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Investigation of the Prevalence of Human Parvovirus B19 DNA in Korean Plasmapheresis Donors

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Background : To ensure the safety of plasma derivatives, some countries have been screening for the human parvovirus B19 (B19V) antigen or DNA in blood donors. We investigated the prevalence of B19V DNA and anti-B19V antibodies in Korean plasmapheresis donors to evaluate the necessity of B19V DNA screening test.

Methods : Plasma samples were collected between March and July 2008 from 10,032 plasmapheresis donors. The B19V DNA test was performed using the LightCycler 2.0 (Roche, Germany) with quantification kits. Anti-B19V IgM and IgG were tested in 928 randomly selected samples from the 10,032 donors using recomWell Parvovirus B19 ELISA IgM, IgG assay (Mikrogen, Germany). RecomLine Parvovirus B19 LIA IgG, IgM assay (Mikrogen, Germany) was used to analyze the epitopes of antibodies in donors showing positive results for B19V DNA and anti-B19V antibodies. DNA sequencing was performed to identify the genotypes.

Results : The prevalence of B19V DNA was 0.1% (10/10,032). Virus titers in B19V DNA positive donors were less than 10^5 IU/mL (range: 2.7×10^1-3.2×10^4 IU/mL) except for 1 donor (1.33×10^8 IU/mL). All the isolated B19V DNAs from 6 donors were identified as genotype I. Nine out of 10 B19V DNA positive donors also possessed anti-B19V IgG only or IgG and IgM. The prevalence of anti-B19V IgG was 60.1% (558/928).

Conclusions : The prevalence of B19V DNA in Korean blood donors was not high and most donors also possessed neutralizing anti-B19V antibodies. Thus, the implementation of a B19V screening test for Korean blood donors does not appear to be imperative. *(Korean J Lab Med 2010;30:58-64)*

Key Words : *Human parvovirus B19, Plasmapheresis donors, Prevalence*

INTRODUCTION

The safety of plasma derivatives has been reinforced with more stringent donor questionnaires, implementation of nucleic acid testing (NAT), and treatment of pathogen inactivation and elimination (PI/PE) by plasma fractionation. However, human parvovirus B19 (B19V) transfusion-transmitted infection cases associated with plasma derivatives have been occasionally reported [1-3], particularly in patients with increased risks of infection, such as infants and immunocompromised or pregnant patients. The prevalence of B19V DNA among blood donors has been reported as 0.003-0.9% [4] and the anti-B19V antibody frequency as 30-75% globally [5-8]. The US Food and Drug administration (FDA) guidelines and the European regulatory requirements recommend that the viral load of B19V in the manufacturing of pooled plasma should not exceed 10^4 IU/mL [5]. The Japanese Red Cross has been screening B19V antigen since 1985 [6] and the German Red Cross implemented B19V mini pool NAT (96 pool) for blood donor screening in 2000 [7]. Additionally, many other

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countries have been implementing B19V large pool NAT for pooled plasma samples and products that are manufactured [8].

In Korea, there is no regulation to ensure the safety of plasma derivatives for B19V. The Korean Food and Drug Administration has been concerned with establishing new guidelines to limit B19V contamination in pooled plasma samples and manufacturing products. Some Korean authors have reported the occurence of B19V antigen in plasmapheresis donors [9] and anti-B19V antibody in Korean patients [10]. We investigated the prevalence of B19V DNA in Korean plasmapheresis donors to evaluate the necessity of B19V screening.

MATERIALS AND METHODS

1. B19V DNA test

Plasma samples were collected between March and July 2008 from 10,032 plasmapheresis donors from 11 Korean Red Cross blood centers. The study was approved by the Institutional Review Board (IRB) and written informed consent was obtained from all the participants. Pooled plasma aliquots of 300 μL from each of the 24 donors were made by automated pooling using the Tecan Genesis RSP 150 (Tecan, Mannedorf, Switzerland). Two hundred microliters of each sample was used for DNA extraction and synthetic nucleic acid was added to the sample as an internal control. MagNa Pure LC (Roche, Mannheim, Germany) and MPLC total nucleic acid isolation kit (Roche) were used for nucleic acid extraction. Real-time PCR was used for the amplification and detection of the amplified products using the Light Cycler 2.0 (Roche) with the Parvovirus B19 quantification kit (Roche). The samples that showed a positive result in the mini-pool screening test were retested individually. The samples that tested positive in the individual tests were tested an additional 3 times.

