**Parvovirus B19 Infection in Patients with Hematological Malignancies Receiving Multiple Courses of Chemotherapy**


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Human parvovirus B19 (B19) exhibits a marked tropism to bone marrow, replicating exclusively in erythroid progenitor cells. Persistent B19 infection tends to occur in immunocompromised hosts and may manifest as pure red cell aplasia and chronic anemia. The aim of the present study was to assess the prevalence of B19 infection in patients with hematological malignancies receiving chemotherapy, and to highlight the relation between B19 infection and unexplained cytopenia(s). The study included 30 adults and 18 children suffering from a variety of hematological malignancies and receiving chemotherapy. In addition, 10 healthy adults and 10 healthy children (age and sex matched as the studied patients) were included as controls. All patients had unexplained anemia, leukopenia, neutropenia and/or thrombocytopenia and had received a minimum of 4 courses of systemic chemotherapy. B19 infection was investigated both by serologic determination of specific IgG and detection of viral DNA in serum by nested PCR. IgG was detected in 24 (80%) adults and 11 (61.1%) children, while viral DNA was found in 16 (53.3%) adults and 5 (27.8%) children. Only one adult patient (3.3%) was positive for viral DNA and negative for IgG denoting an acute infection. Significant association was detected between unexplained anemia and IgG seropositivity in children. Meanwhile, unexplained thrombocytopenia was significantly associated with the presence of viral DNA in adults. In conclusion, patients with hematological malignancies receiving chemotherapy are at particular risk of persistent B19 infection. Moreover, it is important to consider B19 infection as a possible cause of unexplained cytopenia(s) in these patients. In such cases, administration of intravenous human immunoglobulin can reduce the viremia and correct the cytopenia(s). Prospective studies are needed to define the serologic and clinical consequences of B19 infection in this group of patients.

**INTRODUCTION**

Human parvovirus B19 (B19), first recognized in 1974, is a small DNA virus of the family Parvoviridae. The virus is non-enveloped, and its genome consists of a linear, single-stranded DNA molecule of approximately 5600 nucleotides with terminal palindromic inverted sequences of 383 nucleotides at both ends. It has two main open reading frames (ORF) encoding three functional proteins. The first ORF codes for the nonstructural protein NS1, while the second ORF expresses two structural proteins known as viral protein 1 (VP1) and viral protein 2 (VP2). VP2 is the major structural protein, with a molecular mass of 58 kDa, and accounting for 96% of total capsid protein. The minor capsid protein, VP1, is identical to VP2 with the addition of 227 amino acids (termed the VP1 unique region) at the amino terminus and a molecular mass of 84 kDa. It makes up the remaining 4% of the total capsid protein.

In healthy B19-infected individuals the predominant immune response is humoral. The early antibody response consists of immunoglobulin (Ig)M and is directed against VP2-specific epitopes. The appearance of IgM in the serum usually occurs 10 to 12 days post infection, and coincides with the clearance of viremia. IgM usually persists in serum samples for approximately 3 months. By the third week, specific IgG antibodies against the capsid proteins VP1 and VP2 appear in serum. However, most viral neutralizing antibodies that offer life-long protection against reinfection are directed against the unique amino-terminal region of VP1. Thus, VP1 is required for an effective immune response despite its less abundant relative concentration in the virion.

Parvovirus B19 is a global and common infectious pathogen in humans. Epidemiologic studies showed that the prevalence of IgG antibodies directed against B19 increases from 2% to 15% in children 1 to 5 years old to reach more than 85% in geriatric population, but, the presence of viremia is rare. Transmission of infection occurs via the respiratory route, through blood-derived products administered parenterally, and vertically from mother to fetus. The only known natural host cell of B19 is the human erythroid progenitor cell. A natural glycolipid, globoside (also known as erythrocyte P antigen), acts as a cellular receptor, and accounts for the tropism of the virus for erythroid cells.

The pattern of clinical disease is strongly influenced by the age, immunological and hematological status of the host. In immunocompetent individuals, B19 may cause a self-limiting subclinical erythroid aplasia, followed by erythematous maculopapular rash (erythema infectiosum or fifth disease) in children and acute symmetrical polyarthritis or arthralgia that can mimic rheumatoid arthritis in adults, and usually resolves within a few weeks with no joint destruction. Both conditions are mediated by the immune response. Infection during pregnancy can lead to spontaneous abortion, hydrops fetalis or congenital anemia. This is most likely due to erythrocyte aplasia in the affected fetus.
In patients with underlying chronic hemolytic anemia such as those with sickle cell anemia, hereditary spherocytosis, pyruvate kinase deficiency, and transfusion-independent beta-thalassemia, infection can result in a dramatic decrease of hemoglobin, leading to aplastic crisis.\(^{12,13,15}\)

