

Molecular Study of Parvovirus B19 Infection in Children with Acute Myeloid Leukemia

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Abstract

Background: Parvovirus B19 is a common viral infection in children. Nearby evidences are present about its association with acute leukemia, especially acute lymphoblast leukemia. Nevertheless, scanty reports have discussed any role in acute myeloid leukemia (AML). **Purpose:** To evaluate the frequency of virological markers of B19 infection including its DNA along with specific immunoglobulins G (IgG) and M (IgM) among children with newly diagnosed AML. Besides, describing the clinical importance of Parvovirus B19 infection in those patients. **Patients and methods:** A case-control retrospective study was conducted on 48 children recently diagnosed with AML before and during chemotherapy induction and 60 healthy control. Specific serum IgM and IgG levels were determined by enzyme linked immunosorbant assay (ELISA) and DNA detection by polymerase chain reaction (PCR). **Results:** Parvovirus DNA was detected in 20 patients with AML. IgM was found in sera of four patients and one case had positive DNA and IgG (5%). Patients with recent parvovirus B19 infection had a significantly reduced hemoglobin levels, RBCs counts, platelet counts, neutrophil counts and absolute lymphocytosis ($p=0.01$, $p=0.0001$, $p=0.01$, $p=0.02$, $p=0.0003$, respectively). There were no clinical findings with statistically significant association to recent infection. Half of the patients with AML had positive PCR and/or IgM for parvovirus B19. Among children with AML under chemotherapy, there were reduced hemoglobin levels ($P=0.03$), reduced platelet counts ($P=0.0001$) and absolute neutropenia (mean \pm SD, 1.200 ± 1.00) in those with parvovirus B19 infection. More than half of patients with parvovirus B19 (72.2%) had positive PCR and/or IgM and 36.4% of them had positive IgG. **Conclusion:** This study highlights that parvovirus B19 is common in children with AML either at diagnosis or under chemotherapy. There are no clinical manifestations that can be used as markers for its presence, but hematological laboratory findings can provide evidence for infection in the presence of anemia and neutropenia. Detection of parvovirus B19 by combined molecular and serological markers is required in such patients for accurate diagnosis.

Keywords: AML- Parvovirus B19- PCR- IgG- IgM

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Introduction

Hematological malignancies are common among children. They include leukemia (30%) and lymphoma (12%). Acute myeloid leukemia (AML) represents around 15-20% of leukemia in children (De Renzo, et al., 1994). The principle manifestation of hematological malignancies is usually anemia at the start of the diseases or during the chemotherapy courses. Anemia and other forms of cytopenia can be attributed to various factors such as malignant infiltrations of bone marrow, cytotoxic drugs or infections by viruses like cytomegalovirus and Parvovirus B19 (Brown, 2000; Lehmann et al., 2003; Segata et al., 2007; Kanvinde et al., 2013).

Human parvovirus B19 belongs to the genus erythrovirus of the family Parvoviridae. It is a single stranded DNA virus which has wide varieties of clinical manifestations including fever, rash, lymphadenopathy and

rarely hepatosplenomegaly. The pathognomonic laboratory finding of B19 is the presence of megakaryocytes in bone marrow examination. Virological diagnosis of recent infection relies mainly upon detection of specific IgM antibodies or B19 DNA (Kishore et al., 2000).

The association of B19 and leukemia has been postulated to be either as an opportunistic infection or even it may play a putative role in pathogenesis of acute leukemia mainly ALL (Heegaard et al., 2001). Most studies have found the frequency of B19 infection in 8 to 18 percent of cases of ALL (Kerr et al., 2003; ElMahallawy et al., 2004; Lindblom et al., 2008).

The clinical manifestations of B19 infection in patients with leukemia are related to its marked association with anemia due to pure red cell aplasia and to some less frequent extent thrombocytopenia and neutropenia (ElMahallawy et al., 2004). B19 has a great tropism for erythroid progenitor cells (Norbeck et al., 2004).

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Role of B19 infections in patients with myeloid leukemia and lymphoma have rarely been studied (Yetgin et al., 2000; Isobe et al., 2004; Tang et al., 2007; van Dam et al., 2008). Difficulty in the detection of B19 infection is due to limited diagnostic facility mostly confined to central laboratory. Frequency of parvovirus B19 infections and its implications in newly diagnosed cases of pediatric hematological malignancies on induction chemotherapy is largely unknown (Kishore et al., 2011) and even little is known among Egyptian children.