2. Anti-B19V antibody immunoassay

We randomly tested 928 samples from 10,032 donors for the anti-B19V antibody using Behring ELISA Processor III (Dade Behring, Marburg, Germany) with recomWell Parvovirus B19 ELISA IgM, IgG kits (Mikrogen, Neurid, Germany). RecomLine Parvovirus B19 LIA IgG, IgM kits (Mikrogen) were used to analyze antibodies against VP-2, VP-N, VP-1S, VP-2r, VP-C, and NS-1 epitopes in donors showing positive results for both B19V DNA and anti-B19V antibodies. All the tests were performed according to the manufacturers' instruction. The results were interpreted using the criteria outlined in the package insert from the manufacturer (U/mL sample >24, positive; U/mL sample <20, negative; 20≤ U/mL sample ≤24, borderline).

3. Retrospective and follow-up tests

To observe the changes of B19V DNA and anti-B19V antibody titers following B19V infection in donors that showed positive B19V DNA test results, a restrospective study in the 6 repeat donors using repository samples and follow up study in 5 donors, including first time donor were undertaken. Follow-up samples involved subsequent blood collection at 12-24 weeks after the index donation time with 2-18 week intervals.

4. B19V genotyping

B19V DNA sequencing of the samples obtained from the 6 donors was performed because the volume of the sample was insufficient. B19V viral DNA was prepared by nested-PCR amplification of a 1,100 bp region spanning the NS1-VP1u junction with the primers shown in Table 1 [11, 12]. The primary PCR round was performed in a 50 μL-reaction mixture containing 5 μL of DNA, 25 pmol of each primer (e1855f and e2960r), and 3 U of AmplitaqGold polymerase (Applied Biosystems, Foster City, CA, USA). The reaction was performed with 1 cycle at 94℃ for 6 min followed by 30 cycles of 94℃ for 30 sec, 50℃ for 30 sec, and 72℃ for 1 min, and a final extension at 72℃ for 7 min. The primary PCR product (5 μL) was then amplified with primers e1863f and e2953r under the same conditions with

50 cycles of 94℃ for 30 sec, 55℃ for 30 sec, and 72℃ for 50 sec. The PCR products were electrophoresed in agarose gel and purified using the QIAquik gel extraction kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

The purified PCR products were sequenced using a Big Dye Terminator cycle sequencing v3.1 kit (Applied Biosystems) and ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions.

B19V sequences were aligned using the BLAST program (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/blast/blast.cgi). Prototype B19 sequences from GenBank were used as reference sequences (GenBank accession numbers were as follows: genotype 1, M13178; genotype 2, AY044266; genotype 3, AY-083234) [11, 12].

Table 1. Primers used in PCRs and sequencing of human parvovirus B19

Gene	Primer name	Nucleotide sequence (5′-3′)	Location* (nucleotides)
NS1/VP1u	e1855f	CACTATGAAAACTGGGCAA	1855-1873
	e2960r	ACAATTCTTCATCTGCTAC	2960-2978
	e1863f	AAACTGGGCAATAAACTACAC	1863-1883
	e2953r	CTTCATCTGCTACCGTCCAA	2953-2972
	n-P5f	CGTGAACTGTTAGTTGGGGTTG	2355-2377
	n-P5r	AATTGCTGATACACAGCTTTAG	2690-2669

*The location refers to the Pvbaua sequence (accession no. M13178) except for primers n-P5f and n- P5r, which refer to the HV sequence (accession no. AF162273).
Abbreviations: f, forward primer; r, reverse primer.

RESULTS

1. The prevalence of B19V DNA

Ten out of 10,032 plasmapheresis donors showed positive results in B19V DNA test (0.1%). The titers of B19V DNA in 9 of 10 donors were low (range, 27–3.2×10^4 IU/mL), and 1 donor showed high virus titer (1.33×10^8 IU/mL) (Table 2).

