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Case Report

Analysis of Parvovirus B19 Infection in Renal Transplant Recipients

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The clinical manifestation of Parvovirus B19 infection depends on the host's physiologic status and immunologic response. B19 infection can result in serious complications in renal transplant recipients. Participants in this study were 88 kidney recipients who regularly attended the nephrology clinics of a tertiary hospital in eastern Saudi Arabia. Viral-specific IgG and IgM antibodies were measured using a virus-like particle-based ELISA. IgM-positive samples were tested for viral DNA by Real Time Polymerase Chain Reaction (RT-PCR). This cohort of patients comprised 45 males and 43 females, of mean age 41 years (range 17 to 72). IgG antibodies to B19 were demonstrated in majority of patients (66/88; 75%). IgM showed lower frequency (18/88; 20.5%). Using RT-PCR for detection of parvovirus B18 viremia, all IgM-positive samples were found to be positive for parvovirus B19 viral DNA. Relatively more B19 seropositivity was found to be associated with receiving azathioprine (8 out of 30, 26.6 %) as compared with receiving mycophenolate mofetil (9 out of 58; 15.5 %). The seropositivity and viral DNA levels of parvovirus B19 was relatively high in the studied population of renal transplant recipients. Further studies are needed to assess the role of the immunosuppressive therapy on the infection of renal transplant recipients with parvovirus B19.

Key words: Parvovirus B19, renal transplant patients, antibodies, RT-PCR.

INTRODUCTION

Parvovirus B19 (B19) is a small non-enveloped single-stranded DNA virus. The non-enveloped viral particles show icosahedral symmetry, with about 25 nm in diameter and 60 capsomeres containing mainly VP2; VP1 accounts for only about 5% of the capsid protein. Protein folds are created (alpha) on the surface of the assembled capsids where the host's immune system can recognize them as antigenic determinants. The B19 virus has a genome that consists of a single-stranded DNA of 5,596 nucleotides with palindromic inverted terminal that repeats at both ends and forms hairpin structures (Foto et al., 1993). The clinical manifestation of B19 infection depends on the host's physiologic status and immunologic response. B19 virus is usually spread by respiratory secretions, but it can be transmitted transplacentally and by blood or blood components transfusion (Laub et al., 2002).

In immunologically competent subjects, infection may be asymptomatic or acute self-limiting diseases such as erythema infectiosum (fifth disease) in children or arthropathy in adults (Young et al., 2004). It can also cause transient aplastic crisis in patients with chronic hemolytic anemia or non-immunologic fetal hydrops in pregnancy. B19 infection in immunodeficiency states tends to be more fulminant and chronic in nature, which results in failure to produce neutralizing antibodies to B19. Pure red cell aplasia (PRCA) due to persistent B19 infection has been reported in patients with congenital or acquired immunodeficiency syndromes, those receiving chemotherapy or immunosuppressive drugs, and often after organ transplantation. In kidney transplantation (KT), B19 infection presenting as persistent anemia or PRCA was described since mid-1980s (Neild et al., 1986), and at least 21 or more cases have been documented so far (Garewal et al., 2004; Choi et al., 2002). However, only a few studies have been performed

to evaluate the incidence of active B19 infection in KT recipients, which has been reported to be variable (0 to 6.3%) (Gallinella et al., 1999; Zolnourian et al., 2000; Bertoni et al., 1997), but was higher (23%) in patients presenting anemia (Cavall et al., 2003). In addition, the clinical significance of B19 infection in KT recipients is still unclear with a broad spectrum of clinical manifestations from asymptomatic infection (Zolnourian et al.,2000) to anemia, PRCA (Bertoni et al., 1997), collapsing glomerulopathy (Moudgil, et al., 2001), and allograft dysfunction (Yango et al., 2002). In the present study, we evaluated the incidence and clinical significance of B19 infection in 88 consecutive KT recipients.

MATERIALS AND METHODS

Patients

Participants in this study were 88 kidney recipients who regularly attended the nephrology clinics of a tertiary hospital in eastern Saudi Arabia. The main inclusion criterion for participation in this study was that patients should have reached the clinically stable stage following renal transplant surgery, usually six months post-operatively. This cohort of patients comprised 45 males and 43 females, of mean age 41 years (range 17 to 72); they were predominantly Saudi nationals (83), two Yemenis, two Sudanese and an Egyptian. Standard clinical care, including medical checkups and tests for renal function, liver function and hematology parameters, was given to the renal transplant recipients in the stable post-operative stage. At this stage, all patients were receivied a standard immunosuppressive regimen consisting of cyclosporin and prednisolone in combination with either azathioprine or mycophenolate mofetil. The administered dosage of each of these drugs was carefully adjusted as necessitated by the clinical status of each patient. Statistical analysis of the data was performed using the SPSS statistical package (chi-square or Fisher's exact tests) to calculate the *P*-values with $p < 0.05$ as the cut-off level of significance.

