CASE REPORT

PARVOVIRUS B19 INFECTION IN HIV PATIENT WITH PURE RED CELL APLASIA

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Abstract. Anemia in HIV-infected patients is a common clinical manifestation. We report on a 31-year-old Thai male, who had been HIV positive for 6 years, did not harbor any opportunistic infection, and had been receiving Highly Active Anti Retroviral Therapy (HAART) for one month, and who developed severe anemia. Investigation revealed pure red cell aplasia, suspected secondary to parvovirus B19 infection. This diagnosis was confirmed by the detection of parvovirus B19 DNA in his serum. He received blood transfusions for supportive treatment and continued on HAART to improve his immune status and to resolve the anemia. This case suggests that parvovirus B19 infection should be considered as a possible cause of anemia in HIV-infected individuals.

INTRODUCTION

Human parvovirus B19 infection occurs predominantly in pediatric patients, with seroprevalence increasing with age. The overall prevalence in Thailand has been reported to be 20.16% (Poovorawan *et al*, 2000). In healthy children, most cases are asymptomatic. However, some may present with fever and exanthematous rash, otherwise known as fifth disease. In healthy adults, particularly middle-aged woman, this infection may cause clinically significant arthropathy (Naides, 1998; Kerr, 2000; Moore *et al,* 2000).

Pure red cell aplasia is a clinical syndrome defined by the absence of mature erythroid precursors in an otherwise normal bone marrow. This leads to severe normochromic normocytic anemia with low reticulocyte counts, and normal platelet and granulocyte counts. Initial symptoms may include loss of appetite, headaches, poor concentration, and irritability. Subsequently, patients will express symptoms and signs of anemia, such as fatigue, weakness, cold fingers and toes, pallor, dizziness, depression, or heart failure. Pure red cell aplasia can also be a manifestation of persistent parvovirus B19 infection (Young and Brown, 2004). This manifestation is usually found in immunocompromized patients, such as HIV^+/AIDS patients.

In Thailand, HIV infection is highly prevalent, and one of the clinical manifestations of symptomatic HIV is anemia. The overall prevalence of anemia among symptomatic HIV patients is 30.3% and is generally associated with CD4+ T lymphocyte counts below 50 cells/mm^3 (Wills *et al*, 2004). In immunocompromized individuals, anemia usually develops from multiple etiologies, such as anemia of chronic disease, medication-induced bone marrow suppression (*eg*, zidovudine, trimethoprim-sulfamethoxazole, amphotericin), medication-induced hemolysis (*eg*, trimethoprim-sulfamethoxazole, dapsone), and infection-related hypoproduction (*eg*, parvovirus B19, disseminated tuberculosis, histoplasmosis, penicillosis and *Mycobacterium avium* complex (MAC) (Hawkins *et al*, 1986; Frickhofen *et al*, 1990; Mitchell *et al*, 1990). Parvovirus B19 multiplies in the erythroid progenitor cells of the bone

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marrow, so the patient commonly presents with severe anemia (Ozawa *et al,* 1986). Parvovirus B19 infection is one of the important treatable causes of anemia in HIV infected individuals. It can be treated with IVIG or HAART regimen. (Frickhofen *et al*, 1990; Mylonakis *et al*, 1999). Therefore, an awareness and detection of this virus is significant.

Laboratory diagnosis of parvovirus B19 infection relies on serologic and DNA tests. Detectable DNA is required to diagnose persistent viremia, because antibody production is absent or minimal (Zerbini *et al*, 2002). Direct hybridization methods are reliable and detect viral titers of more than 10^6 genome copies (Young and Brown 2004). In Thailand, there are limited data on the parvovirus B19 infection. Our objective was, by reporting a case of an immunocompromised patient, who presented with anemia and was diagnosed with parvovirus B19 infection by polymerase chain reaction (PCR) to detect viral DNA as evidence of parvovirus B19 infection, to make clinicians aware of this condition in immunocompromized patients.

CASE REPORT

The patient was a 31 year-old Thai male, known to have been HIV positive for 6 years. He had never had any opportunistic infection and had been regularly seeing his physician for follow-up. His most recent CD4 count was 53 cells/mm^3 (3%). In June 2004 he started the HAART GPOvir regimen (3TC, d4T, NVP).

Two weeks before this admission, he suffered several episodes of calf pain after walking. Also, he complained of numbness in his toes. He still could exercise regularly but felt much more exhausted than before. He occasionally suffered from syncope and dizziness. Three days before this admission, He had pitting edema in both legs . He denied having orthopnea. He was admitted in order to determine the cause of anemia.

On admission, a complete blood count (CBC) was performed that revealed he was anemic, with a RBC of 1.88x106 cells/mm^3 (Hct 12.5%, Hb 4.8 g/dl, MCV 67 fl, MCH 25.8 pg, and RDW 15.0%). His WBC was 4,660 cells/mm^3 (PMN 41.4%, lymphocytes 37.7%, monocytes 5.9% eosinophils 9.4%, basophiles 1.4%, and LUC 4.3%). His platelet count was 545,000/mm^3. Physical examination revealed he was moderately pale without icteric sclera. His heart was regular sized with normal heart sound. His lungs were clear and devoid of adventitious sound. His liver was just palpable with an 8-cm span. The spleen could not be palpated. His skin and mucosa were normal. Neither petichiae nor ecchymosis was found on his body and extremities; nor were koilonychia or glossitis detected.

