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ORIGINAL ARTICLE

# Detection of Human Parvovirus B19 Infection in Children with Chronic Haemolytic Anaemia in Benha University Hospitals

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***Key words:***
***Parvovirus B19 IgG, chronic hemolytic anemia, transient aplastic crisis, B19-DNA detection by PCR***

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## ABSTRACT

***Background:*** *Due to the tropism of human parvovirus B19 to erythroid progenitor cells, infection in patients with an underlying hemolytic disorder such as thalassemia, hereditary spherocytosis, sickle cell disease and Glucose-6-phosphate dehydrogenase deficiency leads to suppression of erythrocyte formation, referred to as transient aplasia crisis (TAC), which may be life-threatening.* ***Objectives:*** *Detection of parvovirus B19 DNA and its IgG antibodies in the serum of children with chronic hemolytic anemia and in apparently healthy children in Benha University Hospitals.* ***Methodology:*** *The study was conducted on 80 children. Forty of them with chronic hemolytic anemia, they were subdivided into 2 groups, Group (1a) included 20 patients without history of aplastic crisis, Group (Ib) included 20 patients with a history of aplastic crisis and 40 age and sex-matched apparently healthy children representing control (Group II). All patients were subjected to full history taking, clinical examination and laboratory investigations. Parvovirus B19 IgG was measured using anti-parvovirus B19 ELISA kits (SUNRED), and parvovirus B19 DNA was detected by using nested-polymerase chain reaction.* ***Results:*** *The seroprevalence of parvovirus B19 IgG was significantly higher (P value =0.016) in Group Ia (50%) (10 out of 20) and Group Ib (45%) (9 out of 20) than the control group (Group II) (17.5%) (7 out of 40). There was a significant positive correlation between anti-parvovirus B19 IgG and age of all patients, frequency of blood transfusion. The prevalence of parvovirus B19 DNA was 10% (2 out of 20) in group Ia and 30% (6 out of 20) in group Ib and no viral DNA was detected in the controls (P value=0.001). Although 42.3% (11 out of 26) of children with β thalassemia major had a detectable level of antiparvovirus B19 virus IgG antibodies, only (23.1%) (6 out of 26) of them had B19 DNA. Anti-parvovirus B19 IgG antibodies were detected in 4 children out of 5 children of sickle cell anemia (80%) but the the prevalence of Parvovirus B19 DNA was 20% among them.* ***Conclusion:*** *Measures to keep away from iatrogenic and nosocomial infection transmission should be implemented including screening of donated blood for parvovirus B19 especially blood given to patients with blood disorders.* ***Recommendation****: Data from this study support the need for introduction of an approved vaccine that mainly protects children with chronic hemolytic anemia against that infection.*

## INTRODUCTION

Parvoviridae are among the smallest known DNA containing viruses that can infect mammalian cells. The virions are non-enveloped particles about 22 nm in diameter with icosahedral symmetry[1].

Parvovirus B19 has exceptional tropism to human erythroid progenitors, faetal liver and umbilical blood erythroblasts[2].The transmission of parvovirus B19 occurs mainly via respiratory droplets but it can also spread by contaminated blood, organ transplantation and from mother to fetus [3].

Infections with parvovirus B19 are common, mainly in children where the pattern of its clinical diseases is influenced by both hematological and immunological status of the infected individuals. In healthy hosts, B19 virus causes self-limiting asymptomatic erythroid aplasia. Infection may be followed by erythema infectiosum (slapped cheek syndrome or the fifth disease), arthritis, leucopenia, vasculitis, spontaneous thrombocytopenia, anemia, abortion or hydrops fetalis in pregnant women[4].

Acute parvovirus B19 infection progression is characterized by a 5 day phase with high viremia. This viremia is neutralized with antibodies directed against viral protein 1 and viral protein 2[5]. The viremia decreases with the synthesis of IgA and IgM followed by the synthesis of IgG anti- parvovirus B19[6].


