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ORIGINAL ARTICLE

Molecular and serological assessment of parvovirus B-19 infection in Egyptian children with sickle cell disease

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Abstract *Background/Purpose:* Human parvovirus B-19 (PB-19) is a cause of hemolysis, red blood cell aplasia, and severe conditions in patients with sickle cell anemia, but the molecular mechanisms of the infection are still insufficiently understood. This study aimed to detect PB-19 DNA together with its antibodies in the sera of Egyptian children with sickle cell disease and to assess the contribution of this infection, which causes transient cessation of erythropoiesis, in precipitating severe anemia in some cases.
Methods: One hundred children with sickle cell disease seeking medical advice in the pediatric-hematology clinic were recruited. Sera of the patients were compared with those of 60 healthy children regarding the presence of PB-19 immunoglobulin (Ig)G and IgM as well as detection of its DNA by nested-polymerase chain reaction technique.
Results: There were statistically significant differences in the prevalence of PB-19 IgM, IgG, and DNA among patients when compared with controls ($p < 0.001$, $p = 0.001$, and $p < 0.001$ respectively). Acute PB-19 infection detected by positive IgM and DNA was found in 30% of the patients, while chronic PB-19 infection detected by positive IgG and DNA was detected in 24% of the patients. Anemia was worse in children with acute PB-19 infection than in those with chronic infection, while anemia was mild in children with old infection.
Conclusion: PB-19 infection is detected at high rates among Egyptian children with sickle cell disease and it may result in severe anemia. So, PB-19 must be suspected and screened for in such group of patients.
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Introduction

Human parvovirus B-19 (PB-19), a member of parvoviridae family, is a common infectious pathogen in humans primarily found in children.[1] The PB-19 virus has a specific tropism for erythroid progenitor cells and thus can cause a temporary infection of the bone marrow eventually leading to impaired erythropoiesis for 7—10 days and transient arrest for 3—7 days.[2] The effect of this on hemoglobin level varies by individual. In healthy children, a mild decrease in hemoglobin levels might occur,[3] whereas a greater decrease has been described in children with iron deficiency and malaria.[4]

In patients with sickle cell disease and other hemolytic anemia, erythroid progenitor cell formation is increased to compensate for red blood cell lysis, but a precipitous decrease in hemoglobin level can be induced through the combination of a high rate of red blood cell destruction and complete cessation of red blood cell production inducing erythropoiesis suppression caused by PB-19, which is often referred to as *transient aplastic crisis*.[5] These patients are at risk of severe clinical illness and become highly viremic. Close monitoring of such high risk groups for this viral infection is, therefore, of great importance for epidemiologic surveillance and disease prevention.[6]

Traditionally, the diagnosis of acute PB-19 disease has been based solely upon the detection of PB-19 immunoglobulin (Ig)M which appears only on days 8—10 post-infection.[3] Virus-induced bone marrow suppression begins to recover on day 16 and the PB-19 IgM usually becomes undetectable after 2—4 months depending on the initial level of response,[7] although persistence for up to 9 months has been reported.[8] After PB-19 virus infection, IgG persists, which is generally useful for diagnosis of chronic or old infection.[9]

Major advances in diagnosis of PB-19 infection have taken place including standardization of serological and DNA based detection methodology.[10] As there is no reliable immunological method for antigen detection, polymerase chain reaction (PCR) is needed for detecting viremia.[11] Combined use of PCR and enzyme-linked immunosorbent assay (ELISA) are optimal for diagnosis of PB-19 infection.[12]

The aim of this study was to detect PB-19 DNA together with its antibodies in the sera of Egyptian children with Sickle cell disease and to assess the contribution of this infection, which causes transient cessation of erythropoiesis, in precipitating severe anemia in some cases.

Methods

This was a prospective, observational study performed in the Pediatric-Haematology Department of Elmounira Children Hospital, Faculty of Medicine, Cairo University, Cairo, Egypt. It was conducted on 100 Egyptian children with sickle cell disease who were seeking medical advice in the pediatric-hematology clinic; 64 of them were diagnosed as sickle cell anemia (homozygous SS) and 36 as sickle cell trait (heterozygous AS). Thirty patients were presented with acute aplasic crisis (a precipitous drop in hemoglobin level occurs in the absence of adequate reticulocytosis).[5] The mean age was 7.8 $\pm$ 1.7 years, 53% of them were males and 47% were females. Sixty age- and sex-matched normal healthy children living in the same area who came for routine checkup in the pediatric clinic were also included as a control group. Written informed consent was obtained from the parents of all the participants as well as Ethical Committee approval.

All children were subjected to complete history taking and full clinical examination. Routine laboratory investigations were carried out including complete blood picture, reticulocyte count, sickling test, and hemoglobin electrophoresis. Virological study for PB-19 included determination of specific IgG and IgM by ELISA together with viral DNA by nested-PCR were performed.

Detection of PB-19 IgG and IgM by ELISA

Serum samples were obtained for detection of PB-19 specific IgG and IgM antibodies by ELISA using a commercial assay (Parvoscan-B19; Biotrin International, Dublin, Ireland), according to the manufacturer's instructions. This assay detects antibodies directed against viral protein VP2. The IgM enzyme immunoassay (EIA) kit has a reported sensitivity of 86% and specificity of 95% (Parvovirus B-19 IgM Biotrin International) and the IgG EIA has a reported sensitivity and specificity of 100% (Parvovirus B-19 IgG Biotrin International). Positive (reactive) and negative (nonreactive) samples were calculated according to the manufacturer's recommendations.

Detection of PB-19 DNA by nested-PCR

Serum samples were further subjected to DNA extraction using the QIAamp DNA blood kit (Qiagen, Hilden, Germany) as described by the manufacturer. The extracted DNA was subsequently used as a template for the detection of PB-19 DNA by nested-PCR using two primer pairs that targeted the minor (VP1) and major (VP2) capsid protein genes according to Regaya et al.[8] The outer primers were: sense, 5′-CAAAAGCATGTGGAGTGAGG-3′; antisense, 5′-CTACTAACATGCATAGGCGC-3′. The inner primers were; sense, 5′-CCCAGAGCACCATTATAAGG-3′; antisense, 5′-GTGCTGTCAGTAACCTGTAC-3′. Each PCR assay was a 50 μL reaction mixture containing 5 μL of extracted DNA sample, 50mM KCl, 10mM Tris-HCl (pH 8.3), 1.5mM $MgCl_2$, 200μM of each deoxyribonucleoside triphosphate, 1.25 U of Taq DNA polymerase (Promega, Madison, WI, USA), 0.5μM of each outer or inner primers (Biomers, Ulm, Germany), and DNAse free water. Positive control DNA (cloned PB-19 virus) to establish the specificity of the reaction. Internal control for β-globin gene was included for each sample to exclude false negative results. Amplification reactions were performed in a thermocycler program, consisting of one cycle of denaturation at 94°C for 5 minutes, 30 cycles denaturation at 94°C for 1 minute, annealing at 55°C with outer primers or 57°C with inner primers for 2 minutes and extension at 72°C for 3 minutes and a final extension step at 72°C for 5 minutes. Both primary and secondary PCR products as well as β-globin were separately electrophoresed on 2% agarose gel prestained with ethidium bromide and visualized on a UV transilluminator (wave length

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