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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

312 nm) obtaining bands of sizes 398 bp, 288 bp, and 208 bp respectively after extrapolation for mobility with 100 bp ladder DNA markers (Invitrogen, Carlsbad, CA, USA) as shown in Fig. 1.

Statistical analysis

Data were collected and tabulated using Excel version 7 (Microsoft Corporation, New York, USA) and analyzed using SPSS for Windows version 11 (SPSS Inc., Chicago, IL, USA). All data were described in terms of mean and standard deviation. Paired and independent sample Student *t* test was used after assessing the normal distribution of values to compare between the two groups. The χ^2 test, or the Fisher's exact test (2-tailed) if appropriate, was applied for the categorical variables, and Kruskal–Wallis one-way analysis of variance (ANOVA) for continuous variables. A p value < 0.05 was considered statistically significant.

Results

The results of PB-19 IgM and IgG antibodies and its DNA for patients and controls are summarized in Table 1. There were statistically significant differences in the prevalence of PB-19 IgM, IgG, and DNA among the patients when compared with the controls ($p < 0.001$, $p = 0.001$, and $p < 0.001$ respectively). Acute parvovirus infection was detected in 30% of the patients presented with sickle cell disease, while not detected (0%) in the control group ($p < 0.001$), whereas chronic infection was detected in only 24% of the patients versus 3.3% of the controls ($p = 0.015$). Moreover, there was no difference between the two groups regarding the presence of old infection ($p = 0.106$).

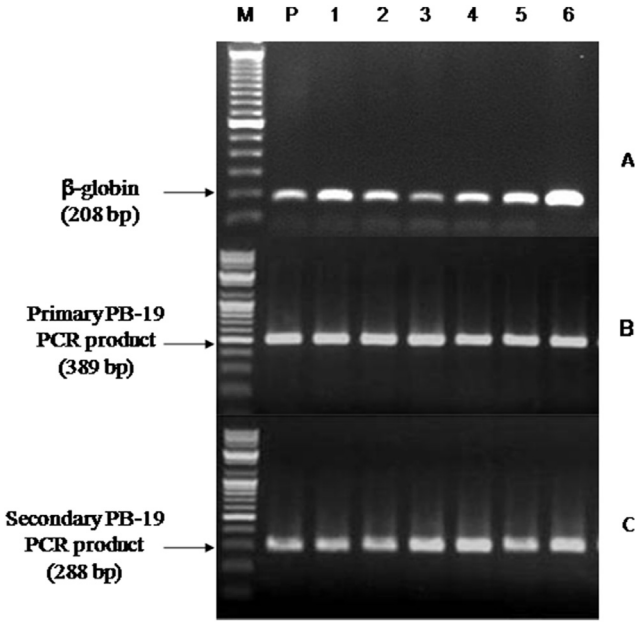

Figure 1. Agarose gel electrophoresis pattern of parvovirus B-19 (PB-19) DNA amplicons in sickle cell disease patients. (A) β-globin DNA bands serves as an internal control. (B) PB-19 DNA bands obtained in the first round of polymerase chain reaction. (C) PB-19 DNA bands obtained in the second round of polymerase chain reaction. Lanes 1–6 = amplicons of sera from Patients 1–6, respectively; M = molecular weight marker (100–1500 bp ladder); P = positive control.

Table 1 Comparison between the children with sickle cell disease and the control group.

Items	Study group ($n = 100$)	Control group ($n = 60$)	p
Age (y)	7.81 ± 1.74	7.14 ± 1.85	0.115
Sex			
Male	50 (50)	26 (43.3)	0.563
Female	50 (50)	34 (56.7)	
Hemoglobin (g%)	8.38 ± 1.39	12.45 ± 1.98	<0.001
RBCs ($\times 10^6/\mu L$)	4.15 ± 0.91	5.16 ± 1.08	<0.001
Hematocrit (%)	25.62 ± 3.97	34.31 ± 2.65	<0.001
IgM positivity	30 (30)	0 (0%)	<0.001
IgG positivity	44 (44%)	6 (10%)	0.001
Parvovirus B-19 DNA detection by PCR	54 (54%)	2 (3.3%)	<0.001
Acute infection (IgM & PCR+ve)	30 (30)	0 (0)	<0.001
Chronic infection (IgG & PCR+ve)	24 (24)	2 (3.3)	0.015
Old infection (Anti-parvovirus B-19 IgG +ve)	20 (20)	4 (6.7)	0.106

Data are presented as *n* (%) or mean ± standard deviation.
Ig = immunoglobulin; PCR = polymerase chain reaction; RBCs = red blood cells.

Table 2 shows comparison between the study population with sickle cell disease according to their clinical presentation whether they came with aplastic crisis (30 patients) or without crisis (70 patients). Acute parvovirus infection was detected in 86.6% of the patients presented with aplastic crisis, while only in 5.7% of those without crisis ($p < 0.001$), whereas chronic and old infections were detected in only 6.7% and 6.7% of the patients with crisis versus 31.4% and 25.7% of those without crisis respectively. These differences were not statistically significant ($p = 0.062$ and $p = 0.123$, respectively).