Detection of anti-parvovirus B19 antibodies and viral DNA

Serum samples were collected from all study participants using a vacuum system. Sera obtained after centrifugation of clotted samples were stored at –80°C until evaluation. Enzyme-linked immunosorbent assay (ELISA – Biotrin International, Dublin, Ireland) was used to detect IgG and IgM antibodies to parvovirus B-19 virus in human serum with a sandwich enzyme immunosorbent assay using B19 virus recombinant VP2 as described previously (Erdman et al., 1991). In brief, the Biotrin International Parvovirus B19 IgG and IgM Enzyme Immunoassay are sandwich enzyme immunoassay for the detection of IgG or IgM class antibodies to Parvovirus B19 VP1 and VP2 proteins in human serum and plasma. The assay was performed according to the manufacturer's recommendations. Result interpretation, that is, whether anti-parvovirus IgG or IgM were present or not, was based on an estimated cut-off value (COV). In the interpretation of absorbencies, samples with mean absorbance equal to or greater than COV x 1.1 were considered reactive (positive) and those with mean absorbance lower than COV x 0.9 were considered non-reactive (negative) to antiparvovirus B19 IgG and IgM. If results fell into the equivocal zone, the assay was repeated and the repeat result was taken as the overall final result for the specimen.

All patients' samples were tested for virus-specific antibodies by the ELISA (IgG and IgM). IgM-positive and discrepant results were tested for viral DNA by the RT-PCR. Detection of B19-viral DNA was done by RT-PCR using Parvovirus B19 Real-TM kit (Sacace biotechnologies, Italy). The sensitivity of the assay was not less than 200 copies/ml.

RESULTS

Patients' data

In the present study, the kidney donors came from Saudi Arabia (35 patients), the Philippines (16), Pakistan (15), Egypt (16), Bangladesh (6), India (6) and Yemen (3) as well as from five other Asian countries (6). The kidney recipients originated from different regions within Saudi Arabia as follows: northern (46 patients), southern (29), western (12) and eastern (3). For the majority (72 out of 88) of renal transplant recipients, hemodialysis was performed for extended periods prior to renal transplantation in five different medical centers in the eastern region of the country. For the rest of patients, hemodialysis was performed in various hospitals of the other regions of the country.

The time that elapsed since the renal transplantation averaged 38 months. The primary cause of renal failure in the renal transplant recipients was unknown (36 out of 88; 40.9 %), hypertension (27 out of 88; 30.7 %), diabetic nephropathy (8 out of 88; 9.1 %), glomerulonephritis (7 out of 88; 8 %), chronic pyelonephritis (4 out of 88; 4.5 %) and other causes related to five different diseases (6 out of 88; 6.8 %).

Detection of parvovirus B19:

Viral-specific IgG and IgM antibodies were measured in serum samples from 88 kidney transplant recipients post transplant period by using a virus-like particle-based ELISA. IgG antibodies to B19 were demonstrated in majority of patients (66/88) (75%), irrespective of clinical category, but titers were highest in patients with viremia. IgM antibodies showed a similar pattern of reactivity but lower frequency of 18/88 (20.5%). Using RT-PCR for detection of parvovirus B18 viremia, all IgM-positive samples were found to be positive for parvovirus B19 viral DNA. A rise in IgG level of >2.1 of the COV units or a rise in IgM level of >1.6 of the COV units was strongly associated with viremia.

Our data shows that 30 kidney transplant recipients received azathioprine and 58 patients received mycophenolate mofetil. Both of these two subgroups of patients received comparable low doses of cyclosporin and prednisolone. Relatively more B19 seropositivity was found to be associated with receiving azathioprine (8 out of 30, 26.6%) as compared with receiving mycophenolate mofetil (9 out of 58; 15.5 %), but this difference was not statistically significant ($P = 0.228$). Among both the aza-

thioprine and myophenolate mofetil subgroups, there were no significant differences between the high daily dose (≥75 and ≥1000 mg, respectively) and low daily dose (≤50 and ≤500 mg, respectively) regimens of the two immunosuppresive drugs used with regard to B19 seropositivity. The present results show that the association of B19 seropositivity in renal transplant recipients was higher (8 out of 30; 26.6%) in patients with hypertension as the primary cause of renal failure when compared with B19 seropositivity in patients with all known causes of renal failure together (4 out of 25; 15%). However, this difference was not statistically significant (*P* = 0.221).

