Original Article

Serological study on parvovirus B19 infection in multitransfused thalassemia major patients and its transmission through donor units

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Abstract:

Background: Human parvovirus B19 (B19) virus is a newly recognized agent for transfusion transmitted diseases. Beta-thalassemia major patients receive a hypertransfusion regimen, hence, are prone to acquire B19 infection; moreover, B19 escapes viral inactivation methods and donor units are not tested for B19, but there are just a couple of studies globally and none from the Asian continent. Hence, a study was designed to find the frequency of B19 infection and its transmission in multitransfused thalassemia patients. **Materials and Methods:** Ninety multitransfused beta-thalassemia major (thalassemia) patients, 32 controls (age, sex matched) without any history of transfusion were enrolled. Besides the donor units were tested in B19 un-infected patients. B19 specific IgG and IgM antibodies in the sera were analyzed by ELISA (in-house), using B19 VPI and VP2 recombinant and purified antigens; additionally HBsAg and anti-HIV and anti-HCV antibodies were tested for coexisting infections. **Results:** Seventy-three (81%) thalassemia patients tested positive for anti-B19 IgG antibodies as compared to seven (21%) in the controls group ($P < 0.01$), while anti-B19 IgM antibodies were detected in 37 (41.1%) compared to two (6.2%) in the controls ($P < 0.01$). Mean age of the thalassemia patient was eight years (range 2 – 18 years) and B19 infection was highest in the six-to-ten year range. Seropositivity increased with the number of transfusions. Two of the four HBsAg positive and five of the seven anti-HCV IgM antibody-positive patients also had anti-B19 IgM. After a six-month follow-up, four (25%) of the 16 seronegative patients seroconverted and anti-B19 IgM antibodies were detected in their donor units. **Conclusions:** Most of multitransfused thalassemics were B19 seropositive or had anti-B19 IgM; in the remaining uninfected group, B19 got transmitted through infected / IgM-positive donor units.

Key words:

B19, blood transfusion, parvovirus, seroconversion, thalassemia

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Introduction

Parvovirus B19 (Latin Parvum means small) is a newly emerging DNA virus discovered by an Australian virologist, working in London, when she was testing donor sera for hepatitis B virus, but found the B19 virus in the sera, numbered 19 in row B, hence, she named it B19. Later, the B19 virus was placed in the genus *Erythrovirus* of the family *Parvoviridae.* B19 is the causative agent of erythema infectiosum or fifth disease in children, and is associated with a wide range of clinical diseases.[1,2] Transmission of B19 occurs mainly through the respiratory droplets of patients, transfusion of B19 infected blood and blood products, and finally transplacentally during maternal B19 infection.[1,2] B19 is a non-enveloped DNA virus, hence, is thermostable and escapes the currently applied viral inactivation methods. Therefore, the issues on safety of blood and blood products were raised.[3-7] Furthermore, it has added a new dimension to the need for prevention of this transfusion-transmitted disease.

Beta-Thalassemia major (thalassemia) patients, owing to chronic hemolytic disease, are on a hyper-transfusion regimen, hence, are at high risk of acquiring transfusion transmitted B19 virus, but have seldom been studied by detecting antibodies to B19.[10-12] A sudden worsening of anemia, reticulocytopenia, and cessation of erythropoiesis in the bone marrow characterize the transient aplastic crisis. It is possible that B19-induced aplastic crisis may often be wrongly diagnosed as a complication of the underlying disease.[2] In addition, these patients are at high risk of acquiring other transfusion-transmitted diseases also. Similarly, B19 infection has also been reported from multitransfused hemophiliacs, recipients of Factor VIII concentrates, and children with congenital coagulation defects.[13-16] Furthermore, the problem of B19 transmission gets aggregated by the frequent contamination of coagulation factor concentrates.[16] Such a transmission of B19 infection leads to red cell aplasia, neutropenia, and thrombocytopenia.[1,17] This underlines the need to screen the donor blood units being given to the susceptible recipients. At present, widespread

donor testing for B19 has not been indicated as a routine test. Furthermore, B19 has been documented to be transmitted by transfusion of blood or its components.[8-10]

Few studies have shown antibodies to B19 in thalassemia major patients and on its transmission, but none from South East Asia. Hence, the present study was undertaken to find the seroprevalence of B19 by detecting anti-B19 IgG antibodies, recent transmission / infection as evidenced by IgM antibodies in multitransfused thalassemia major patients from North India, besides its transmission through donor units in serologically naïve patients. Additionally thalassemia patients were screened for HBsAg, human immunodeficiency virus (HIV) antibodies, and hepatitis C virus (HCV) antibodies, to find coinfections with B19.