2. The prevalence of anti-B19V antibodies

Nine of the 10 donors who showed positive results in B19V DNA test possessed high titers of anti-B19V IgG, and 7 of them also showed anti-B19V IgM. The rate of positive identification of anti-B19V antibody (IgG only or IgG and IgM) among the donors was 60.1% (558 of 928 donors). The anti-B19V antibody levels in elderly donors were higher than those of the young donors (Table 3). The pattern of reactivity to B19V antigen epitopes in the blot assay (line-immuno assay, LIA) of the 8 donor samples revealed a variable status in B19V infection (Table 4). Donors 8 (D8) and 9 (D9) presented strong reactivity to the VP2r and VPc antigen epitopes with high titer of B19V IgM, thereby suggesting an early stage of B19V infection.

Table 2. Quantitative PCR results of 10 confirmed human parvovirus B19 positive cases

Concentration of DNA (IU/mL)	Donor ID	Gender	Age	B19V DNA (IU/mL)		Anti-B19V (U/mL*)	
				Mean	95% CI	IgM	IgG
10^1-10^2	Donor1	M	29	2.72×10^1	0.96×10^1-5.23×10^1	Negative	147.34
10^2-10^3	Donor2	F	28	1.39×10^2	0.51×10^1-2.78×10^2	38.86	392.83
	Donor3	M	25	4.25×10^2	1.15×10^2-7.36×10^2	Negative	151.54
	Donor4	F	25	4.60×10^2	4.06×10^2-5.15×10^2	27.01	163.72
	Donor5	M	24	8.01×10^2	6.55×10^2-9.47×10^2	83.61	162.81
	Donor6	F	27	8.15×10^2	2.15×10^1-1.61×10^3	96.04	153.13
10^3-10^4	Donor7	M	42	1.85×10^3	1.53×10^3-2.17×10^3	123.44	156.00
	Donor8	M	31	2.33×10^3	1.35×10^3-3.31×10^3	124.98	140.14
10^4-10^5	Donor9	F	24	3.20×10^3	2.70×10^4-3.69×10^4	128.31	77.36
$\geq10^5$	Donor10	F	24	1.33×10^8	1.05×10^8-1.62×10^4	Negative	Negative

*U/mL sample >24, positive; U/mL sample <20, negative; 20≤ U/mL sample ≤24, borderline.
Abbreviations: B19V, human parvovirus B19; CI, confidence interval.

In contrast, Donors 1 (D1), 3 (D3), and 4 (D4) showed reactivity to the NS-1 antigen epitopes with high titer of anti-B19V IgG, thereby suggesting latent or past stage B19V infection.

3. Retrospective and follow-up study

The results of the retrospective and follow-up test are shown in Table 5. B19V DNA titers reduced at the following donation in most donors. The examination of high anti-B19V IgG titers in D1, D2, and D3 revealed steadiness of the antibody titer and low viral loads. Donor 10 (D10) showed the highest titer of B19V DNA (1.33×10^8 IU/mL) but did not possess any B19V antibodies at the index time. However, at the time of follow-up, 13 weeks after the index time, the virus titer abruptly decreased to 2.7×10^2 IU/mL and revealed the presence of anti-B19V IgG. D8 and D9 did not show evidence of B19V infection in archived samples, which suggested recent infection stage at the index time. D2, D5, and D8 showed concurrent decreasing titers of B19V DNA and anti-B19V IgM. The results of the retrospective and follow-up studies revealed the presence of long-lasting neutralizing anti-B19V IgG in spite of the presence of B19V DNA (Table 4).

Table 3. Prevalence of human parvovirus B19 antibodies in plasmapheresis donors

Age	N of donors	Only IgG positive		IgG and IgM positive	
		Number	%*	Number	%*
16-19	30	15	50.00	0	0.00
20-29	630	356	56.90	5	0.78
30-39	164	106	64.85	1	0.61
40-49	58	37	64.41	1	1.69
50-59	35	27	77.14	0	0.00
≥60	11	10	90.91	0	0.00
Total	928	551	59.38	7	0.75

*proportion (%) of antibody positively identified within the same age group.

