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Original Article

Seroprevalence of human parvovirus B19 in healthy blood donors

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ABSTRACT

Background: Human parvovirus B19 is an emerging transfusion transmitted infection. Although parvovirus B19 infection is connected with severe complications in some recipients, donor screening is not yet mandatory. To reduce the risk of contamination, plasma-pool screening and exclusion of highly viraemic donations are recommended. In this study the prevalence of parvovirus B19 in healthy blood donors was detected by ELISA. **Methods:** A total of 1633 samples were screened for IgM and IgG antibodies against parvovirus B19 by ELISA. The initial 540 samples were screened for both IgM and IgG class antibodies and remaining 1093 samples were screened for only IgM class antibodies by ELISA.

Results: Net prevalence of IgM antibodies to human parvovirus B19 in our study was 7.53% and prevalence of IgG antibodies was 27.96%. Dual positivity (IgG and IgM) was 2.40%.

Conclusion: The seroprevalence of human parvovirus B19 among blood donor population in our study is high, and poses an adverse transfusion risk especially in high-risk group of patients who have no detectable antibodies to B19. Studies with large sample size are needed to validate these results.

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Introduction

Human blood and its components are widely used as life saving therapy in hospital practices. However, there is always an associated risk of transfusion reactions due to viral transmission via contaminated blood. Due to the high frequency of human parvovirus B19 in blood donors and pooling of large

number of blood donations (>5000) used in a plasma pool to produce a batch of components like clotting factor concentrate, a large number of batches could be potentially B19 infected.

Human erythrovirus (parvovirus) B19 causes a wide range of diseases, such as erythema infectiosum or fifth disease, a common illness in children, aplastic crisis, chronic pure red

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cell aplasia, fetal hydrops and fetal death. The virus is associated with arthropathies, hepatitis and various other syndromes and diseases.¹ Specific immunoglobulin M (IgM) and IgG antibodies are produced following experimental² and natural³ B19 infection. Infection follows a biphasic clinical course: One week after intranasal inoculation with B19 in healthy adult volunteers, viraemia is detected in seronegative individuals accompanied by a mild illness with pyrexia, malaise, myalgia, itching, and excretion of virus from the respiratory tract. About 17–18 days after infection, a second phase of symptoms commenced and was characterized by rash, itching, or arthralgia. Recovery involves production of IgM antibody 10–12 days post-infection, coinciding with a peak in virus level. IgM usually persists in serum samples for approximately 3 months but may be found for several months.⁴ IgG antibody is detectable in volunteers about 2 weeks after inoculation and persists giving lifelong immunity protecting against secondary infections. IgA may also be detected and probably plays a role in protection against infection by the natural nasopharyngeal route.⁵ Several studies have reported the presence of a persistent B19 low level viraemia beyond 6 months post-infection with a degree of immunodeficiency.⁶ More recent data using highly sensitive molecular detection methods suggest that viral DNA may persist in the circulation of immunocompetent individuals.⁷

Though incidence and prevalence of parvovirus B19 infection in blood donors has been documented in western literature, till date there is no reliable data of the in blood donors of our country. Thus, there is a need to explore the prevalence of parvovirus B19 in blood donors, and thereby, prevent and/or minimize its transmission in various clinical setting as a result of transfusion. The aim of our study was to detect antibodies against parvovirus B19 in blood units collected at the Blood Bank, Armed Forces Medical College, Pune.

Material and methods

In this study a total of 1633 samples were screened for IgM and IgG class antibodies in human serum against parvovirus B19 during the period October 2007 till February 2008. Ethical clearance and informed consents were obtained.

The initial 540 consecutive samples were screened for both IgM and IgG class antibodies (Serion classic ELISA IgG/IgM, Germany) and remaining 1093 samples were screened for only IgM class antibodies by ELISA (Novalisa IgM ELISA Parvovirus B19, Germany).

The blood donor samples which tested positive for antibodies for parvovirus B19 by ELISA were further selected for PCR analysis. Isolation of parvovirus B19 viral nucleic acid from subject samples was done using QIAamp Blood DNA extraction kit (Qiagen, Valencia, USA). The final eluate volume was stored at –20 °C till further use. The extracted DNA samples were subjected to polymerase chain reaction (PCR) targeting the Delta (δ) V region of parvovirus B19 using nested PCR primers.⁸ The primers used were (δ) AV FI – GGTTGATTATGTGTGGG (2193–2209), (δ) AV BI – ACTGAAGTCATGCTTGG (3119–3135) and (δ) V F2 – TGTGTGTTGTGTGCAAC (2229–2245), (δ) V B2 – CAAACTTCCTTGAAAATG (3065–3082) as first and second

Table 1 – Analysis of first 540 samples which were screened for IgM.