Materials and Methods

Patients

Ninety beta-thalassemia major patients registered in a tertiary care institute from northern India (SGPGIMS, Lucknow) were randomly included in the study. All patients were under a hypertransfusion regimen and treated with regular transfusion of packed red blood cells, at an interval of about three weeks. From a routinely drawn pre-transfusion blood sample an aliquot of sera was preserved for the present study, after obtaining informed consent from the parents of the patients, and a detailed history of the patient was documented in a structured questionnaire at each visit.

Control group

The control group comprised of 32 children (age- and sex-matched), without any history of transfusion of blood or blood products and without any hematological disorders. Sera were similarly collected after obtaining informed consent, but only once. All the sera samples were stored at - 30°C until tested.

Donor units

From a routinely drawn pre-transfusion blood sample an aliquot of sera was preserved at – 40°C and analyzed for B19 antibodies, when required.

Methods

Anti-B19 IgM and IgG antibodies were analyzed as indicators for B19 infection by previously standardized in-house indirect enzyme-linked immunosorbent assay (ELISA), at the Department of Microbiology.[18] Briefly, the ELISA technique used was comprised of B19 VPI and VP2, cloned and baculovirus expressed, and purified antigen (kindly donated by Y Matsunaga NIID, Tokyo, Japan), while the sera were tested at 1 : 400 dilutions, incorporating a set of negative and positive control sera. Optical densities (O.D.) were read at 450 nm with a reference filter of 620 nm in an ELISA reader. Index values more than or equal to 1.1 were taken as positive for either anti-B19 IgM or IgG antibodies.

In addition, documenting the transmission of B19 was also planned for the B19 uninfected group in anticipation, and the donor unit's sera were also analyzed for the presence of B19 IgM and IgG antibodies. Furthermore, the samples were also screened for HBsAg, anti-HIV, and anti-HCV, using commercial ELISA kits, to find the co-existing infections in the study group.

Statistical methods

A computerized analysis of the data was carried out using m-stat and SPSS programs. 'Chi square' and 'Fisher's exact Z' tests were applied for statistical evaluation.

Results

The mean age of the Beta-thalassemia major patient was eight years (range 2 to 18 years), of which 29 (32.2%) patients were from the age group of less than one year to five years of age, 42 (46.7%) were in the six to ten year age group, 11 (12.2%) patients were in the age group of 11 – 15 years, and eight (8.9%) were in the 16 – 20 year age group, respectively. Predominance of male patients was observed in this randomized group and 55 (61%) patients were males. Out of 90 thalassemia major patients tested by in-house ELISA, anti-B19 IgM antibodies were detected in 37(41.1%) thalassemia patients in comparison to two (6.2%) of the controls ($P < 0.01$), while 73 (81%) tested seropositive for anti-B19 IgG antibodies as compared to seven (21.1%) subjects from the control group ($P < 0.01$). According to gender, 65% (24 of 37) males and 35% (13 of 37) females were positive for anti-B19 IgM antibodies, while 83% (46 of 55) males and 77% (27 of 35) females tested positive for anti-B19 IgG. The prevalence of IgG antibodies was 100, 75, 83, and 80% in males and 66, 77, 100, and 100% in females of age groups 1 – 5, 6 – 10, 11 – 15, and 16 – 20 years, respectively. The anti-B19 IgM antibody positivity was 25, 46, 66, and 60% in males and 25, 39, 50, and 66% in females in the age groups shown in Table 1. Although percentage positivity for either of the B19 antibodies increased with increasing age, the differences were not statistically significant ($P > 0.05$).