He underwent further examinations. The reticulocyte count was 0.1%, and his peripheral blood smear showed moderate hypochromia and minor microcytosis. Nucleated red blood cell and polychromasia were not found (Fig 1A). A liver function test showed total bilirubin 0.15 mg/dl, direct bilirubin 0.03 mg/dl, SGOT 27 U/l (0-38), SGPT 46 U/l (0-38), ALP 124 U/l (39-117), and albumin 3.7g/dl. A renal function test revealed BUN 12 mg/dl, and Cr 0.8 mg/dl. His LDH was 468 U/l (230-460). Serum ferritin was 414.8 ng/ml (30-400). Stool occult blood was negative. Hemoglobin typing showed HbA 61.1% and HbA2/E 38.9%, which is compatible with HbE trait. A bone marrow biopsy was done. Trephine bone marrow biopsy demonstrated slightly decreased cellularity with essentially normal myeloid cells and megakaryocytes. There was a marked deficiency of erythropoietic cells, especially normoblasts (Fig 1B). Occasional giant pronormoblasts were present (Fig 1C and 1D) along with mildly increased interstitial small lymphoid cells.

He was diagnosed with Pure Red Cell Aplasia (PRCA). He received blood a transfusion with 2 units PRC and was discharged. After one month, he reported for follow-up as scheduled. A CBC revealed he was still anemic with Hb 3.9g/dl, Hct 11.6%, MCV 67 fl, MCH 22.6 pg, and RDW 16.3%. He was admitted for further investigation and discussion about his treatment; although his clinical status was stable.

Parvovirus B19 detection

To detect parvovirus B19, we obtained serum from the patient and extracted DNA, applying the alkaline extraction method adjusted for an initial volume of 15 µl. For parvovirus B19 DNA

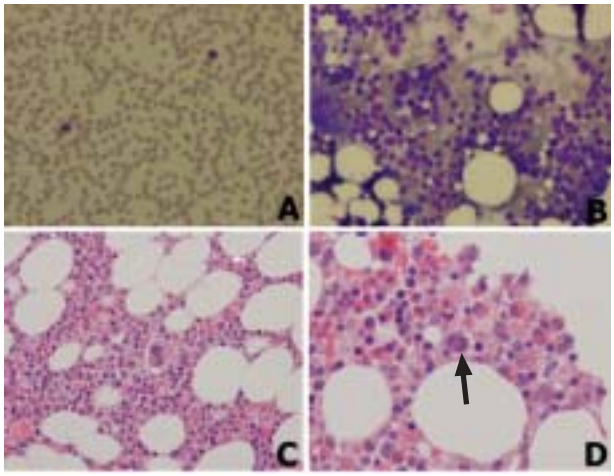

Fig 1–A : Peripheral blood smear shows microcytic anemia and mild anisocytosis. B : Bone marrow (Wright stain, 40x) shows mildly hypocellular bone marrow with marked erythroid hypoplasia. Normal myeloid cells and megakaryocytes are noted. C. Trephine biopsy of bone marrow showing usual amount and appearance of myeloid cells and megakaryocytes, but absence of normoblasts with some small lymphocytes in the interstitium. D. Trephine biopsy of bone marrow exhibiting a giant pronormoblast (Arrow).

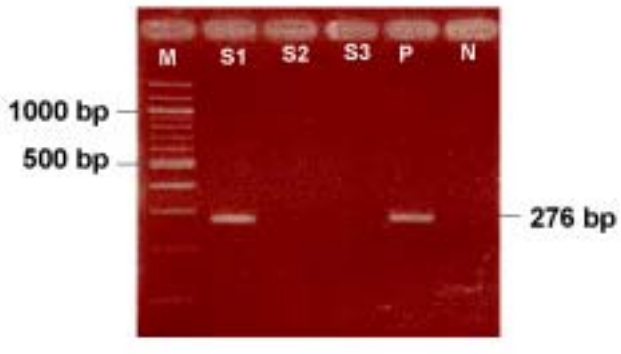

Fig 2–Agarose gel electrophoresis of PCR amplification products of Parvo virus B19 DNA stained with ethidium bromide and photographed under UV light. M denotes the molecular base pairs markers; S, specimen (S1, patient's specimen); P, positive control; N, negative control.