Donation details	Number (n)	IgM	
		Positivity	Negativity
Male	529	53	476
Female	11	1	10
Total	540	54 (10%)	486
Fischer exact P = 1.000, odds ratio = 1.11(0.14–23.70%).			

round primers respectively. There was no positive control of parvovirus B19 DNA that was available for the PCR assay. The first and second round PCR were carried out as per steps described previously.⁸ Briefly, the thermal cycling conditions were as follows: Initial denaturation at 94 °C for 4 min; followed by 35 cycles of (a) denaturation at 94 °C for 1 min, (b) annealing at 55 °C for 1.5 min, (c) Extension at 72 °C for 2 min; and final extension at 72 °C for 7 min. The reaction mix was held at 4 °C on completion of PCR till the time of removal of the reaction tubes from the thermal cycler.

Results

A total of 1633 samples were screened for detection of antibodies in human serum against parvovirus B19. In our study, predominant population was males, in both voluntary and replacement donations. Initial 540 samples were screened for both IgM and IgG class antibodies and remaining 1093 samples were screened only for IgM class antibodies. In first 540 samples, 54 samples were screened positive for IgM class antibodies and 151 samples (27.9%) were screened positive for IgG class antibodies respectively [Tables 1 and 2]. In 1093 samples which were screened only for IgM class antibodies 69 samples were screened positive [Table 3]. Therefore, total number of samples screened positive for IgM antibodies was 123 (including 54 samples which were positive out of 540 initial samples which were screened for both class of antibodies). Net prevalence of IgM antibodies to human parvovirus B19 in our study was 7.53% and prevalence of IgG antibodies was 27.96% (151 samples were positive out of 540 tested). Dual positivity (IgG and IgM) was 2.40% (13 out of 540).

Out of total 261 samples tested positive for parvovirus B19 through ELISA [IgG positive only (138) + IgM positive only (110) + IgG & IgM both positive (13)], none were tested positive for parvovirus B19 DNA by nested PCR.

Table 2 – Analysis of first 540 samples which were screened for IgG.

Donation details	Number (n)	IgG	
		Positivity	Negativity
Male	529	150	379
Female	11	1	10
Total	540	151 (27.96%)	389
Fischer exact P = 0.306, odds ratio = 3.96(0.52–83.36%).			

Table 3 – Analysis of 1093 samples which were screened for IgM.

Donation details	Number (n)	IgM	
		Positivity	Negativity
Male	1068	65	1003
Female	25	4	21
Total	1093	69(6.31%)	1024

Fischer exact P = 0.067, odds ratio = 0.34(0.11–1.21).

Discussion

Infections with parvovirus B19 are quite common, particularly in children. In otherwise healthy individuals, the course of infection is usually indolent. B19 infections can result in serious complications in certain high-risk population. The major high-risk groups are pregnant women, patients with underlying haematological problems and immune-deficient patients who constantly are multi-transfused. But yet donor screening is not mandatory. Current attempts, using sensitive screening tests, to improvise blood safety focus entirely on eliminating the risk of transmitting infectious agents. Besides the primary respiratory route of transmission, hospital acquired transmission may occur between family members, from patient to patient and from patient to health care workers.⁹ These findings have lead to the hypothesis that B19 may also have been transmitted for some years through the administration of blood products to susceptible patients.¹⁰ Of potential concern for blood safety is increasing evidence of long term B19 persistence in the circulation and tissues of not only immunocompromised but also immunocompetent individuals.

With the detection of newer B19 variants, subsequently classified as B19 genotype 2 and 3, as well their infectivity and pathogenesis has raised concern. At this stage, it appears that the risk and the frequency of transfusion-associated transmission of these newly discovered variants are similar to those posed by classical B19 genotype1. More studies are required to assess the role of B19 variants in transfusion. However, such diversity has an impact on B19 detection by genomic amplification because commercial nucleic acid testing (NAT) assays might, be unable to detect new B19 variants.

Studies suggest that chronic carrier state of B19 is probably more frequent than initially believed.^{11,12} Most donors have low viral loads and antibodies likely to protect against post-transfusion disease. The implications of these chronic infections for recipients of blood products, especially at-risk patients such as pregnant women or immunocompromised recipients, have not been studied.