In patients with underlying immunodeficiency status, persistent B19 infection may occur. It is thought that these patients fail to mount a neutralizing antibody response to the virus due to a persistent bone marrow insufficiency. Therefore, they usually lack the immune complex-mediated symptoms of erythema infectiosum and arthropathy. Chronic anemia and pure red cell aplasia due to chronic B19 infection have been documented in congenital immunodeficiency, children with lymphoblastic leukemia, patients with immunodeficiency syndrome (AIDS), and recipients of solid organ transplants.\(^{5,16,17}\)

In chronically infected patients, the anemia may be intermittent, with periods of relapse (associated with viremia) and remission (associated with spontaneous disappearance of the virus from the circulation). Clinically, the degree of anemia may be severe.\(^{12,13}\)

The aim of the present study was to assess the prevalence of B19 infection in patients with hematological malignancies receiving multiple courses of chemotherapy. Also, to highlight the relation between B19 infection and the development of unexplained anemia, leukopenia, neutropenia, or thrombocytopenia either separately or concomitantly in these immunocompromised patients.

**PATIENTS AND METHODS**

The study included 30 adult patients suffering from a variety of hematological malignancies and receiving multiple courses of chemotherapy. They were selected from those admitted to the Hematology Unit or attending the Hematology and Bone Marrow Transplant Outpatient Clinic, Internal Medicine Department, Main Alexandria University Hospital, during the period from January 2007 till August 2007. The age of these patients ranged from 18 to 65 years with a mean of 48.6 years. They were 18 males and 12 females.

The study also included 18 children with acute lymphoblastic leukemia (ALL) in complete remission (CR) on maintenance chemotherapy. They were selected from those attending the Pediatric Hematology Outpatient Clinic of the Alexandria University Children Hospital during the same period. Their ages ranged from 3 to 9 years with a mean of 5.8 years. They were 11 boys and 7 girls.

All patients were suffering from unexplained anemia, leukopenia, neutropenia and/or thrombocytopenia. Unexplained cytopenia(s) were defined as a sudden drop of hemoglobin concentration, total leukocytic count, absolute neutrophilic count, and/or platelet count without a readily attributable etiology, such as acute and chronic blood loss, accelerated peripheral destruction, iron deficiency, folate or vitamin B\(_12\) deficiency, renal insufficiency, drug-induced marrow suppression, and tumor involvement of the marrow.\(^{13}\)

In addition, 10 healthy adults and 10 healthy children with matched age and sex as the studied patients were included as controls. The study was approved by the Research Ethics Committee (REC) of Alexandria University Hospital and a written informed consent was obtained from the adult patients and the parents of all children participating in the study.

**All patients included in this study were subjected to:**

1. Thorough medical history with emphasis on the presence of fever, joint symptoms, symptoms of anemia, infection or bleeding manifestations. Also, meticulous review of the patients’ records to determine the number of previous chemotherapy cycles and the onset of unexplained cytopenia(s).
2. Systematic clinical examination searching for signs of infection, bleeding or organomegaly.
3. Routine laboratory investigations including liver and kidney function tests.
4. Hematological investigations including complete blood count (CBC) and bone marrow (BM) aspiration (when indicated).

Serum samples were collected from patients and healthy controls. Samples had been stored at -20°C until tested for parvovirus B19 specific IgG antibody, and DNA.

**Serological examination:**

Serum samples were used to detect parvovirus B19 IgG-class antibodies using an enzyme-linked immunosorbent assay (ELISA) (NovaTec, Immundiagnostica GmbH) according to the manufacturer’s instructions. The assay was an antigen capture sandwich enzyme immuno-assay, and the antigen used was purified parvovirus B19 recombinant VP1 and VP2 capsid proteins. Following a wash step, peroxidase-labelled rabbit anti-human IgG was added and bound to the human parvovirus B19 IgG present. The whole complex was then detected by the addition of a substrate (tetramethylbenzidine), which turns blue in the presence of peroxidase. The absorbance at 450 nm was measured and the results were interpreted as positive, equivocal, or negative according to the stated cutoff values.\(^{18}\)