4. B19V sequence analysis

The genotypes of B19V DNA taken from the 6 donors were identified as the G1 genotype.

DISCUSSION

The safety of plasma derivatives with regard to B19V has been a major concern to date. Contamination with a high titer of B19V can cause serious problems in patients with an increased risk of infection such as infants and those who are immunocompromised or pregnant. There were some reports of transfusion-transmitted B19V infec-

Table 4. The results of the confirmatory test for the positive donors in both anti-B19V and B19V NAT

Donor ID	Ab	Antigen band reading						Status
		VP-2p	VP-N	VP-1S	VP-2r	VP-C	NS-1	
Donor1	IgG	2+	3+	3+	2+		1+	Past infection month to year
Donor2	IgM	2+	3+	3+	2+			Status after infection (weeks to months)
	IgG	2+	3+	3+	3+	1+		
Donor3	IgG	2+	3+	3+	1±		1+	Long past infection
Donor4	IgM	±	3+	2+				Status after infection(weeks to months)
	IgG	2+	3+	3+	3+	1+	1+	
Donor5	IgM	2+	3+	3+	2+			Status after infection (weeks to months)
	IgG	3+	3+	3+	3+	2+		
Donor6	IgM	2+	3+	1+	2+			Status after infection (weeks to months)
	IgG	1+	3+	3+	2+	1±		
Donor8	IgM	2+	3+	3+	3+	2+		Acute infection (infection within the last 12 weeks)
	IgG	3+	3+	2+	3+	2+		
Donor9	IgM	2+	3+	2+	3+	1+		Status after infection (weeks to months)
	IgG	2+	3+	3+	3+	1+		

Abbreviations: B19V, human parvovirus B19; NAT, Nucleic Acid Testing.

Table 5. B19V DNA and anti-B19V antibodies in retrospective and follow-up specimens

Donor ID	Donation date	B19V DNA con (IU/mL)	Anti-B19V†	
			IgM (U/mL)	IgG (U/mL)
Donor1	2008-05-04*	2.72×10^{1}	Negative	147.34
	2008-08-02		Negative	358.36
	2008-10-23	7.19×10^{1}	Negative	145.26
Donor2	2007-04-17	Negative	ND	ND
	2007-05-26	Negative	ND	ND
	2007-10-23	Negative	ND	ND
	2007-11-09	Negative	ND	ND
	2007-12-06	Negative	ND	ND
	2007-12-20	Negative	ND	ND
	2008-01-11	7.29×10^{4}	160.20	152.45
	2008-03-08	6.36×10^{1}	88.72	434.46
	2008-04-02	5.34×10^{1}	52.88	396.47
	2008-04-16*	1.39×10^{2}	38.86	392.83
	2008-05-05	2.96×10^{1}	Negative	350.31
	2008-07-14	1.39×10^{2}	Negative	329.31
	2008-09-29	Negative	Negative	162.28
Donor3	2007-12-23	1.13×10^{2}	Negative	159.42
	2008-01-06	1.28×10^{2}	Negative	160.24
	2008-01-27	8.87×10^{1}	Negative	156.70
	2008-02-17	1.24×10^{2}	Negative	155.36
	2008-03-16*	4.25×10^{2}	Negative	151.54
	2008-03-30	9.44×10^{1}	Negative	149.97
	2008-08-17	2.24×10^{2}	Negative	135.26
Donor5	2007-05-18	Negative	ND	ND
	2007-06-01	Negative	ND	ND
	2007-06-15	Negative	ND	ND
	2008-03-06	5.78×10^{4}	104.40	161.61
	2008-03-22*	8.01×10^{2}	83.61	162.81
Donor8	2008-01-15	Negative	ND	ND
	2008-03-15	Negative	ND	ND
	2008-05-19*	2.33×10^{3}	124.98	140.14
	2008-08-06	7.14×10^{2}	71.84	153.08
	2008-10-29	3.03×10^{2}	Negative	144.47
Donor9	2007-08-12	Negative	ND	ND
	2007-10-06	Negative	ND	ND
	2007-11-10	Negative	ND	ND
	2008-02-02	Negative	ND	ND
	2008-02-24	Negative	ND	ND
	2008-04-10	Negative	ND	ND
	2008-04-27	Negative	ND	ND
	2008-07-15*	3.20×10^{4}	128.31	77.36
Donor10	2007-11-23	Negative	ND	ND
	2008-02-19	Negative	ND	ND
	2008-03-27*	1.33×10^{8}	Negative	Negative
	2008-07-08	2.68×10^{2}	Negative	266.54
	2008-09-24	2.85×10^{2}	Negative	158.66