Among the 90 thalassemia major patients had received blood transfusion ranging from 10 to 360 (mean 93.9) units in their life time. Ten (83.3) patients from a group of 12, who received more than 200 transfusions, were positive for both anti-B19 IgG and IgM antibodies, indicating definite B19 infection, and when compared with the group that received less than 200 units transfusion [Table 2], it was found to be positive in 79% (67 of 84) and 39% (33 of 84) of the patients, and it was significantly high

Table 1: Prevalence of anti-B19 antibodies in thalassemia major patients according to age and gender

Age Distribution Groups in Years	Sex Distribution			Positive (%) Anti B-19 IgG			Positive (%) Anti B-19 IgM		
	Total	Male	Female	Male	Female	Total	Male	Female	Total
1 – 5	28	16	12	16 (100)	8 (66)	24	4 (25)	3 (25)	7
6 – 10	46	28	18	21 (75)	14 (77)	35	13 (46)	7 (39)	20
11 – 15	8	6	2	5 (83)	2 (100)	7	4 (66)	1 (50)	5
15 – 20	8	5	3	4 (80)	3 (100)	7	3 (60)	2 (66)	5
Total	90	55	35	46	27	73	24	13	37

As the age increased, the positivity for anti B-19 IgM increased in both sexes. Alhough the prevalence of antibodies (IgG and IgM) were higher in males, the age-specific prevalence did not differ significantly in males and females: ($P > 0.05$)

($P < 0.01$). None of the study and control group patients tested positive for anti-HIV antibodies. Co-existence of B19 infection along with hepatitis B virus (HBV) and HCV was seen in four (4.4%) and seven (7.7%) thalassemia patients, who were positive for HBsAg and anti-HCV antibodies, respectively. Two of four (50%) of the HBsAg positive and five of seven (71%) of the anti-HCV positive thalassemia patients were also positive for anti-B19 antibodies (IgG). One each of the HBsAg and anti-HCV positive patients tested positive for both the anti-B19 IgG and IgM antibodies. Only one child from the control group tested positive for HBsAg and was also positive for B 19 anti-IgG antibodies. None of the children in the control group tested positive for the anti-HCV antibodies [Table 3].

In 16 thalassemia patients, neither anti-B19 IgG nor IgM antibodies were detected by ELISA, therefore, they were considered to be serologically naïve. All these 16 patients were followed up for six months together with their donor units and tested for B19 IgG and IgM, to look for donor-related transmission. Four (25%) patients seroconverted, of whom three patients seroconverted at two months and one patient at three months post transfusion follow-up, and their corresponding donor units were found to have anti-B19 IgM antibodies.

Discussion

Multitransfused beta-thalassemia major patients are the most susceptible population to acquire transfusion-related infections, including B19, owing to regular transfusions of one to three units of blood every three to four weeks, which amounts to 12 – 51 units / year. As the mean age of our patients was eight years (range 2 – 18 years) it can be estimated that the number of donor units transfused at this age ranged from 96 – 408 units. As the probability of acquiring transfusion-transmitted diseases (TTDs) is related to the probability of being exposed to the infected units of blood,[4] which in turn depends on the prevalence of asymptomatic viremic blood donors in the population and the number of units transfused,[5] hence, the magnitude of risk of TTDs can be appreciated.

The present study revealed a high prevalence of anti-B19 IgG (81%) and IgM (41.1%) antibodies in multitransfused thalassemia major patients, which was statistically significant in comparison to the controls ($P < 0.01$). None of the cross-sectional studies were reported from India or Asian countries and only one study and a case report has dealt with the problem of B19 infection and its transmission in thalassemia patients. Globally only one study and a case report have highlighted the concern of B19 infection in thalassemics. One is a study by Siritantikorn *et al.*,[19] from Thailand, of 60 thalassemia major patients, where he found anti-B19 IgG in 38% and anti-B19 IgM in only 4% of these positive anti-parvovirus B19 IgG patients, and concluded that acute and chronic persistent parvovirus B19 infection was found in the thalassemic Thai patients. Contrary to our findings, blood transfusion had a significant influence in increasing the prevalence of parvovirus B19 infection in thalassemic patients. In one case report Zanella *et al.*,[10] found B19 transmission due to single-donor transfusion, in a 22-year-old female thalassemia major patient, who presented with an aplastic crisis; this was followed one week later by transitory heart failure and acute tricuspid incompetence. The patient's sera had both anti-B19 IgM antibodies and B19 DNA in the acute phase by polymerase chain reaction and remained detectable up to four months after diagnosis. High titer B19 IgM antibodies and anti-B19 DNA were also found in the serum samples collected at the time of donation from one of the donors before the onset of clinical symptoms. Owing to the paucity of reports the problem of B19 transmission due to prolonged multiple transfusions can be understood, by comparing results obtained from hemophilia patients, who are also dependent, life long, on multiple transfusions with plasma-derived products, especially factor VIII, made from large pools of donors, and hence, are the epitome of patients at risk for developing blood-borne infections. In a study from the USA among hemophiliacs, B19 infection was observed in 97% of the population.[5] Williams *et al.*, have reported a prevalence of 89% in the United Kingdom.[20]

