hybridization (FISH) assay. Their results showed that the prevalence of B19 DNA was 22.4% (18). The other study in Brazil has shown that the prevalence of human parvovirus B19 on UCB and placenta by nested PCR method was 3/138 (~2.2%) (37). Based on Finnish experiences, they provided 3.5% human Parvovirus B19 DNA in 311 umbilical cord blood samples, which was detected by the PCR method (38). On the other hand, two studies in Korea and Austria, respectively, used DNA-Chip and PCR methods reported no existence of parvovirus DNA in UCB samples (39, 40). A review article surveyed the relationship between recurrent abortion and human Parvovirus B19 DNA existence in Umbilical Cord Blood. Overall, they reported 10.9% in normal full-term pregnancies, in comparison with other sources such as placental tissue (8.3%), amniotic fluid (5.7%) (41). In our study, human Parvovirus B19 DNA was detected in 2 out of 691 UCB samples and made the prevalence of 0.29%. Parvovirus B19 viremia was detected in the serum of these 2 samples, which explains the active infection of infants. These results for UCB plasma coincide with those of each UCB buffy coat.

In a case-control study with cases of perinatal death and controls with live birth, nested in the Norwegian Mother and Child Cohort Study, a high prevalence of PCR positive B19 virus was detected in both cases and control groups (24% and 28.2%; respectively). However, studying the cord samples showed a vertical transmission of the B19 virus in 9.1% of cases and 11.9% of the control group. Also, this study questions the clinical importance of exclusive detection of viremia, as the rate of B19 positivity was not significantly different between study groups.

In Mexico, the study reported 379 cord blood of pregnant women, and just one human parvovirus B19 DNA was detected in cord blood that equals 0.26% incidence (42). A serology study was done in South-West of Finland on the serum of pregnant women as well as their cord blood and leads in 58% seropositivity for B19, and 3 out of 558 women had shown seroconversion during pregnancy, and IgM antibody for B19 was detected in 2 out of 3 seroconverted women's cord blood serum by ELISA technique (43). Several studies have been performed on UCB and B.M. to compare the risk of transmission of infection to recipients. Behbahani et al. reported in Shiraz a 2.9 percent prevalence of parvovirus B19 in cord blood by PCR (44) which is higher than our findings. According to Sohrabi et al.'s serological investigation, B19 specific IgG antibodies in pregnant women were lower in Tehran (55%) than in Shiraz (69.56%) (45). Viral replication in UCB recipients is possible. Due to active hematopoiesis and abundance of erythroid progenitor cells.

To summarize, the immunocompromised status of transplant recipients prior to cord blood transplantation was linked to an increased prevalence of infections. In this regard, designing and validating a quantitative PCR assay for detection, quantification, and discrimination of Human B19 DNA genotypes in cord blood donors is recommended to enhance the safety of UCB samples as a life-saving alternative source of hematopoietic stem cells.

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