*index donation; †U/mL sample >24, positive; U/mL sample <20, negative; ≥20 U/mL sample ≤24, borderline.
Abbreviations: B19V, human parvovirus B19; ND, not done.

tion through the use of tainted plasma products [13, 14]. In our study, the prevalence of B19V DNA in healthy Korean plasmapheresis donors was 0.1%, which was not high compared with that of other countries such as United States (0.83–0.88%), and Germany and Austria (0.27%) [7, 15]. Additionally, the donors had low viral loads (range, 10^{2}–10^{3} IU/mL), except for one donor. These results are consistent with those of previous reports [15].

Recently, the significance of the neutralizing effect of anti-B19V IgG has been considered in B19V infection. The anti-B19V IgG in donated blood or recipients can neutralize the virus and prevent B19V infection. Some reports indicate that 11 U/mL of anti-B19V antibody can neutralize 4.3 log of B19V DNA [16].

Kleinman et al. [17] reported that minimum infectious doses of B19V were 2×10^{4} IU/mL (in the coagulation factor) or 10^{7} IU/mL (in the other plasma products) for recipients who did not possess neutralizing antibodies. They also insisted that the blood products containing virus titers below 10^{4} IU/mL were not infectious [17].

A recent survey reported that the global prevalence of anti-B19V antibody was 30–60% in adults. Most of the B19V DNA positive donors and 60% of the recipients showed anti-B19V IgG [15]. The Transfusion Related Infection Prospective Study (TRIPS) and Retrovirus Epidemiology Donor and Recipient (RADAR) study showed that the prevalence of B19V DNA was 0.8% in blood donors and 1.7% in recipients in the United States. In these studies, all the B19V DNA positive donors showing a low viral load of less than 10^{2} IU/mL also possessed neutralizing anti-B19V antibodies. They also reported that 21 recipients who did not possess neutralizing antibodies and transfused B19V DNA-tainted blood components did not show any evidence of B19V infection [15]. The neutralizing anti-B19V antibodies contained in the donor blood may have neutralized B19V.

Data from German Red Cross blood centers (GRCBC) that have been screening mini-pool B19V NAT in all blood donors revealed that virus titers below 10^{5} IU/mL in pooled samples released to hospitals, except in patients with special risks [5]. The reason B19V DNA positive blood components were released was because of the simultaneous presence of neutralizing antibodies (to VP1 and

VP2) in the blood products. GRCBC reported that 75% of the B19V DNA negative donors possessed neutralizing antibodies, which could protect against B19V infection.

In our study, 90% of the B19V DNA positive plasmapheresis donors and 60% of the 928 randomly selected plasmapheresis donors possessed anti-B19V IgG. However, the blood components containing high titers of B19V (1.33×10^8 IU/mL) could lead to infection in patients with risk factors. The clinical progression following B19V infection would vary according to factors such as the patient's immune status and the presence of neutralizing anti-B19V IgG. Japanese groups [18, 19] have reported that most of the transfusion transmitted B19V infected patients show only mild symptoms such as a fever or rash.

Recently, new pathogen elimination techniques such as nanofiltration (20-35 nm pore size) have been introduced and the Japanese Red Cross has been using this method during fractionation.

In conclusion, the prevalence of the B19V DNA in Korean plasmapheresis donors was not high and more than 60% of the plasmapheresis donors possessed neutralizing anti-B19V antibodies, thereby suggesting that the possibility of transfusion transmitted B19V infection in Korea is not serious. Therefore, the implementation of B19V DNA screening for all blood donors does not seem to be warranted at this time.

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