It is of utmost importance to determine the appropriate pool size to be tested (taking into account parameters such as prevalence, viral load, test sensitivity, and the efficacy of inactivation procedures) and to correlate viral loads with the serological status of donors as regards antibodies against different viral proteins.^{11,12}

Total prevalence of IgM antibodies to human parvovirus B19 in our study [Tables 1 and 3] was 7.53% (123 samples were positive out of total 1633 tested) [Fig. 1]. The prevalence of IgM

Fig. 1 – Distribution of IgM positive samples according to age (total no of samples screened were 1633 in this 123 were positive for IgM).

to B19 in blood donors or in other healthy populations is usually below 2%, but it can be higher depending on the time of study in relation to the epidemic cycle. In temperate climates most infections occur in the spring, with mini-epidemics occurring at regular intervals several years apart. These donors in our study were probably in a relatively late stage of infection, that is, within the first several months of acquiring infection & hence might have tested negative for DNA in PCR.

Total prevalence of IgG antibodies to human parvovirus B19 in our study [Table 2] was 27.96% (151 samples were positive out of total 540 tested) [Fig. 2]. We believe either that these IgG-positive donors were at the tail end of resolving their B19 viraemia or that some of these donors may have had very-low-titre B19 DNA that persisted for longer than predicted by the standard natural history model. Future longitudinal studies will be needed to distinguish these possibilities. The prevalence of B19 IgG seropositivity in our study though low as compared to other donor cohorts, but were relatively significant.^{13–15} Donations with both IgM and IgG B19 antibody (2.40%) most likely represent acute resolving infection, whereas those with IgG but no IgM are most consistent with a more chronic and possibly persistent phase of B19 infection. At present, interventions for preventing B19 transfusion from blood components have not been implemented in the vast

Fig. 2 – Distribution based on gender of the first 540 study samples screened for both IgG and IgM antibodies against human parvovirus B19.

majority of developed countries, due in part to the prevailing view that blood components with low levels of B19 DNA will not transmit B19 infection.

Genome amplification assays are the most sensitive diagnostic tools to detect B19 DNA. Most published PCR assays are able to detect viral DNA at 1–100 copies/ml.¹⁶ The nested PCR can disclose between 10 and 100 copies of B19 DNA in a sample, with sensitivity at least 10–100 times higher than that achieved by amplification with a single primer set. A major potential problem associated with nested PCR, however, is the risk of contamination due to carry-over between samples, which can occur during sample handling from the first reaction to second round amplification.¹⁷ In the present study the tested samples were completely negative by the very sensitive and specific nested PCR. Although, this seems quite reassuring, these results cannot be generalized as they represent testing of small batches and continuous monitoring is recommended. Other researchers from different countries have been able to detect parvovirus B19 DNA in 1 percent of all blood cell preparations and blood products applied to the patients on a haematologic ward, in 0.9 percent of standard blood components (in 2.0 percent of pooled plasma products and in 0.7 percent of single donor products),¹⁸ in 0.006 percent of blood donations, in 0.14 percent of single-donor blood products,¹⁹ in 0.16 percent of plasma samples,¹⁵ in 0.6–1.3 percent of blood donors.¹³ Given these data, it is apt that screening of blood donors for parvovirus B19 is now in prospect. Although the prevalence & seasonality of B19 appear to vary between different donor populations & geographic locations, the fact remains that B19 is found consistently within blood donor population, throughout the year, & throughout the world. Candotti et al tested 1000 UK whole-blood and platelet donors in minipools of 10 donation specimens and found a prevalence of 0.9 percent with a nested PCR with a 95 percent LOD of 25 IU per mL; this prevalence was very similar to that found in our study.¹³

Among the donors testing negative for B19 DNA, a high percentage are positive for anti-B19 IgG antibodies. This suggests that a majority of donors have had contact with the virus and have recovered. Such individuals are probably immune. In our study, the parvovirus B19 DNA was not detected by PCR in the 261 samples which had tested positive for IgM or IgG ELISA. Previously, Mendonça et al have analyzed 526 sera collected from symptomatic patients (exanthema, fever, adenomegaly, and arthralgia) from Brazil, by IgM-enzyme-linked immunosorbent assay and polymerase chain reaction, and reported 8.8% simultaneous positivity by the two methods in such symptomatic patients.²⁰ Vallerini et al reported single sample positivity for B19 DNA after testing 100 blood donor samples by nested PCR.²¹ The incidence of B19 DNA positivity in Germany and Austria has been found to range from 12.7 to 261.5 per 100,000 blood donations depending on virus loads equal to or above 10^5 and below 10^5 IU per mL, respectively.²² B19 DNA prevalence of 0.88 percent has reported from USA with a real-time B19 DNA polymerase chain reaction in stored plasma aliquots from 5020 donations collected between 2000 and 2003.²³ This study suggested that the DNA-positive donations with both IgM and IgG B19 antibody were likely represent acute resolving infection, whereas those with IgG but no IgM are most consistent

with a more chronic and possibly persistent phase of B19 infection.²³ In view of these findings, our study findings are in consonance with the previous studies wherein the overall PCR positivity of B19 amongst blood donor samples has been below 1%, with IgM-ELISA and PCR positivity of less than 10% in symptomatic individuals. In addition, there is a chance that as a result of possibly lower viral loads amongst our study population, and the use of nested PCR instead of real time PCR may have lead to non-detection of parvovirus B19 DNA.