detection, we amplified the viral protein 2 (VP2) gene by nested PCR in an automated thermocycler (Perkin Elmer Cetus, Norwalk, USA). Briefly, 5 µl of the respective DNA samples were added to a reaction mixture containing 1 unit Taq polymerase (QIAGEN, Hilden, Germany), 20 mM dNTP, primer pair KO1 (located at position 3,187; 5′-CAAAAGCATGTGGAGTGAGG-3′), and KO2 (located at position 3,583; 5′-TACTAACATGCATAGGCGCC-3′) for the first amplification round; and primer pair KI1 (located at position 3,286: 5′-TAAGGTGTTTTCTCC CGCAG-3′) and KI2 (located at position 3,561: 5′-GTAGTGCTGTCAGTAACCTG-3′) for the second amplification round. Each primer was at a 0.75 µM concentration, 5 µl 10x buffer and 2 mM $MgCl_2$ (QIAGEN, Hilden, Germany) at a final volume of 50 µl. The amplification reaction included an initial denaturation step at 94ºC for 5 minutes, followed by 40 cycles of a 30-second denaturation step at 95ºC, a 30-second annealing step at 55ºC, and a 50-second extension step at 72ºC, each. The amplification was concluded by a final 10-minute elongation step at 72ºC. For the second amplification round, 2 µl of the first PCR product was added to the reaction mixture, and amplification was performed in a manner identical to the first round. Ten µl of each amplified DNA sample was loaded on a 1.5% agarose gel stained with ethidium bromide on preparation. Electrophoresis was performed with a known 276 base-pair parvovirus B19 positive DNA sample (AY 251006) as a positive control (Fig 2). Sterile water served as the negative control. The sensitivity of the test for parvovirus B19-DNA detection in our laboratory was 10^4 copies/µl.

To confirm the PCR results, the first round PCR product was purified from the agarose gel using the Gel Extraction Kit (Perfectprep Gel Cleanup, Eppendorf, Hamburg, Germany) according to the manufacturer's instructions. The purified product was subjected to automated sequencing (ABI-Prism 310, ABI, Fostercity, CA). The sequence was analyzed by a sequence navigator program and submitted to the GenBank database (accession number AY701188). The nucleotide sequence obtained was further confirmed by comparison with the sequences previously published in GenBank, applying the BLAST program (www.ncbi.nlm.gov/BLAST).

Clinical course

He received 2 units of PRC, before being discharged with Hct 22%. For the next three

weeks he appeared weekly at the hematology clinic for follow-up. Although he was still pale, his clinical status was stable. He exercised regularly and had no obvious anemic symptoms. On the fourth visit, he complained about his tiredness (Hct 10.3%); therefore, he was given a blood transfusion. The following week he came to see his physician and had no further complaints. His latest Hct was 16.8%, with a CD4 count of 149 cells/mm^3 3 months after having started HAART. His viral load was less than 50 copies/ml. He was continuing to be monitored by the doctor to see whether the PRCA would improve after the reconstitution of his immune status.

DISCUSSION

Among healthy individuals in Thailand, immunity to parvovirus B19 increases with age, with the overall prevalence of parvovirus B 19 infection being 20.16% (26/129) (Poovorawan *et al*, 2000). It has been reported at 16.0 % (17/106) among immunocompromized children in Thailand (Suandork *et al*, 2000).

This patient presented with severe anemia without evidence of blood loss or hemolysis in either his history or clinical findings. The peripheral blood smear (PBS) revealed that he had microcytic hypochromic anemia. Serum ferritin and physical examination excluded iron deficiency as its cause. The hemoglobin typing revealed that he had HbE trait, which could not explain severe anemia in this patient. The reticulocyte count was <0.1% that indicated a hyporesponsiveness of bone marrow to anemia. A bone marrow biopsy was performed and revealed decreased erythroid progenitor cells, with others compatible with pure red cell aplasia. Persistent parvovirus B19 infection was considered among the most plausible causes of pure red cell aplasia in an immunocompromized host, especially an HIV-positive one. (Abkomiz *et al*, 1997).

The presence of parvovirus B19 infection may also be hinted at by the histopathological aspect of the bone marrow: overall hypocellularity and the presence of giant pronormoblasts with finely granulated cytoplasm and glassy, variably eosinophilic, intranuclear inclusions with a clear central halo (lantern cells) are thought to be indicative of parvovirus B19 infection (Koduri *et al*, 1998). Confirmation of parvovirus B19 infection typically relies on the identification of a specific antibody or viral DNA (Portmore *et al,* 1995). With immunocompromized patients, serological methods of diagnosis are acknowledged as being somewhat imprecise (Zonourian *et al*, 2000). In this case, the diagnosis was confirmed by parvovirus B19-DNA detection after provisional diagnosis based on clinical parameters and bone marrow biopsy.

The management of chronic parvovirus B19 infection has been a subject of controversy as intravenous immunoglobulin therapy is expensive and should not be used simply on an empirical basis or as a therapeutic test (Frickhofen *et al*, 1990). Also, the effect of some therapies used to treat anemia in AIDS patients, for example, erythropoietin can be harmful in B19-infected patients, as they may increase the substrate for viral replication. However, the resolution of anemia can be achieved by the reconstitution of immunity by HAART (Mylonakis *et al*, 1999). In this case, the treatment was supported by blood transfusion and continued HAART in order to increase immunity and thus encourage remission of parvovirus B19 infection.

ACKNOWLEDGEMENTS

We would like to express our deep gratitude to The Thailand Research Fund for supporting our group research, including the laboratory diagnosis. We also would like to thank the entire staff, who have been taking care of this patient and Ms Pisanee Saiklin for reviewing the manuscript.

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