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In patients with blood disorders (sickle cell disease, thalassemia and hereditary spherocytosis) erythroid progenitor cell formation is increased to compensate for red blood cell lysis, B19 infection can suppress erythropoiesis and induce transient aplastic crisis [7].

A clinical diagnosis of B19V infection can only be clinically suspected. Infections can be asymptomatic, or present with nonspecific symptoms, while the typical symptoms attributed to B19 infection can be caused by other infective processes. Then, a laboratory diagnosis is required in the existence of a specific or generic request [8].

Combined use of PCR and enzyme-linked immunosorbent assay (ELISA) are optimal for diagnosis of parvovirus B19 infection [5].

The present work aims to detect the parvovirus B19 DNA and its IgG antibodies in the serum of children with chronic haemolytic anaemia and in apparently healthy children in Benha University Hospitals.

## METHODOLOGY

This work was carried out in Medical Microbiology & Immunology Department, Faculty of Medicine, Benha University in the period from January 2018 to March 2020.This study was approved by Benha University Ethical Committee and an informed consent was obtained from parents of children enrolled in the study.

**Subjects and samples**

This study was carried out on 40 children with chronic hemolytic anemia including {thalassemia, hereditary spherocytosis, sickle cell disease and Glucose-6-phosphate dehydrogenase deficiency}.They were selected from the Hematology Outpatient Clinic and Inpatient Unit of Pediatrics Department at Benha University Hospital. As well as 40 apparently healthy children matched for patient age and sex with patients and representing the control group.

Children included in this study were classified according to clinical findings into 3 groups as the following: *Group Ia* included 20 patients with chronic hemolytic anemia without a history of aplastic crisis. It included 12(60%) males and 8 (40%) females. *Group Ib* included 20 patients with chronic hemolytic anemia with a history of aplastic crisis. It included 14 (70%) males and 6 (30%) females. *Group II* included 40 apparently healthy children matched for age and sex with patients. It included 24 (60%) males and 16 (40%) females.

***Inclusion criteria:***

- Children in the first 12 years of life.
- All the selected patients were presented with chronic hemolytic anemia documented by hemoglobin electrophoresis.
- All the selected patients received blood transfusion.

All children were subjected to:

Full history taking, full clinical examination, routine laboratory investigations including CBC.

Only for patients; bone marrow aspiration, smear and special laboratory investigations were done to diagnose patients with hemolytic anemia, including estimation of osmotic fragility test for diagnosis of hereditary spherocytosis, G6PD activity and screening for sickle cell anemia by demonstrating sickling of RBCs under reduced conditions. Hemoglobin electrophoresis was also performed to confirm the presence of Hbs in sickle cell anemia and for the diagnosis of thalassemia.

Detection of anti parvovirus B19 IgG by ELISA and parvovirus B19 DNA by PCR were carried out for all children.

**Blood sampling:** Venous blood samples (5mL) were taken under sterile conditions in serum separator tubes from each participant. Samples were centrifuged for 15 minutes at 1000 x g. The samples were divided into two aliquotes for anti-parvovirus B19 IgG antibodies quantification and nested PCR analysis and stored at -80°C until further use.

**Quantification of anti-parvovirus B19 IgG antibodies serum levels:**

Anti-parvovirus B19 IgG antibodies serum levels were quantitated by using SUNRED human antiparvovirus B19 IgG ELISA Kit and procedure was done according to the manufacturer's instructions.

**Detection of parvovirus B19-DNA by nested PCR:**

The primers used for amplifications are shown in Table 1.