When the patients with sickle cell disease were classified according to PB-19 infection into acute infection (IgM positive and DNA detection by PCR), chronic infection (IgG positive and DNA detection by PCR), old infection (IgG positive only), and no PB-19 infection (IgM, IgG negative and no DNA detection by PCR), as shown in Table 3, statistically significant differences between the three groups regarding hemoglobin level, red blood cells count, and hematocrit values were found ($p = 0.006$, $p = 0.016$, and $p = 0.003$ respectively). Anemia was worse in children with acute PB-19 infection than in those with chronic infection, while anemia was mild in children with old PB-19 infection.

Discussion

Parvovirus B-19 infection in humans is distributed worldwide. Certain populations are vulnerable to complications

Table 2 Comparison between patients with sickle cell disease with and without acute aplastic crisis.

Items	Sickle cell patients with acute aplastic crisis ($n = 30$)	Sickle cell patients without acute aplastic crisis ($n = 70$)	p
Hemoglobin (g%)	6.41 ± 0.36	8.91 ± 1.34	<0.001
RBCs ($\times 10^6/\mu L$)	3.81 ± 0.35	4.13 ± 0.61	0.021
Hematocrit (%)	20.07 ± 1.09	27.88 ± 1.76	<0.001
Acute infection (IgM & PCR+ve)	26 (86.6)	4 (5.7)	<0.001
Chronic infection (IgG & PCR+ve)	2 (6.7)	22 (31.4)	0.062
Old infection (Anti-parvovirus B-19 IgG +ve)	2 (6.7)	18 (25.7)	0.123

Data are presented as n (%) or mean ± standard deviation.
Ig = immunoglobulin.

Table 3 Comparison between patients with acute, chronic, old and no parvovirus B-19 infection.

Items	Acute infection ($n = 30$)	Chronic infection ($n = 24$)	Old infection ($n = 20$)	No infection ($n = 26$)	p
Hemoglobin (g%)	6.16 ± 1.98	8.42 ± 1.09	9.33 ± 1.99	10.41 ± 1.25	0.006
RBCs ($\times 10^6/\mu L$)	3.19 ± 0.89	3.95 ± 0.64	4.01 ± 0.39	4.92 ± 0.68	0.016
Hematocrit (%)	20.86 ± 2.63	27.83 ± 3.46	29.82 ± 2.97	31.28 ± 3.74	0.003

Data are presented as mean ± standard deviation.

as a result of depressed erythropoiesis that arises with PB-19. These populations include people with hemolytic disorders that cause an increased rate of erythrocyte destruction.[13] Some epidemiological studies of several countries showed that the prevalence of PB-19 infections varied among countries and populations.[6,14]

Miron et al,[15] Zimmerman et al,[16] and Salvov et al[17] studied acute PB-19 in hospitalized children and demonstrated that both serum PB-19 IgM and detection of PB-19 DNA by PCR are informative and that the evaluation of both is necessary to achieve a high level of diagnostic accuracy. In other studies, it was suggested that chronic PB-19 infection was demonstrated by the presence of PB-19 DNA and anti-PB-19 IgG in patients' sera in the absence of anti-PB-19 IgM.[12,18]

Our study demonstrated that 30% of the studied patients with sickle cell disease have acute PB-19 infection and 24% of them have chronic infection, while 20% of them have an old infection. Almost similar rates were recorded by studies done in USA and Guinea on patients with sickle cell anemia.[1,3] Higher seroprevalence of anti-PB-19 IgG in patients with hematological disorders including thalassemia and sickle cell disease were reported in Taiwan, Sweden, and Nigeria.[12,19,20] In agreement with the current data, the prevalence of IgG antibodies in patients with several types of chronic hemolytic anemia was 56% in Egypt,[21] 40% and 68% in Saudi Arabia,[2,22] and 56.5% in Tunisia.[8]

The present study also revealed that PB-19 infection, especially acute infection, is a major contributor to severe anemia in children with sickle cell disease. Anemia was worse in children with acute PB-19 infection than in those with chronic infection, while anemia was mild in children with old PB-19 infection. This finding is reminiscent of other researches[3,17,23] that reported a serological association between acute PB-19 infection and severe anemia. These reports also stated that children with hemolytic disorder would have an even larger decrease in hemoglobin levels if they developed the PB-19 infection and they might be even more prone to PB-19 induced severe anemia.

The strength of association between PB-19 and severe anemia observed in the present study indicates that the prevention of PB-19 infection is likely to result in burden of severe anemia in children with sickle cell disease in Egypt. Efforts to develop a safe and effective vaccine against PB-19, which is already being investigated, should be strengthened by the view of its possible use in the prevention of severe anemia among children with chronic hemolytic anemia.[21,24]

In conclusion, PB-19 infection is detected in high rates among Egyptian children with sickle cell disease and it may result in severe anemia. Therefore, PB-19 must be suspected and screened for in such group of patients. Screening of blood for PB-19 may be helpful in understanding the epidemiology of infection with this virus. The direct detection of DNA by PCR in sera needs to be coupled with serology for a more reliable diagnosis of PB-19 infections in these children. Further studies for genotyping and quantification of the virus will be more useful for diagnosis and staging of infection.

Conflicts of interest

The authors state that there are no conflicts of interest.

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