However, minipool whole-blood screening may be planned for the future in blood centres. Screening of source plasma for parvovirus DNA is currently recommended in North America and Europe as an in-process control to ensure that levels of parvovirus B19 DNA do not exceed 10,000 IU per ml in the manufacturing pool.¹⁹

Due to the general introduction of screening tests for hepatitis B virus, hepatitis C virus and HIV, there has been a sharp fall in the risk of these viruses being transmitted via blood products. It would also be possible, but expensive, to test all blood products for the presence of emerging pathogens, the transmission of which is a risk to only a part of those using these products. A less expensive option is the risk-group approach, in which only selected groups of patients receive tested blood products. In this way, patients for whom infection with B19 could cause problems will be given maximum safety blood products. This approach is in keeping with measures previously used in blood transfusion medicine with respect to cytomegalovirus transmission. By definition 'B19-virus safe' cellular blood products from a donor in which IgG antibodies against B19 have been detected in two separate blood samples, one taken at least six months after the other. Safe cellular blood products are to be administered to pregnant women (except in the case of transfusions given during birth), patients with congenital or acquired haemolytic anaemia who have no detectable antibodies to B19 and patients with cellular immunodeficiency who have no detectable antibodies to B19.²⁴

It is still unclear whether B19 NAT screening of blood products should be envisaged. Future studies of transmissibility of B19 by transfusion should, when possible, take into account not only the level of B19 in the blood product, its overall transfused dose, but also, equally importantly, the presence of anti-B19 antibodies, their potency and titre in the blood product, the immune status of the recipient, and recipient's B19 infection history, anti-B19 status and viral persistence. Samples collected for blood transfusion should be screened for parvovirus B19 DNA using IgM ELISA, and be supplemented sensitive nucleic acid testing methods such as real time PCR. Technical developments, such as the inactivation of microorganisms and the use of nanofiltration to cut down the number of viral particles in the final product, can lead to other options for making blood products B19-virus safe.

Conclusion

The seroprevalence of human parvovirus B19 among blood donor population in our study is high, and poses an adverse transfusion risk especially in high-risk group of patients. The

high risk-group approach, which includes pregnant women, Rh isoimmunised pregnancies requiring intrauterine transfusion, patients with congenital or acquired haemolytic anaemia and patients with cellular immunodeficiency who have no detectable antibodies to B19, should receive tested blood products. This could serve as an option of choice and is recommended by this study to improvise blood safety. Safe cellular blood products as recommended by the present study are to be administered to all high risk group patients. Physicians should distinguish between patients for whom a B19 infection represents a health risk and patients for whom such infections pose no serious problems. Patients other than those in the high-risk groups should continue to receive blood products that have been produced in accordance with current safety criteria.

Units should be screened for parvovirus B19 using IgM ELISA, and may be supplemented through sensitive nucleic acid testing methods such as real time PCR. Technical developments may produce more direct methods for rendering blood products B19-virus safe, such as the inactivation of microorganisms and the use of nanofiltration (in plasma products) to cut down the number of viral particles in the final product.

Intellectual contribution of each author

Study concept: Lt Col Satish Kumar, Col RM Gupta.

Drafting & Manuscript revision: Lt Col Satish Kumar, Col Sourav Sen, Brig RS Sarkar.

Statistical analysis: Col Atul Kotwal, SM, Col J Philip.

Study supervision: Lt Col Satish Kumar, SH Sumathi.