**Table1: Sequences of the primers used in nested PCR for detection of B19:**

| | |
|---|---|
| First Round | Forward 5′-CAAAAGCATGTGGAGTGAGG-3′<br>Reverse 5′-CTACTAACATGCATAGGCGC-3′ |
| Second Round | Forward 5′-CCCAGAGCACCATTATAAGG-3′<br>Reverse 5′-GTGCTGTCAGTAACCTGTAC-3′ |

The first amplification run was performed in a total volume of 50µL consisting of 25µL of EasyTaq® PCR SuperMix ,4µL of template DNA, 1µ of the first and second primers, water (nuclease free) to a final volume of 50 µL. Reaction was carried out in Thermal Cycler (Biometra,Germany) with the following steps: Initial denaturation step at 95°C for 5 minutes, Thirty five repeated cycles of: denaturation at 94°C for 1minute, annealing at 60°C for 1 minute and extension at 72oC for 2 minute followed by final extension step at 72˚C for 5 minutes then hold at 4ºC.The second amplification run of nested PCR was performed in a total volume of 50µL consisting of 25µL of EasyTaq® PCR SuperMix, 4 µL of amplified DNA, 1µL of the third and fourth primer,

water (nuclease free) to a final volume of 50 µL using the same amplification condition of the first run. Ten µL of each amplified DNA and 100 bp ladder (molecular weight marker) (Fermentas, Germany) were separated by electrophoresis using 1.5% agarose gel containing 2 µl of ethidium bromide. The bands were visualized by using UV transilluminator **(Figure 1).**

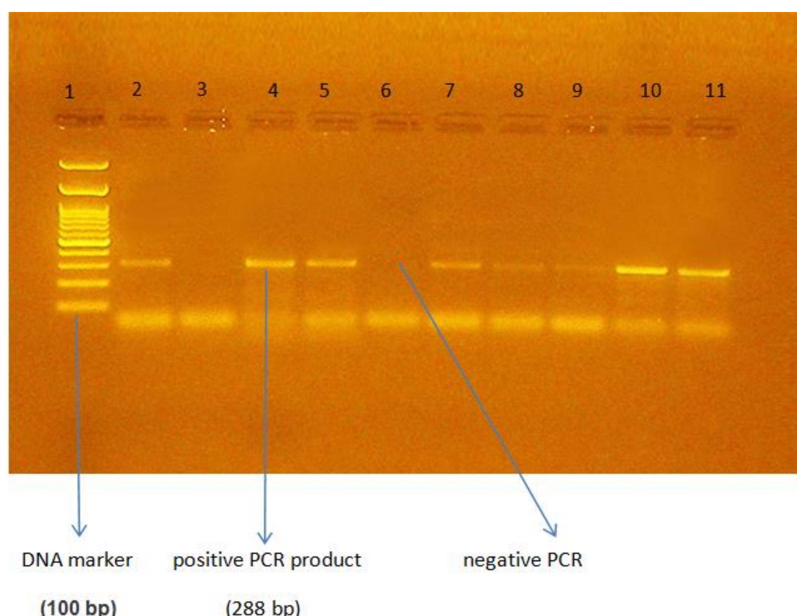


**Fig. 1:** Gel electrophoresis of final nested PCR product (288bp) of parvovirus B19

**Statistical analysis:**

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

## RESULTS

Table (2) shows age and sex distribution in children under study.

**Table 2: Age and sex distribution of the three groups:**

| Parameters | Cases | | Control Group II (no=40) | Test of significance | P-value |
|---|---|---|---|---|---|
| | Group Ia without aplastic crisis (no=20) | Group Ib with aplastic crisis (no=20) | | | |
| **Age** | | | | | |
| Mean±SD | 5.7±2.9 | 7.2±2.9 | 6.4±3.0 | **F- test** | 0.26 |
| Range | (1-11) | (1.5-11) | (0.8-11) | =1.35 | NS |
| **Sex (%)** | | | | | |
| Male | 12(60%) | 14(70%) | 24(60%) | **Chi$^2$-test** | 0.06 |
| Female | 8(40%) | 6(30%) | 16(40%) | =5.4 | NS |

*NS : non significant difference (p>0.05).*

There was no statistical significant difference between all studied groups (P value >0.05) regarding the age and sex.