DISCUSSION

Parvovirus B19 infection is common throughout the world. The percentage of people with measurable levels of B19-specific IgG increases with increasing age, with most individuals becoming infected during their school years. During school outbreaks, 25 to 50% of students and 20% or more of susceptible staff may become infected. More than 70% of adults have measurable B19-specific IgG antibodies (Cohen et al., 1988., Kerr et al., 1999). Analyzing sera for the presence of B19-specific IgM antibodies is a practical and common approach for diagnosing acute infection in the immunocompetent individual (Erdman et al., 1997). Detectable levels of B19-specific IgM can be found within 7 to 10 days of virus exposure and remain measurable for several months before diminishing (Erdman et al., 1991). In some patients, B19-specific IgM antibodies can persist for six months or more. Therefore, the presence of these antibodies, especially at low titers, is suggestive but not conclusive proof of recent infection. Acute infection can also be diagnosed by demonstrating a fourfold or greater rise in serum B19-specific IgG antibody titers. However, since this procedure requires two separate time points for sample collection, it is considered impractical in most clinical situations. In this study, we used the ELISA method which concurrent analyses for both B19-specific IgM and IgG antibodies from a single blood sample.

B19 infection can be a serious complication of in KT recipients, which may result from nosocomial spread of respiratory secretions (Anderson et al., 1985) or the administration of contaminated organ (Yango et al., 2002).

Although many cases of B19 infection in KT recipients have been reported, few studies were performed to assess the incidence of clinical significance of B19 infection in KT recipients. Gallinell et al. (1999), evaluated the occurrence of B19 in a total of 212 recipients who underwent solid organ or bone marrow transplantation and found only one liver transplant with positive B19 IgM. Among 59 KT recipients, none had positive B19 IgM. Zolnourian et al. (2000), also reported a very low frequency of B19 infection in KT among 110 KT recipients, only two recipients showed positive B19 IgM. On the other hand, Cavallo et al. (2003), evaluated the incidence of B19 in KT recipients presenting anemia and reported that 11 of 48 (23%) were positive PCR.

In the present study, we collected a total of 88 samples from KT recipients and found that The B19 IgM and viral DNA positive rate was 20.5% (18/88). It is not clear why our subjects showed such a higher incidence of B19 infection than those in the previous reports. However, a possible explanation could be an outbreak of B19 infection during the period of the study. Lui et al. (2001), confirmed the nosocomial transmission of B19 by molecular epidemiologic studies in KT recipients showing that three recipients had genetically identical B19 strains. Considering the high infection rates reported among exposed susceptible individuals, the unusually high incidence of positive B19 PCR during the period of seven months in our study is presumed to be attributable to an outbreak of B19 infection in the KT recipients.

The role of immunosuppressive therapy was observed; those who received azathioprine showed more B19 seropositivity (26.6%) when compared with mycophenolate mofetil (15.5%). However, this was not statistically significant (*P* = 0.228) and that might be to the low number of screening samples

Although there were many reports of B19 infection in KT recipients, the clinical significance of B19 infection is still controversial. Usually, B19 infection is associated with transient or chronic anemia, which can be cured by IVIG treatment (Choi et al., 2002; Liefeldt et al., 2002). However, direct renal involvement leading to various forms of glomerulopathy and allograft dysfunction has been reported (Moudgil et al., 2001; Yango et al., 2002; Barsoum et al., 2002; Moudgil et al., 1997).

From this study, it is possible to conclude that B19 infection is common in KT recipients and the clinical infection should be suspected when a recipient had two or more positive B19 IgM or PCRs. In addition, B19 infection is significantly associated with low Hb levels and PRCA after KT. However, there is no evidence of association between B19 infection and graft dysfunction. Further studies are needed to address the question whether B19 PCR testing should be used or not as a confirmation of positive IgM result for KT recipients.

Conclusions

The seropositivity and viral DNA levels of parvovirus B19 was relatively high in the studied population of renal transplant recipients. Further studies are needed to assess the role of the immunosuppressive therapy on the infection of renal transplant recipients with parvovirus B19.

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