Brief Original Article

Molecular and serological assessment of parvovirus B19 infections among sickle cell anemia patients

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Abstract

Introduction: Parvovirus B19 is a cause of hemolysis and red blood cell aplasia in patients with sickle cell anemia. The present study aimed to assess parvovirus B19 infection among sickle cell anemia patients.

Methodology: All patients (n = 138) included in the study were sickle cell anemia patients. Blood donors were used as a control group. Assessment of parvovirus B19 antibodies and viral DNA was performed using established methods of detection and B19 recomBlot assay.

Results: Detectable levels of parvovirus B19 IgG were found in 52 samples (37.6%) whereas anti-parvovirus B19 IgM antibodies were detected in four (2.89 %) patients of the sickle-cell anemia group. Anti-B19 IgM-positive samples contained B19-viral DNA. These four patients presented with fever, malaise, pallor and no cutaneous rash. Anti-parvovirus B19 antibodies were detected in 22 (39.3%) of the control blood donors group. Anti-parvovirus B19 IgM antibodies were not detected in the control group. Using the recomBlot assay, 58 test samples (42%) were found to contain detectable levels of Parvovirus B19 antibodies. All the samples that were positive for parvovirus B19 IgG by the ELISA were also positive by the recomBlot assay. Six samples were only positive by the recomBlot assay and not by the ELISA. Two of these six samples were positive for B19 viral DNA.

Conclusions: Establishing the extent of parvovirus B19 infection in sickle cell anemia patients will help in proper management of aplastic crisis in such patients. The B19 recomBlot assay may be suitable as a confirmatory assay.

Key words: parvovirus B19; sickle cell anemia; antibodies; RT-PCR; recomBlot

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Introduction

Parvovirus B19, a small, single-stranded, non-enveloped DNA virus, is a human pathogen that can result in a wide array of clinical findings [1,2]. It is resistant to heat, cold, and solvents. The B19 virus was initially discovered in 1975 by Cossart *et al*. [3], whilst investigating hepatitis B virus laboratory assays [4].

Infection with parvovirus is ubiquitous and occurs worldwide. It is mildly contagious, relatively common, and may occur sporadically or in epidemics. The peak incidence rates occur in 6- to 14-year-old children. The immunity prevalence to parvovirus increases with age and may be greater than 60% by adulthood [1]. Infection is most frequently found in the late winter or early spring [5]. As parvovirus DNA has been found in respiratory secretions at the time of viremia, the most common route of transmission appears to be respiratory. The virus is most probably transmitted by close contact. The rate of transmission is nearly 50% in household contacts and varies from 10% to 60% in school and daycare exposure [6,7]. Nosocomial, transplacental, tissue transplantation, and blood product transmission can also occur [1].

Parvovirus B19 has a specific tropism for erythroïd progenitor cells and thus can cause a temporary infection of the bone marrow eventually leading to a transient arrest in erythropoiesis [7]. Patients with hematological disorders are at risk of severe clinical illness especially in chronic hemolytic anemia such as sickle cell disease [9,10], thalassemia [11], and hereditary spherocytosis [12]. In these diseases erythroid progenitor cell formation is increased to compensate for red blood cell lysis and B19 infection can suppress erythropoiesis and induce acute erythroblastopenia, which is often referred to as transient aplastic crisis [13]. The patients usually become highly viremic and pose an increased risk of virus transmission. Close monitoring of such high-risk groups for this viral infection is, therefore, of great importance for epidemiologic surveillance and

Table 1. Anti-parvovirus B19 antibodies (IgM and IgG) in patients with sickle cell anemia and control group using ELISA method

Group tested	B19-IgM antibodies positive n (%)	B19-IgG antibodies positive n (%)	B19-antibodies detected by recomBlot n (%)	B19 viral DNA n (%)
Sickle cell anemia n = 138	4 (2.89%)	52 (37.6)	58 (42%)	4 (2.89%)
Control n = 56	0	22 (39.3)	25 (44.6%)	0

disease prevention. To the best of our knowledge, four epidemiological studies on human parvovirus B19 infection in Saudi Arabia have been reported. The present study aimed to assess parvovirus B19 infection among sickle cell anemia patients using established methods of detection and B19 recomBlot assay.

Methodology

Patients and sera

The study was conducted in a tertiary hospital in Eastern Saudi Arabia over a period of one year (2009-2010). All sickle cell anemia patients attending the hematology clinics of the hospital were included in the study (n = 138). Sickle cell disease was confirmed by high-performance liquid chromatography (HPLC). The age of the patients included in the study ranged from 6 months to 61 years (mean 28 years). Both sexes were included (77 males and 61 females). Blood donors were used as a control group (n = 56). They included 50 males and 6 females with a mean age of 29 years.

Clinical assessment (history and physical examination) was conducted for all patients.