The number and percentage of anti-parvovirus B19 IgG positive subjects in the three groups were studied. In group Ia; anti-parvovirus B19 IgG antibodies were detected in 10 patients (50%), nine patients in group Ib (45%) and 7 children (17.5%) in group II. There was a statistical significant difference (p=0.016) among groups (Table 3).

**Table 3: Seroprevalence of anti-parvovirus B19 IgG positive children among the studied groups:**

| IgG | Cases | | Control Group II (N=40) | $Chi^2$- test | p-value |
|---|---|---|---|---|---|
| | Group Ia Without aplastic crisis (N=20) | Group Ib With aplastic crisis (N=20) | | | |
| **Positive(26)** | 10(50%) | 9(45%) | 7(17.5%) | 8.31 | 0.016* S |
| **Negative(54)** | 10(50%) | 11(55%) | 33(82.5%) | | |
| **Total** | 20(100%) | 20(100%) | 40 (100%) | | |

There were no statistical significant differences between different types of anemia regarding prevalence of anti-parvovirus B19 IgG (Table 4).

**Table 4: Distribution of positive anti-parvovirus B19 IgG cases among different types of anemia:**

| IgG | Type of hemolytic anemia | | | | | $Chi^2$-test | P value |
|---|---|---|---|---|---|---|---|
| | **B-thalassemia major** (No.=26) | **Thalassemia intermedia** (No.=3) | **Hereditary spherocytosis** (No.=2) | **Sickle cell anemia** (No.=5) | **G6PD** (No.=4) | | |
| **Positive** | 11(42.3%) | 1(33.3%) | 1(50%) | 4(80%) | 2(50%) | 1.1 | 0.91 NS |
| **Negative** | 15(57.7%) | 2(66.5%) | 1(50%) | 1(20%) | 2(50%) | | |
| **Total** | 26 | 3 | 2 | 5 | 4 | | |

*NS: non significant difference (p>0.05).*

In group Ia; DNA was detected in 2 patients (10%). Six patients in group Ib out of 20 (30%) had detectable DNA, while the control group showed; no detectable DNA. There were highly statistical significant differences among the three groups (P value=0.001) (Table 5).

**Table 5: Number and percentage of parvovirus B19 infection among three groups using PCR:**

| DNA | Cases | | Control | FET | p-value |
|---|---|---|---|---|---|
| | Group Ia Without aplastic Crisis | Group Ib With aplastic crisis | | | |
| **Positive** | 2(10%) | 6(30%) | 0(0%) | 13.3 | 0.001** HS |
| **Negative** | 18 (90%) | 14(70%) | 40 (100%) | | |
| **Total** | 20 (100%) | 20(100%) | 40 (100%) | | |

*FET.... Fisher Exact test*      *** .... Highly significant*

There were no statistical significant differences in number of PCR positive patients among different types of anemia (Table 6).

**Table 6: Prevalence of parvovirus B19 infection through detection of DNA among different types of anemia**

| DNA | Type of hemolytic anemia | | | | | FET | P value |
|---|---|---|---|---|---|---|---|
| | **B-thalassemia major** (No.=26) | **Thalassemia intermedia** (No.=3) | **Hereditary spherocytosis** (No.=2) | **Sickle cell anemia** (No.=5) | **G6PD** (No.=4) | | |
| **Positive** | 6(23.1%) | 0(0%) | 0(0%) | 1(20%) | 1(25%) | 1.46 | 0.94 NS |
| **Negative** | 20(76.9%) | 3(100%) | 2(100%) | 4(80%) | 3(75%) | | |
| **Total** | 26 | 3 | 2 | 5 | 4 | | |

*NS: non significant difference (p>0.05).*

## DISCUSSION

In this study, althoug there was no significant difference in sex distribution of all groups, percentage of males are higher than females (60% versus 40% in group Ia and 70% versus 30% in group Ib).This was in agreement with that reported by Palit et al.[9] in Bangladesh who found that percentage of males (60.4%) were more than females patients (39.6%).