**DNA extraction**

**Viral** DNA was extracted from serum samples by using the QIAamp DNA Mini Kit (Qiagen,
GmbH, Hilden, Germany) according to the manufacturer's recommendations. Briefly, 200 µl of sample were added to a 1.5 ml microcentrifuge tube containing 20 µl proteinase K. Then, a second lysis buffer (buffer AL) was added, and mixed thoroughly by pulse-vortexing for 15 seconds, and incubated at 56 °C for 10 minutes. Next, 200 µl absolute ethanol was added and mixed again by pulse-vortexing for 15 seconds. The mixture was then loaded on the QIAamp spin column which holds a silica membrane, and centrifuged at 6000 xg for 1 minute. The filtrate was discarded. The column material was washed twice with each of the two provided washing buffers (AW1, AW2) by centrifugation at 6000 xg for 1 minute, and 20,000 xg for 3 minutes respectively. The membrane bound DNA was eluted by centrifugation with 50 µl of buffer (AE) after 5 minutes incubation at room temperature. The resulting DNA extracts were stored at -20 °C until analysis assessment.

**DNA amplification and gel electrophoresis**

For amplification of parvovirus B19 DNA, a nested PCR technique was used. In the first round of amplification, 0.2 µM of oligonucleotide primers corresponding to nucleotides 2381-2400 (B19SI) and 2781-2800 (B19ASI) (5’-CCTTTTCTGCTAACCTGC-3’ and 5’-CCCAGGCTTTGTGTAAGTCTT-3’ respectively) were used. Five microliters of each sample extract were used in a 50 µl reaction containing 5 µl of 10x PCR reaction mix. The second-round reaction mix contained the same constituents as the initial mix, but the first-round primers were substituted by 0.2 µM of each oligonucleotide primer corresponding to nucleotides 2429-2448 (B19SII) and 2730-2751 (B19ASII) (5’-AAAGCTTTGTAGATTATGAG-3’ and 5’-GGTTCTGACATGCTATGG-3’ respectively). Then 25 cycles of amplification were performed using the cycling parameters described. Subsequently, the nested PCR products of size 322 base pairs were resolved by 1% (wt/vol) agarose gel electrophoresis and were visualized after ethidium bromide (0.5mg/ml) staining, using an ultraviolet transilluminator. Negative controls were also included in each PCR reaction. 

**Statistics**

The Statistical Package for Social Science (SPSS) computer program was utilized for data analysis. Fisher's Exact Test was used. The level of significance used was the 0.05 level.

**RESULTS**

The 30 adult patients with hematological malignancies included in this study were suffering from acute myeloid leukemia (8 patients), acute lymphoblastic leukemia (6 patients), myelodysplastic syndrome (2 patients), blastic crisis of chronic myeloid leukemia (4 patients), Hodgkin's lymphoma (2 patients), and non-Hodgkin's lymphoma (8 patients). The number of previous chemotherapy courses taken by these patients ranged from 4 to 13 with a mean of 5.8 cycles. Meanwhile, all the 18 pediatric patients included, had acute lymphoblastic leukemia in complete remission (16 were of the B immunophenotype, and 2 had T phenotype). All had received 4 previous chemotherapy courses. Table (1) shows the prevalence of different cytopenia(s) in the studied patients.

Parvovirus B19 infection was detected in 83.3% (25/30) of adult patients by demonstrating viral DNA and/or specific IgG. Eighty percent (24/30) of adult patients were positive for B19 IgG, and 53.3% (16/30) were positive for viral DNA detected by PCR. Only one adult patient (a 43-year-old male with non-Hodgkin's lymphoma of diffuse large cell type who received 11 chemotherapy cycles) was positive for viral DNA and negative for IgG denoting acute B19 infection. This patient had total leukocyte count of 2.4x10⁹/L, absolute neutrophil count of 0.3x10⁹/L, platelet count of 65x10⁹/L, but his hemoglobin concentration was 12g/dl.

In pediatric ALL patients, B19 specific IgG was detected in 61.1% (11/18). Meanwhile, 27.8% (5/18) of children were positive for both IgG and viral DNA. The relation between B19 DNA and IgG seropositivity and the presence of different cytopenia(s) in adult and pediatric patients included in this study was summarized in tables (2) and (3) respectively. Significant association was detected between unexplained anemia and IgG positivity in children (p= 0.043). Meanwhile, unexplained thrombocytopenia was significantly associated with the presence of viral DNA in adults (p= 0.007).