In the present study no significant correlations ($P > 0.05$) could be derived in terms of parvovirus prevalence with age and gender of the 90-thalassemia major patients. However, the fact is, with advancing age the prevalence of B19-specific IgM antibodies increased, which could be attributed to the low socioeconomic status or a higher number of transfusions received by these patients. This fact could also explain the very high prevalence of B19 IgG antibodies in the multitransfused group of patients in our study. In a study from northern India the increase of HCV infection with gradually increasing age has been documented.[21]

In the present study, a positive correlation between the number of units transfused and the prevalence of anti-B19 antibodies was observed. In a study from eastern India, 70 thalassemics and 20 hemophiliacs who received periodic transfusions of packed cells and components, like fresh frozen plasma, were reported to have an increased prevalence of TTD markers with increasing number of transfusions.[6] Considerable overlapping of TTD viruses like HCV and HBV in high-risk groups of thalassemics and hemophiliacs is expected and it is possible that simultaneous infections with these two viruses may occur. In the present study also B19 co-infections were seen in seven (7.8%) thalassemics with HBsAg or anti-HCV IgM positivity. Furthermore, serological monitoring of the susceptible group of B19 naïve patients showed B19 IgG seroconversion in 25% of such thalassemics with anti-B19 IgM detected in their donor units, indicating and confirming B19 transmission. It may be noted that only donors or donor units

Table 2: Anti B-19 antibodies and the total number of transfusions received by thalassaemia major patients

Number of transfusion	Total Patients	Positive (%) Anti B-19 IgG	Positive (%) Anti B-19 IgM
0 – 100 units	67	54 (80)	25 (37)
101 – 200 units	17	13 (76)	8 (61)
201 – 300 units	4	4 (100)	3 (75)
300 units	2	2 (100)	1 (50)

The prevalence was higher in patients who had received > 200 transfusions

Table 3: Transfusion-transmitted disease markers and anti B19 antibodies in multitransfused thalassaemia major patients

TTD (marker)	Total (%)	Positive (%) Anti-B19 IgG	Positive (%) Anti-B19 IgM	Positive (%) Anti-B19 IgG and IgM (Both)
HBsAg	4 / 90 (4.4)	2 / 4 (50)	1 / 4 (25)	1 / 4 (25)
Anti HCV	7 / 90 (7.7)	5 / 7 (71)	1 / 7 (14)	1 / 7 (14)

found positive for anti-B19 IgG antibodies are safe, as risk of B19 transmission arises when donors are either viremic, that is, B19 DNA positive and the incidence of such a viremic donor has been estimated to range from 1 : 30,000 to 1 : 3,000 and is now 0.88%, depending on the sensitivity of the detection method used[22] or else has anti-B19 IgM antibodies. Donor unit linkage[23] has also been reported recently and we have also found B19 transmission through transfusion of B19 IgM antibody-positive donor units in four (25%) of the 16 seronegative patients. B19 IgM antibody-positive donor units denote a recent infection or a persistent infection in the donor. Hence, such donor units may be regarded as potentially infective, as viremia may still persist, besides, a negligible chance of false positivity cannot be entirely ruled out. After controlling transfusion-transmitted viral infections, such as, HIV, HBV, and HCV, blood establishments need to be vigilant for new threats, for the safety of blood supply. Now many agents have fulfilled the broad definition of emerging blood-transmitted infections, including parvovirus B19, dengue, and West Nile virus (WNV).[4]

However, serological monitoring is needed for a longer duration, and a larger number of patients should be included to establish definite correlations. Hence, to lower the incidence of B19 transmission and safety of the blood or its components, improvements are required in pathogen inactivation. Of late, nano-filtration of the factor IX concentrate has been tried in Holland, in patients who need lesser number of transfusions to eliminate the B19 virus.[24] Furthermore, a multi-pathogen microarray technology has to be developed to near-zero infectious disease risk, for emerging and re-emerging pathogens, including the B19 virus.

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