There was also no significant difference in age distribution among the patients and controls but the mean age of patients in group Ib is higher than group Ia. This finding was in agreement with Bukar et al.[10] who reported that the incidence of aplastic crisis increased with age due to needs of the multiple blood transfusions.

In the present study, it was found that the prevalence of anti-parvovirus B19 IgG in children with chronic hemolytic anemia without a history of aplastic crisis was 50%. This finding was comparable to that reported by; Regaya et al.[11] in Tunis Azzazy et al.[12] in Egypt who found that the incidence of anti-parvovirus B19 IgG was 61% ,52% and 62% respectively.

In our study, the prevalence of anti-parvovirus B19 IgG in children in group Ib was 45%. Zaki et al.[13] reported nearly similar anti-parvovirus B19 IgG positivity which was 50%. But Azzazy et al.[12] reported a lower IgG positivity (34%) in patients with aplastic crises in Egypt. These differences may be primarily due to the difference in the sample size and differences in the specificity and sensitivity of the assays used [14].

In this study the prevalence of parvovirus B19 IgG is significantly higher (p=0.016) in patients groups (group Ia and group Ib) (50% and 45% respectively) than the control group (17.5%). This result is supported by Kishore et al.[15] and Makled et al.[5] who found that that the IgG positivity in the control group was 21% and 20% respectively. The higher seroprevalence in children with hemolytic anemia may be due to the history of repeated blood transfusion.

In this study, there was a significant positive correlation between anti-parvovirus B19 IgG and the age of all patients (with or without aplastic crisis) (r=0.228) (P value = 0.04). This result was in agreement with that reported by Cennimo,[16] in New Jersey; Eid et al.[17] and Green & Fraire[18] who found that the seropositivity rates were 5-10% among young children aged 2-5 years, increased to 50% by the age 15 years and reached to 60% by the age 30 years. A small percentage of adults acquire infections every year leading to a seropositivity of 90% in those older than 60 years.

On the contrary, Obeid,[14] in Saudi Arabia, Kishore et al.[15] in India, Musa et al.[19] in Nigeria and Urio et al.[20] in Tanzania; found that there was no correlation between viral specific antibody positivity and age.

Marano et al.[21] showed that the range of the occurrence of anti B19 virus IgG in blood donor populations was from 6% to 79.1%. This percentage may be due to that the virus was present in blood and in plasma products, transmitted through transfusion of infected blood donated by apparently healthy and asymptomatic blood donors, can circulate at extraordinarily high titres and infect recipients.

Parvovirus B19 associated crisis in children with thalassemia, sickle-cell anemia, spherocytosis, and glucose-6-phosphate dehydrogenase deficiency are typically managed by simple erythrocyte transfusions [22]. In this study, there was a highly significant positive correlation between frequency of blood transfusion and prevalence of anti B19 virus IgG (r=0.472) (P value = 0.000).

Supporting this correlation was positive, studies reported by Kishore et al.[15] in India and Ghwass et al.[23] in Fayoum who noticed that the seropositivity of anti B19 virus IgG increased with increasing the number of blood transfusions as the transfused anemia patients may receive packed red cells containing parvovirus B19.While Siritantikorn et al.[24] in Thailand found that the incidence of anti-parvovirus B19 IgG in multi-transfused thalasemic patients was not higher than the non-transfused patients. This might be due to the lower incidence of infections in populations in Thailand including adult blood donors.

Multi-transfused β-thalassemia major patients are the most susceptible to acquire transfusion-related infections, including B19 virus, due to regular transfusions of one to three units of blood every three to four weeks, which amounts to 12–51 units/year[25]. In this study, Twenty six patients out of 40 (65%) in all patients were categorized as β-thalassemia major. Eleven patients out of 26 β-thalassemia major children (42.3%) had a detectable level of IgG antibodies. This result was matched with Al-Kasaby et al.[25] in Mansoura who reported that (42.8%) of β-thalassemia major patients were positive for anti-parvovirus B19 IgG antibodies. On the other hand, a lower rate of anti-B19 virus IgG seropositivity (18.2%) among thalassemia major patients was reported by Ghwass et al.[23] in Fayoum.