Therefore, this work was conducted to study the frequency of positivity of parvovirus B19 virological markers including its DNA detection by polymerase chain reaction (PCR) along with detection of specific immunoglobulins G (IgG) and M (IgM) among children with newly diagnosed AML and during induction chemotherapy. In addition, the current work aimed to study the clinical significance of B19 infection in those patients.

Materials and Methods

A case-control retrospective study was conducted on children recruited from Mansoura University Children's Hospital (MUCH) and Oncology Center of Mansoura University (OCMU), Egypt from December 2014 till June 2016. Children recruited were those recently diagnosed to have AML before chemotherapy induction (32 children), and children with AML under induction chemotherapy during the same duration (16 children). Inclusion criteria for patients under induction chemotherapy were Prolonged unexplained anaemia defined as anaemia of more than 2 weeks duration with haemoglobin levels < 10 g/dl or sudden drop of haemoglobin level of 2.5 g/dl without a readily attributable etiology (Kishore et al., 2011). In addition, 60 healthy children with matched age and sex were included as control group. The study was approved by ethical committee of Faculty of Medicine, Mansoura University, Egypt. Informed written consent was obtained from the guardian of each participant child.

The included children were subjected to full clinical history and complete clinical examinations.

Six milliliter blood samples were obtained from each child and divided into two vacutainers one with EDTA for complete blood counts and the other was plain vacutainer for virology markers for parvovirus B19. The second samples were centrifuged and sera were separated and kept frozen at -20 °C for further analysis of parvovirus B19.

Parvovirus B19 study was performed by determination of specific IgM and IgG by enzyme linked immunosorbant assay (ELISA) and DNA detection in serum by PCR.

Qualitative Assessment of Parvovirus B19 IgM and IgG with ELISA (I B L –international, GMB, D-22335 Hamburg-Germany)

These kits are based upon qualitative ELISA determination of specific IgM and IgG antibodies against Parvovirus B19.

Microtiter strip wells are coated with specific parvovirus B19 antigens to bind corresponding antibodies either IgM or IgG of the sided sera samples. After

incubation, washing is performed to the wells and horseradish peroxidase (HRP) labeled anti-human IgM conjugate or anti-human IgG conjugate according to the kits is added. The conjugate binds to the captured parvovirus B19 specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product and after the period of incubation, the reaction is stopped by adding sulfuric acid. The intensity of the product is proportional to the amount of specific IgM or IgG to parvovirus B19 specific IgM or IgG antibodies in the samples. The optical density of the product is measured at 450 nm using an ELISA microwell plate reader.

PCR for Parvovirus B19

Parvovirus B19 DNA extraction from sera samples was performed by Qiagen DNA extraction blood kit (GmbH-Hilden, Germany).

Four primers were used in the current work for PCR amplification as shown in a previous study (Clewley, 1989). Amplification was done in 50 µL reaction volume using Qiagen amplification mixture. The reaction was carried on thermal cycler according to the following amplification protocol: 95 °C for 5 min, 50 °C for 30 s followed by 35 cycles (91 °C for 1 min, 50 °C for 1 min and 67°C for 3 min.

Gel electrophoresis was performed using 1.5% gel and positive band was considered at (218 bp).

Statistical Analysis:

Data were analyzed by the use of the statistical package SPSS version 16. Descriptive statistics were used such as mean ± SD values for quantitative data and percentage for qualitative data. Statistical differences between groups were tested using Chi Square test for qualitative variables, independent sample test and ANOVA (analysis of variance). P value ≤ 0.05 was considered significant.

Results

Among studied subjects, Recent Parvovirus B19 infection was diagnosed by positive IgM and /or PCR results while past infection was diagnosed by single positive IgG result. The majority of patients with AML had evidence of parvovirus infection either at start of diagnosis (87.5%) or under induction of chemotherapy (68.7%). In control group, the majority of children have no evidence of recent or past infection of parvovirus B19 (85%). The results was statistically significant (p=0.0001) Table 2.