Among controls, B19 specific IgG was detected in 70% (7/10) of adults, and 20% (2/10) of children. None of the controls was positive for viral DNA by PCR.
Table (1): Prevalence of different cytopenia(s) in the studied patients

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DISCUSSION

Unexplained cytopenia(s) developing in patients with hematological malignancies receiving ongoing chemotherapy poses a significant problem. Parvovirus B19 infection has been recognized as an important cause of severe anemia in immunocompromised patients, including organ transplant recipients, patients with acquired immunodeficiencies, and leukemia patients receiving maintenance or consolidation chemotherapy. In patients with hematological malignancies, B19 infection could mimic a leukemic relapse or therapy-induced cytopenia and led to hospital admission, frequent blood sampling, renewed bone marrow aspirates, multiple transfusions of red cells or platelets, and cessation of chemotherapy for several weeks. For all these reasons and because B19 infection in immunosuppressed patients usually presents with nonspecific symptoms and signs, we conducted the present study in order to highlight the association between B19 infection and unexplained cytopenia(s).

In the current study, B19 specific IgG was detected in 61.1% (11/18) of sera of children with ALL on maintenance chemotherapy, while viral DNA was found in 27.8% (5/18), all of them were also seropositive. Our results were higher than that reported by other investigators. Zaki 2007 reported the detection of B19 IgG in serum of 40% of children with ALL and receiving maintenance chemotherapy, meanwhile, B19 DNA was found in 20% of studied cases. Also, El Mahallawy et al 2004 found B19 DNA in 22% (11/50) ALL children with anemia, and B19 specific IgG in a total of 19 (38%) cases with B19 DNA present in 6 of them. The higher prevalence rate of B19 infection among ALL children detected in this study may be due to the fact that all our cases had cytopenia(s); either anemia,
leukopenia, or thrombocytopenia at the time of sampling.

In the present study, significant association was found between IgG seropositivity in children with ALL on maintenance chemotherapy and the occurrence of unexplained anemia. This may be attributed to the marked tropism of the virus to human erythroid progenitor cells and because children with ALL share the chief age of B19 infections and may be particularly vulnerable to the ill effects of the virus due to immunosuppression.\textsuperscript{(25)} The same was reported by other investigators who documented B19 seroconversion in ALL children on maintenance chemotherapy.\textsuperscript{(16)}

In adults with hematological malignancies, B19 specific IgG was found in 80\% (24/30) of cases and viral DNA was detected in 53.3\% (16/30). Again, our results were slightly higher than those reported by other investigators.\textsuperscript{(26,27)} Unexplained anemia was found in 83.3\% (20/24) of B19 seropositive cases and 87.5\% (14/16) of those with viremia. This was in accordance with Kuo et al 2002\textsuperscript{(26)} who studied young adult cancer patients (below 40 years) receiving ongoing chemotherapy, and found significantly higher prevalence of unexplained anemia among B19 seropositive patients (63.2\%; 12/19) than among B19 seronegative patients (15\%; 3/20).

One of the most important findings in this study was the highly significant association between B19 viremia and the occurrence of unexplained thrombocytopenia in adults with hematological malignancies. The thrombocytopenia associated with B19 infection seems to consist of a central and a peripheral type. The first is due to BM suppression, as viral NS1 protein has been found to inhibit the megakaryocytic colony formation.\textsuperscript{(28)} This indicates tissue tropism of B19 beyond the erythroid progenitor cell and shows that viral proteins may be toxic to cell populations that are nonpermissive for viral DNA replication. The second may result from immunologically mediated antiplatelet antibody production with subsequent excessive platelet clearance in the reticuloendothelial system.\textsuperscript{(29)}

In the present work, no significant difference, regarding the prevalence of B19 IgG seropositivity, was found between adult patients (80\%) and their controls (70\%). This could be attributed to the fact that the majority of the patients and controls were older than 40 years. So, the already much higher rate of B19 seropositivity, found between IgG seropositivity in children with ALL and their controls (20\%). This indicates that these ALL children are particularly vulnerable to B19 infection, probably because of two reasons: first, all these children had prolonged periods of hospital stay and hence an increased risk of nosocomial infection with B19 by airborne transmission and person-to-person contact. Second, these children have been exposed to multiple transfusions of blood products and thus prone to another source for B19 infection.\textsuperscript{(31,32)}