Higher rate of anti- parvovirus B19 IgG in β-thalassemia major patients was obtained by Ragni et al.[27] in USA and Kishore et al.[15] in India with percentage of 81% and 89% respectively.

There is a vicious circle between parvovirus B19 and aplastic crisis as parvovirus B19 causes aplastic crisis which in turn increase the need for blood transfusion which in turn increases the hazard of transmissible infection including parvovirus B19 [10].

In the present study, five children with sickle cell anemia were only in chronic hemolytic anemia with a history of aplastic crisis by a percentage of 12.5%. Anti-

parvovirus B19 IgG antibodies were detected in 4 children out of 5 children of sickle cell anemia (80%).

A lower prevalence of anti-parvovirus B19 IgG antibodies in sickle cell anemia patients was detected by Abraham et al.[28] in India (50%); by Regaya et al.[11] in Tunisian (56.5%) and Iheanacho et al.[29] in Nigeria 56.72%.

According to this study, the parvovirus B19 specific nucleotide sequences was present in 8 out of 40 (20%) patients; Out of the 20 cases of patients without aplastic crisis crisis, two cases (10%) had parvovirus B19 DNA and six cases (30%) out of the 20 cases of patients with aplastic crisis had parvovirus B19 DNA. In a study done by Makled et al.[5] in Egypt, the viral DNA was detected in 14.3% of cases; two cases (8%) out of 25 cases of patients without aplastic crisis and five cases (25%) out of 20 cases of patients with aplastic crisis.

In another study done in Egypt by Azzazy et al.[12] they reported that the incidence of parvovirus B19 DNA in children with chronic hemolytic anemia not in aplastic crisis was 4% and 46% in patients with hemolytic anemia in aplastic crisis.

Regaya et al.[11] in Tunisia reported that the viral DNA was in only 8.7% of the patients. The high prevalence parvovirus B19 DNA in the present study may be attributed to high frequency of blood transfusion in all patients.

In another studies done in Turkey done by Us et al.[30] and Gupta et al.[31] in India; the prevalence of viral DNA was 29.1% in all patients with hematological disorders and 27.3% of patients with aplastic anemia respectively.

In patients with β-thalassemia major, Parvovirus B19 DNA was found in only six out of 26 (23.1%) cases. A relatively similar prevalence was recorded by Nikoozad et al.[7] in Isfahan (20%) and Al-Kasaby et al.[25] (19.04%) in Mansoura University Children Hospital. A lower prevalence (13%) was found by Siritantikorn et al. [24] in Thailand.

A much lower prevalence (4%) was noticed by Arabzadeh et al.[32] in Iran. This difference may be due to the larger study population (150 thalassemic patients).

In this study, the prevalence of Parvovirus B19 DNA was 20% among sickle cell disease patients which is similar to another study done in Egypt by Makled et al.[5].This result is also comparable to that of Makhlouf et al.[33] in Egypt who reported that the prevalence of Parvovirus B19 DNA among SCD patients was 24% and Urio et al.[20] in Tanzania who reported a prevalence of 29% among SCD patients.

On the other hand, a much lower prevalence (2.89%) was reported by Obeid, [14] in Eastern Saudi Arabia. The difference may be due to the wider range of age group of study participants (from 6 months to 61 years of age) compared to the age range of patients enrolled in this study (1-11years).

The wide variation of Parvovirus B19 prevalence in different studies may be due to the differences in the number of patients and age group. Overcrowding and geographical distribution may have effective influence on B19 seroprevalence. Also presence of high medical care in blood banks service and screening of blood components in these areas which leads to a lower prevalence of blood born infections including parvovirus B19 [21].

## CONCLUSIONS

Direct detection of DNA by PCR should be performed with serology in these children. Screening of patients with a high risk of parvovirus B19 infection can considerably reduce the incidence and prevalence of Parvovirus B19 infection.

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- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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