In recent parvovirus B19 infection among children with AML, hematological markers were the most significant

Table 1. Sequences of the used primers for PCR of parvovirus B19

Pair A	F-(5/-TGT GGT AAG AAA AAT AC-/3) R-(5/-TCA TTA AAT GGA AAG TTT-/3)
Pair B	F-(5/-GGA ACA GAC TTA GAG CTT ATTC-/3) R-5/-GCT TGT GTA AGT CTT CAC TAG -/3)

Table 2. Distribution of Parvovirus B19 Infections among Studied Subjects

Parameter	Patients with acute myeloid leukemia (n=32)	Patients under chemotherapy for acute myeloid leukemia (n=16)	Control group (n=60)	p value
Recent Infection	28(87.5%)	11(68.7%)	2 (3.3%)	0.0001
No infection	1(3.1%)	1(6.3%)	51 (85%)	0.0001
Past infection	2(6.3%)	4(25%)	7 (11.7%)	0.0001

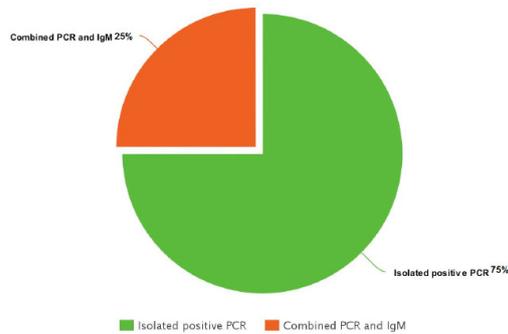


Figure 1. Distribution of IgM Positive Patients Within Positive PCR in Patients with Acute Myeloid Leukemia

findings; reduced hemoglobin level, RBCs counts, platelets counts, reduced neutrophil counts and absolute lymphocytosis among patients with recent parvovirus B19 infection ($p=0.01$, $p=0.0001$, $p=0.01$, $p=0.02$, $p=0.0003$ respectively). There were no clinical findings with statistically significant association with recent infection Table 3.

Among children with AML under chemotherapy, there were reduced hemoglobin levels ($p=0.03$), reduced

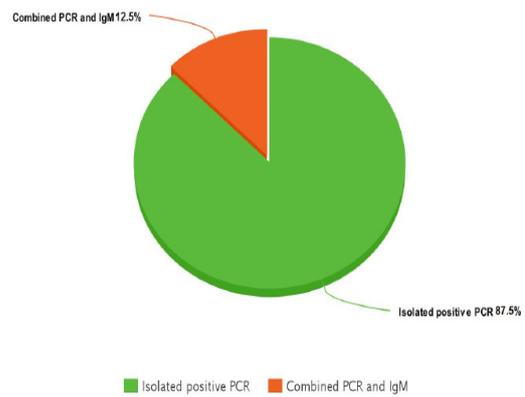


Figure 2. Distribution of IgM Positive Patients with Positive PCR in Patients with Acute Myeloid Leukemia under Chemotherapy

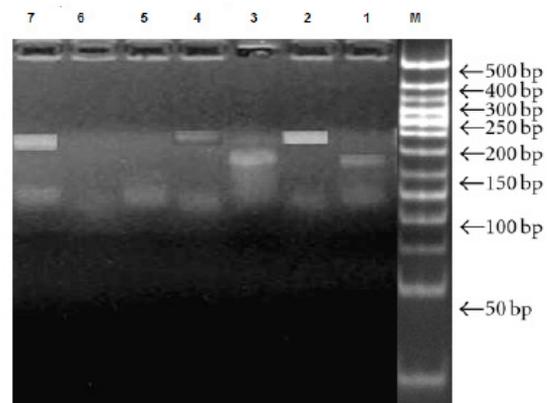


Figure 3. Marker, Positive and Negative PB19 Samples by PCR. Ethidium bromide- stained agarose gel electrophoresis; M: marker; lanes 2, 7 positive samples for parvovirus B19; Lanes 1, 3,4,5,6 negative samples

Table 3. Comparison of Demographic, Clinical, Hematological and Virological Markers Parameters among Patients with Acute Myeloid Leukemia According to Presence of Parvovirus B19 Infection