Conflicts of interest

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REFERENCES

1. Heegaard ED, Brown KE. Human parvovirus B19. *Clin Microbiol Rev.* 2002;15:485–505.
2. Anderson MJ, Higgins PG, Davis LR, et al. Experimental parvoviral infection in humans. *J Infect Dis.* 1985;152:257–265.
3. Saarinen UM, Chorba TL, Tattersall P, et al. Human parvovirus B19-induced epidemic acute red cell aplasia in patients with hereditary hemolytic anemia. *Blood.* 1986;67:1411–1417.
4. Anderson LJ, Tsou C, Parker RA, et al. Detection of antibodies and antigens of human parvovirus B19 by enzyme-linked immunosorbent assay. *J Clin Microbiol.* 1986;24:522–526.
5. Erdman DD, Usher MJ, Tsou C, et al. Human parvovirus B19 specific IgG, IgA, and IgM antibodies and DNA in serum specimens from persons with erythema infectiosum. *J Med Virol.* 1991;35:110–115.
6. Blaaser F, Kelly M, Siegrist K, et al. Critical function of the CD40 pathway in parvovirus B19 infection revealed by a hypomorphic CD40 ligand mutation. *Clin Immunol.* 2005;117:231–237.
7. Cassinotti P, Siegl G. Quantitative evidence for persistence of human parvovirus B19 DNA in an immunocompetent individual. *Eur J Clin Microbiol Infect Dis.* 2000;19:886–887.
8. Hemauer A, von Pöblitzki A, Gigler A, et al. Sequence variability among different parvovirus B 19 isolates. *J Gen Virol.* 1996;77:1781–1785.
9. Bell LM, Naides SJ, Stoffman P, Hodinka RL, Plotkin SA. Human parvovirus B19 infection among hospital staff members after contact with infected patients. *N Engl J Med.* 1989;321:485–491.
10. Paton G, Pillay D, Myint S, Pattison J. Characterization of nested polymerase chain reaction assay for detection of parvovirus B19. *J Clin Microbiol.* 1993;31:540–546.
11. Jordan J, Tiangco B, Kiss J, Koch W. Human parvovirus B19: prevalence of viral DNA in volunteer blood donors and clinical outcomes of transfusion recipients. *Vox Sang.* 1998;75:97–102.
12. Weimer T, Streichert S, Watson C, Groner A. High-titer screening PCR. A successful strategy for reducing the parvovirus B19 load in plasma pools for fractionation. *Transfusion.* 2001;41:1500–1504.
13. Candotti D, Etiz N, Parsyan A, Allain JP. Identification and characterization of persistent human erythrovirus infection in blood donor samples. *J Virol.* 2004;78:12169–12178.
14. Zaaijer HL, Koppelman MH, Farrington CP. Parvovirus B19 viraemia in Dutch blood donors. *Epidemiol Infect.* 2004;132:1161–1166.
15. Thomas I, Di Giambattista M, Gerard C. Prevalence of human erythrovirus B19 DNA in healthy Belgian blood donors and correlation with specific antibodies against structural and non-structural viral proteins. *Vox Sang.* 2003;84:300–307.
16. De Jong EP, de Haana TR, Kroes ACM, Beersma MFC, Oepkes D, Walther FJ. Parvovirus B19 infection in pregnancy. *J Clin Virol.* 2006;36:1–7.
17. Zerbini M, Gallinella G, Cricca M, Bonvicini F, Musiani M. Diagnostic procedures in B19 infection. *Pathol Biol.* 2002;50:332–338.
18. Plentz A, Hahn J, Knöll A, Holler E, Jilg W, Modrow S. Exposure of hematologic patients to parvovirus B19 as a contaminant of blood cell preparations and blood products. *Transfusion.* 2005;45:1811–1815.
19. Koppelman MH, Cuypers HT, Emrich T, Zaaijer HL. Quantitative real-time detection of parvovirus B19 DNA in plasma. *Transfusion.* 2004;44:97–103.
20. Mendonça MCL, Ribeiro SB, Couceiro JNSS, von Hubinger MG. Parvovirus B19 infections in state of Rio de Janeiro, Brasil: 526 sera analyzed by IgM-enzyme-linked immunosorbent assay and polymerase chain reaction. *Mem Inst Oswaldo Cruz, Rio de Janeiro.* December 2005;100(8):847–852.
21. Vallerini Daniela, Barozzi Patrizia, Quadrelli Chiara, et al. Parvoviruses in blood donors and transplant patients, Italy. *Emerg Infect Dis.* January 2008;14(1):185–188.
22. Schmidt M, Themann A, Drexler C, et al. Blood donor screening for parvovirus B19 in Germany and Austria. *Transfusion.* 2007;47:1775–1782.
23. Kleinman SH, Glynn SA, Lee TH, et al. National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study (REDS-II); prevalence and quantitation of parvovirus B19 DNA levels in blood donors with a sensitive polymerase chain reaction screening assay. *Transfusion.* 2007;47:1756–1764.
24. Kerr S, O'Keefe G, Kilty C, Doyle S. Undenatured parvovirus B19 antigens are essential for the accurate detection of parvovirus B19 IgG. *J Med Virol.* 1999;57:179–185.