Methodology

Sickle cell anemia patients were chosen for laboratory assessment of parvovirus B19-specific IgG and IgM antibodies using type-specific ELISA (Parvoscan-B19, Biotrin International, Ireland). The Biotrin International Parvovirus B19 IgG/IgM Enzyme Immunoassay is a sandwich enzyme immunoassay for the detection of IgG/IgM class antibodies to parvovirus B19 VP1 and VP2 proteins in human serum. Positive (reactive) and negative (non-reactive) samples were calculated according to the manufacturer's recommendations. Equivocal results were defined as values between 1.2 and 0.8.

Samples were further analyzed using Parvovirus B19 recomBlot assay (Mikrogen, Munich, Germany) according to the manufacturer's recommendations. The assay detects antibodies to VP-N, VP-C, VP-1S and NS-1 viral proteins. The result is regarded as positive when the VP-N and the VP-C are detected.

Detection of B19-viral DNA was performed by real time PCR using a Parvovirus B19 Real-TM kit (Sacace Biotechnologies, Como, Italy). The sensitivity of the assay is not less than 200 copies/ml.

All patients and control samples were tested for virus-specific antibodies by the ELISA (IgG and IgM) and recomBlot. IgM-positive and discrepant results were tested by RT-PCR.

Erythrocyte count, reticulocyte count, sickling test, and hemoglobin electrophoresis were also performed. Written informed consent was obtained from all individuals included in this study or their parents.

Results

Samples of sickle cell disease patients (n = 138) were analyzed for IgG and IgM antibodies against parvovirus B19 using ELISA. A total of 52 samples (37.6%) were found to have detectable levels of parvovirus B19 IgG. Anti-parvovirus B19 IgM antibodies were detected in only four (2.9%) patients of the sickle-cell anemia group. The anti-B19 IgM-positive samples were further analyzed by real-time PCR for B19 viral DNA. All four samples were found to contain B19-viral DNA (CT values ranged between 0.00-38.02). The four patients were two males and two females and their ages were 44, 14, 27 and 36 years. All four patients were also positive for anti-B19 IgG (Table 1). All four patients presented with no symptoms of acute erythroblastopenia and they all had transfusion history within the last six to eight months. They presented with fever, malaise, pallor and no cutaneous rash. Laboratory evaluation showed anemia (Table 2). All the IgG positive/IgM negative patients presented with no symptoms of acute erythroblastopenia. Anti-parvovirus B19 IgG

Table 2. Hematological data for the four parvovirus B19-infected sickle-cell anemia patients (n = 4)

Patient	Gender/Age	Anti-parvovirus B19IgM IgG	Parvovirus B19 viral DNA	Hemoglobin concentrations g/dl (normal value)	Erythrocyte count millions/ul (Normal value)	Reticulocyte % (normal value)
1	M/ 44 Y	+ +	+	8.7 (13-18)	3.75 (4.5-6.5)	3.5 (0.5-2)
2	F/ 14 Y	+ +	+	9.4 (11-15)	3.5 (4-5.6)	3 (0.7-2.8)
3	M/ 27	+ +	+	11.5 (13-18)	3.58 (4.5-6.5)	0.2 (0.7-2.8)
4	F/ 36 Y	+ +	+	11 (11.5-16.5)	3.4 (4.5-6.5)	0.4 (0.7-2.8)

antibodies were detected in 22 (39.3%) of the control blood donors group. Anti-parvovirus B19 IgM antibodies were not detected in the control group.

When the test samples (n = 138) were analyzed for antibodies against parvovirus B19 using a western blot assay (recomBlot assay), 58 samples (42 %) were found to contain detectable levels of parvovirus B19 antibodies. All the samples that were positive for parvovirus B19 IgG by the ELISA were also positive by the recomBlot assay. Six samples were only positive by the recomBlot assay and not by the ELISA. Two of these six samples were positive for B19 viral DNA. These two patients were asymptomatic and they have no hematological abnormalities apart from the sickle cell anemia. Sixteen samples that gave equivocal results by the IgG ELISA were negative by the recomBlot assay. Using the rcomBlot as a gold standard, the sensitivity of the ELISA method used in this study was found to be 89.7% and specificity was found to be 83.3%.

Discussion

Sickle cell anemia is a highly prevalent disease in eastern Saudi Arabia. The Hb S, alpha- and beta-thalassaemia gene frequency range is 0.005-0.145, 0.01-0.40 and 0.01-0.15 respectively in various areas of Saudi Arabia [14]. There is no published data on the prevalence of parvovirus B19 infection among sickle cell anemia patients in eastern Saudi Arabia. The present study aimed to assess parvovirus B19 infection among these patients using established methods of detection including the B19 recomBlot assay.