Parameter	Recent Infection (n=28)	Past Infection group (n=3)	No infection (n=5)	p value
Sex				
Male	14 (50%)	3 (100%)	3 (60%)	0.4
Female	10 (35.7%)	0 (0%)	2 (40%)	
Age	6.8±3.4	8.00±3.00	5.8±3.7	0.7
Rash	17 (60.7%)	1 (33.3%)	3 (60%)	0.8
Fever	20 (71.4%)	3 (100%)	3 (60%)	0.8
Hepatosplenomegaly	24 (85.7%)	3 (100%)	3 (60%)	0.5
Lymphadenopathy	20 (71.4%)	3 (100)	3 (60%)	0.5
Hemoglobin g/dl	4.2±0.1	6.3±2.2	7.2±1.5	0.01
RBCs x 10 ⁶ /l	1.25±0.5	2.3±1.7	3.01±0.5	0.0001
WBC x 10 ³ /mm ³	27.5±1.5	29.6±1.4	30.2±9.8	0.5
Lymphocytes x 10 ³ /mm ³	19.50±9.50	9.00±1.50	10.00±1.00	0.0003
Neutrophils x 10 ³ /mm ³	5.40±1.50	10.20±120	18.5±10.50	0.02
Platelets x 10 ³ /mm ³	78.28±7.2	105.00±50.000	123.2±60.1	0.01
IgM	12 (42.8%)	0 (0%)	0 (0%)	0.04
PCR	16 (57.1%)	0 (0%)	0 (0%)	0.04
IgG	8 (28.6%)	3 (100%)	0 (0%)	0.7

Table 4. Comparison of Demographic, Clinical, Hematological and Virological Markers Parameters Among Patients with Acute Myeloid Leukemia after Chemotherapy Induction According to Presence of Parvovirus B19 Infection.

Parameter	Recent Infection (n=11)	Past Infection group (n=4)	p value
Sex			
Male	8 (72.2%)	3 (75%)	0.1
Female	3 (36.4%)	1 (25%)	
Age	6.5±3.7	5.8±3.7	0.9
Rash	7 (63.7%)	0 (0%)	0.02
Fever	4 (36.4%)	0 (0%)	0.3
Hepatosplenomegaly	4 (36.4%)	0 (0%)	0.3
Lymphadenopathy	3 (27.3%)	4 (100%)	0.02
Hemoglobin g/dl	10.2±0.6	11.5±4.00	0.03
RBCs x 10 ⁶ /l	3.45±0.2	3.5±1.0	0.2
WBCs x 10 ³ /mm ³	12.0±1.4	29.00±4.00	0.1
Lymphocytes x 10 ³ /mm ³	9.450±1.20	25.0±10.0	0.03
Neutrophils x 10 ³ /mm ³	1.2±1.0	2.9±1.5	0.005
Platelets x 10 ³ /mm ³	54.5±28.6	168.4±4.00	0.0001
IgM	8 (72.7%)	0 (0%)	0.04
PCR	8 (72.7%)	0 (0%)	0.04
IgG	4 (36.4%)	4 (100%)	0.7

platelets counts (p=0.0001) and absolute neutropenia (mean± SD, 1.200± 1.00) in patients with parvovirus B19 infection Table 4.

The distribution of Parvovirus B19 virological markers among studied subjects is illustrated in Table 5. In patients with AML, recent infection was determined by positive PCR in 16 patients among them only 4 (25%) had also positive IgM Figure 1. In children with AML under chemotherapy, recent infection was determined by positive PCR in 8 patients with one patient had also positive IgM (12.5%) Figure 2.

Discussion

The putative role assumed to be played by parvovirus B19 in the pathogenesis of acute leukemia in children is thought to be explained by delayed infection hypothesis with the two-step mutation model (Kerr and Matthey, 2015). Previous studies have described strong associations between acute lymphoblastic leukemia and parvovirus B19 infection in various populations (Kishore et al., 2011; Nunes et al., 2016). However, association between parvovirus B19 and AML was less frequently studied in sufficient number of patients.

In the current study, parvovirus B19 infection was associated in the majority of patients with AML either at the start of diagnosis (87.5%) or under induction chemotherapy (68.7%). A previous study reported the presence of parvovirus B19 in 50 per cent of AML patients (Kerr et al., 2003). The presence of parvovirus B19 during induction chemotherapy may represent activation of infection partly controlled by preexisting immunity that is suppressed by chemotherapy (Lindblom et al., 2008).