Laboratory diagnosis of B19 infection relies primarily upon serological methods to demonstrate an antibody response. IgM assays will reliably detect a current infection in immunocompetent persons.\textsuperscript{(6)} but in immunocompromised patients who may not be able to produce IgM, PCR-based methods to detect viral DNA are used to diagnose acute or persistent infections.\textsuperscript{(33)} Past infection is diagnosed by detecting B19 IgG antibodies which persist for years and, perhaps, for life.\textsuperscript{(34,35)} Interestingly, in the present study, almost all patients (adults and children) with B19 viremia were also B19 IgG seropositive. This may be explained by the presence of an aberrant immune response to B19 in patients with hematological malignancies, as the presence of neutralizing antibodies to B19 did not clear the virus or its effects.\textsuperscript{(23)} Only one adult patient (3.3\%) showed B19 viremia and was negative for B19 IgG denoting acute B19 infection. Gallinella et al 2003\textsuperscript{(35)} reported that PCR was fundamental for the diagnosis of B19 disease in 32\% (50/152) of patients with documented infections, both in acute infections at the onset of symptoms before the appearance of immunologic response and during the course of persistent B19 infections in which IgM had cleared. Moreover, immunocompromised patients have been shown to suffer from prolonged viral infections often without detectable immune response. Here, chronic infections with low virus levels can be frequently observed and viral DNA can be detected by PCR.\textsuperscript{(33)} Further studies to detect the presence of B19 DNA in bone marrow are required to prove persistent B19 infection in immunocompromised individuals with negative viremia.

In conclusion, patients with hematological malignancies receiving chemotherapy are at particular risk of persistent B19 infection. Moreover, there may be an aberrant immune response to B19 in these patients as the presence of IgG antibodies failed to clear the virus. It is important to consider B19 infection as a possible cause of unexplained cytopenia(s) in immunocompromised patients. In these cases, administration of intravenous human immunoglobulin can reduce the viremia and correct the cytopenia(s). Prospective studies are needed to define the serologic and clinical consequences of B19 infection in this group of patients.
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العدوى بفيروس البارفو بـ ۱۹ في مرضى سرطات الدم تحت العلاج الكيميائي ذي المنظومات المتعددة

تهدف هذه الدراسة إلى استقصاء العلاقة بين البارفو بـ ۱۹ والنزيف غير المبرر لخلايا الدم الحادة في مرضى سرطات الدم تحت العلاج الكيميائي وقد استنمت السرائدة على ثلاثين شخصًا بالغًا يعانون من سرطات الدم وثمانية عشر طفلا مصابين بالكيميائية المขวาة تحت العلاج الكيميائي المستمر وكذلك على عشرة أشخاص بالغين وعشرة أطفال طبيعين كمجموعة مضريبة. وعاني كل المرضى من واحد أو أكثر من العلامات المرضية الأخرى: نقص الدم - نقص كرات الدم البيضاء - نقص الصفائح الدموية كما تلقوا في المتوسط أربعة برامج من العلاج الكيميائي. وتمت دراسة عدد فيروس البارفو بـ ۱۹ عن طريق الكشف المقصى عن الأجسام المضادة (IgG) لهذا الفيروس في الكشف الجزيئي عن باعطة التفاعل البوليميرازي المتسلسل في عينات الدم الطفويل.

وقد كان ۸۰% من المرضى البالغين ايجابيين للاجسام المضادة (IgG) بينما كان ۵۳.۳% منهم ايجابيين بواسطة التفاعل البوليميرازي المتسلسل. وقد وجدنا سطحي كلاً واحداً (۶.۳% ايجابياً بواسطة التفاعل البوليميرازي المتسلسل على الرغم من كونه سلبياً للاجسام المضادة (IgG) وهو ما يوسع باحتمال اصابتهم بالعدوى الحادة. وفسي المرضاطي الأطفال كلاً معدل الاجسام المضادة (IgG) يساوي ۱۱.۰% ومعدل الاجسام المشتركة لكل من الأجسام المضادة (IgG) والتفاعل البوليميرازي المتسلسل يساوي ۲۷.۸%. وقد وجدنا ارتباطا معاونياً بين فقر الدم غير المبرر والإجابة المصاحبة للإجاع المضادة (IgG) في مرضاطي الأطفال بينما كان نقص الصفائح الدموية غير المبرر مرتبطا معاونيا بعدي فيروس البارفو بـ ۱۹ المكشف عنه بواسطة التفاعل البوليميرازي المتسلسل في عينات الدم الطفويل.

وخلص من هذه النتائج أنه أن مرضى سرطات الدم تحت العلاج الكيميائي معرضون على وجه الخصوص للعدوى المستمرة بفيروس البارفو بـ ۱۹، ومن الأهمية بمكان أن نأخذ في الاعتبار قياس البارفو بـ ۱۹ كسبب محتمل للفشل في علاج الدم غير المبرر في هؤلاء المرضى. وفي هذه الأحوال فإن الجلوبولينات المناعية البشرية الوريدية قد تكون من كمية الفيروس في الدم وتساعد في علاج نقص خلايا الدم. و نحتاج إلى دراسات أخرى للتحديد الدقيق للعلاقة بين الحالة المصلية لهذا الفيروس و الاستجابة الاكلينيكية للمرضى.