The prevalence of anti-B19 IgG antibodies among patients and the control group (37.6 and 39.3%) is considerably higher in our study than that in previous reports of B-19 antibodies in the general Saudi population [15]. This could well be due to differences in the specificity and sensitivity of the assays used. Anti-B19 antibodies reflect previous exposure to the virus. B19 virus infection is common, and 40% to 60% of adults have antibodies against the virus due to previous exposure. Epidemiologically, infection rates increase every three to four years, but there can be longer cycles, with viral activity increasing every four to seven years [16-18]. The overall prevalence of IgG antibodies to parvovirus B19 in healthy adults is 50% in the United States and Japan [19,20]; 60%-70% in England and Wales [21]; 50% in India [22]; and 53.2% in Spain [23]. Several authors reported an increase in the parvovirus B19-specific antibodies with increase in age [1,29]. There was no correlation between viral-specific antibody positivity and increase in age in our test and control groups.

The published data on parvovirus B19 infections among Saudi patients is scanty. Alfadley *et al*. [24] described the occurrence of papular-purpuric "gloves and socks" syndrome and demonstrated the seroconversion of human parvovirus B19 in a Saudi mother and her daughter. Badr [25] analyzed human parvovirus B19 infection among patients with chronic blood disorders and with immunosuppression. He found that 78% of B19-infected patients had a chronic blood disorder while 22% had immunosuppression.

Al-Frayh *et al*. [15] detected IgG and IgM antibodies to human parvovirus B19 in the serum of patients with a clinical diagnosis of infection with the virus and in the general population of Riyadh, Saudi Arabia. Specific IgM antibodies were detected in 94% of specimens collected one week after the onset of illness and could be detected for up to two months. On the other hand, specific IgG antibodies were detected in 85% of patients from whom acute- and

convalescent-phase serum samples were collected. The overall prevalence of IgG parvovirus in the general population in Riyadh was 19.0% [26,27]. Earlier reports on the prevalence of parvovirus B19 infection may have been underestimated. Availability of newer assays that include more viral proteins from different genotypes could be related to the increased detection rate of antibodies.

In this study, B19 infection (viral IgM antibodies and viral DNA) was demonstrated in four sickle cell disease patients but not in the control group. These patients showed no symptoms of acute erythroblastopenia in spite of the anemia confirmed by the laboratory data. The high tolerance of sickle cell patients to low levels of hemoglobin is well established [27].

Acute erythroblastopenia is the first disease to be associated with parvovirus B19 [28]. In predisposed patients such as those affected with chronic hemolytic anemia, 70% to 80% of erythroblastopenia cases are caused by infection with this virus. The infected patients usually become highly viremic and pose an increased risk of B19 virus transmission [28]. Close monitoring of such high-risk groups is required to acquire data on parvovirus infection for the formulation of epidemiological surveillance programs and prevention strategies.

The B19-infected patients in this study presented with symptoms of fever and pallor. This could be due to infection with the B19 virus. Most persons with parvovirus B19 infection are asymptomatic or exhibit mild, nonspecific, cold-like symptoms that are never linked to the virus. However, clinical conditions associated with the infection include erythema infectiosum; arthropathy; transient aplastic crisis; chronic red cell aplasia; papular, purpuric eruptions on the hands and feet ("gloves and socks" syndrome); and hydrops fetalis. Conditions postulated to have a link to parvovirus B19 infection include encephalopathy, epilepsy, meningitis, myocarditis, dilated cardiomyopathy, and autoimmune hepatitis.

Parvovirus B19 frequently causes transient red cell aplasia (TRCA) in children with sickle cell disease [30]. Other parvovirus B19-related complications include acute splenic sequestration, hepatic sequestration, acute chest syndrome, nephrotic syndrome, meningoencephalitis, and stroke. These complications or untreated severe anemia could result in chronic medical conditions or death [30].

Six samples were only positive by the recomBlot assay and not by the ELISA. This could be due to the fact that recopmBlot detects antibodies to VP-N, VP-C, VP-1S and NS-1 viral protein, whereas the ELISA detects antibodies to VP1 and VP2 viral proteins [31]. Of those, two were viral-DNA-positive. These two patients could be in the early stage of B19 infection where there is viremia and the antibody level is too low to be detected by the ELISA. Sixteen samples that gave equivocal results by the IgG ELISA were negative by the recomBlot assay. This data clearly shows that the recomBlot assay is a sensitive and specific assay. RecomBlot assay is a western blot assay. In general, western blot assays allow the detection of antibodies directed against the different proteins of a particular microbe. It may be suitable as a screening as well as confirmatory assay. Recombinant immunoblot assay (RIBA) is routinely used by many laboratories worldwide as a subliminal or confirmatory test for hepatitis C virus infection. Western blot is also used in the confirmation of HIV infection. It may be argued that the western blot-based assays and/or the RT-PCR could be used in the confirmation of acute parvovirus B19 infection. The sensitivity and specificity of the ELISA used in this study are comparable with those of previous reports [1,29-30]

The discrepancy between the antibody-positive and DNA-positive samples can also result from the ability of real-time PCR assays to detect different genotypes of parvovirus B19 equally [31]. Different genotypes were shown to circulate in different countries of Europe and Africa [32-33]. Future work may include genotyping of parvovirus B19 isolates from Saudi Arabia.

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