Table 5. Distribution of Parvovirus B19 Virological Markers Among Studied Subjects

	Patients with acute myeloid leukemia (n=32)	Patients under chemotherapy for acute myeloid leukemia (n=16)	Control group (n=60)	p value
IgM	12 (37.5%)	8 (50%)	2 (3.3%)	0.0001
PCR	16 (50%)	8 (50%)	0 (0%)	0.0001
IgG	11 (34.4%)	8 (50%)	7 (11.7%)	0.0001

Parvovirus has been reported in previous studies to precede development of ALL (Petrella et al., 1992; Heegaard et al., 2001). In the present study in the series of children with AML, it appears that this is a common event also in AML. The pathogenic role of parvovirus B19 in development of acute leukemia was assumed to be through development of preleukemic clones in utero with subsequent genetic and/ or proliferative events during early childhood. The host response to infection may play an additional role in allowing proliferation of the premalignant clone (Lee et al., 1994; Kerr et al., 2003).

It is clear from our preliminary data that parvovirus B19 infection is significantly common in children with AML rather than in healthy children as in the control group, the majority of children have no evidence of recent or past infection of parvovirus B19 (85%). This finding may support the hypothesis of association of parvovirus B19 with development of AML.

In recent parvovirus B19 infection among children with acute AML, hematological markers were the most significant findings. It is reported that the classical manifestations of clinical signs of erythema infectiosum are usually absent in immunocompromized patients and only hematological parameters especially cytopenia can point out to the presence of hidden parvovirus B19 infection. Unfortunately, the infection with parvovirus B19 is not considered as a differential diagnosis in cases of unexplained cytopenia (Wiemels et al., 1999).

In the patient with AML and parvovirus B19 infection, there were reduced hemoglobin level, RBCs counts, platelets counts, reduced neutrophils counts with absolute lymphocytosis among patients (p=0.01, p=0.0001, p=0.01, p=0.02, p=0.0003 respectively). Neutropenia and lymphocytosis combined with reduced haemoglobin levels and red blood cell counts in children with ALL have been also described previously (Wiemels et al., 1999, Kerr et al., 2003). Thus, it appears that parvovirus B19 infection has also the same hematological effects in children with AML.

The neutropenia and even cytopenia may be associated with reduced immune control and lead to uncover underlying malignant diseases (Jitschin et al., 2011; Munthe-Kaas et al., 2015).

Among children with AML under induction chemotherapy, there were reduced hemoglobin levels (p=0.03), reduced platelet counts (p=0.0001) and absolute neutropenia (mean± SD, 1.200± 1.00) in patients with parvovirus B19 infection.

Parvovirus B19 occurrence during induction phase

of chemotherapy may affect the induction therapy by its cytopenic effects and by decreasing the tolerance to the cytostatic drugs, causing a need to reduce the dose (Kerr et al., 2003).

It is reported that transient interruptions of chemotherapy, though cause delay in the treatment, can be beneficial in regression of parvovirus B19 infection in certain patient groups (Zaki et al., 2006). Specific treatment of parvovirus B19 relies upon intravenous immunoglobulins in immunosuppressed patients on the other hand have been proved to be of value (Young and Brown, 2004; Kerr and Modrow, 2006; Zaki and Ashray, 2010), although some studies (Broliden et al., 2006) could not document a clear benefit. However, even if the infection with parvovirus B 19 is not completely cleared, a decrease in viral load is beneficial and may increase the blood cell count (Kurtzman et al., 1988).

In the present study, PCR for detection of parvovirus B19 has proven to detect many patients. Acute parvovirus B19 infection is known to be associated with intense viraemia; hence, PCR for parvovirus DNA detection is a valuable method of laboratory diagnosis. In addition, detection of IgM antibodies is a reliable indicator of recent infection especially in case of disappearance of parvovirus B19 DNA from serum as lasts for 2 to 3 months or longer. The use of PCR for detection of free DNA in serum denotes an active infection; besides, it is important in immunocompromized patients like leukemia as patients may not produce detectable amounts of virus specific IgM (Kishore et al., 2011). Moreover, it has been claimed that parvovirus B19 can precede the clinical presentation of ALL even by five months (Azzi et al., 1989; Mihal et al., 1996) making it a good target for follow up of patients with unknown etiology of cytopenia that may be proven to be acute leukemia later on.

This study highlights that parvovirus B19 is common in children with AML either at start of diagnosis or under chemotherapy. There are no clinical manifestations that can be used as marker for its presence, while hematological laboratory findings can give an evidence for such infection by presence of anemia and neutropenia. Detection of B19 by combined molecular and serological markers is required in those patients for accurate diagnosis.

Conflicts of Interests

The authors declare no conflicts of interest.

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