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ORIGINAL ARTICLE

Molecular and Serological Assessment of Chronic Parvovirus B19 among Chronic Hemolytic Anemia Children

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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: *Parvovirus B19 infections can suppress erythropoiesis and induce acute erythroblastopenia which is often referred to as transient aplastic crisis with hematological disorders especially chronic hemolytic anemia.* ***Objectives:*** *to detect the presence of parvovirus B19 (B19) DNA together with its IgG antibodies in the sera of children with chronic hemolytic anemia and in apparently healthy children in Menoufia University Hospitals and to detect the prevalence of chronic parvovirus B19 infection through detection of PCR among positive anti-parvo virus B19 IgG in different types of anemia.* ***Methodology:*** *The study was conducted on 60 children with chronic hemolytic anemia (40 children with chronic hemolytic anemia without a history of aplastic crisis and 20 children with chronic hemolytic anemia with a history of aplastic crisis) and 20 age and sex-matched apparently healthy children. All patients were subjected to full history taking, clinical examination and laboratory investigations. The presence of B19 IgG levels were measured by using anti-parvovirus B19 ELISA kits (EUROIMMUN), as well as detection of its DNA by nested-polymerase chain reaction technique.* ***Results:*** *Anti-parvovirus B19 IgG antibodies were detected in 62.5% and 100% of patients in group I and group II respectively. Only 20% in control group had a detectable level of anti-parvovirus B19 IgG. There were significant positive correlations between age of patients, frequency of transfusion, and transfusion index in both groups and the level of anti-parvovirus B19 IgG. Seven B19 IgG seropositive cases (14.3%) had B19 DNA. Although78% of children with β thalassemia major had a detectable level of anti-parvovirus B19 virus IgG antibodies, only 15.4% of them had B19 DNA. Children with sickle cell anemia were presented only in group II with aplastic crisis by percentage of 25%, where the prevalence of anti-B19 IgG antibodies among them was 100% but only one child had both anti-B19 IgG antibodies and B 19 DNA (20%).Patients who had anti-parvo virus B19 IgG and those who had both B19 DNA anti-parvo virus B19 IgG cases had a higher transfusion index compared to negative cases.* ***Conclusion:*** *All children with hematological disorders must be screened for B19. Direct detection of DNA by PCR needs to be performed along with serology in these children. Genotyping and quantification of the virus can be more useful for diagnosis and staging of infection.*

INTRODUCTION

Human parvovirus B19 is a small single-stranded, non-enveloped DNA virus belonging to the genus Erythrovirus within the Parvoviridae family and solely infects humans which encodes one non-structural protein, namely NS1, and two viral capsid proteins, VP1 (83 kDa) and VP2 (58 kDa)[1]. Parvovirus B19has a tropism to the progenitor cells of erythrocytes and replicates in erythrocyte precursor cells in the bone marrow through high molecular weight intermediate linked through hairpin structures because of the presence of genomic palindromes[2]. Parvovirus B19 infections have been reported as a nosocomial infection with transmission via blood products, respiratory droplets as well as organ transplantation and vertical transmission from mother to fetus[3]. The small size of B19 makes its removal by filtration with virus-removal membranes impossible[4].

Parvovirus B19 causes a variety of disease syndromes, determined by the age, immunological and hematological status of the host including erythema infectiosum, transient aplastic crisis, persistent infection manifesting as pure red cell aplasia in immunocompromised individuals, nonimmune hydrops fetalis, and arthritis[5]. In patients with hematological disorders especially those with chronic hemolytic anemia such as sickle cell anemia, thalassemia and hereditary spherocytosis; erythroid progenitor cell formation are increased to compensate for red blood cell lysis. B19 virus infections can suppress erythropoiesis and induce acute erythroblastopenia which is often referred to as transient aplastic crisis[6].

Children with chronic hemolytic anemia under a hyper-transfusion regimen, are at high risk of acquiring parvovirus B19.A sudden worsening of anemia, reticulocytopenia, and cessation of erythropoiesis in the bone marrow characterize the transient aplastic crisis. Parvovirus B19 induced aplastic crisis may often be wrongly diagnosed as a complication of the underlying disease[7].

Acute parvovirus B19 infection evolution is characterized by a 5-day phase with high viremia. This viremia is neutralized with antibodies directed against VP1 and VP2. The viremia decreases with the synthesis of IgA and IgM, followed by the synthesis of IgG anti-B19V[8].The prevalence of immunity to parvovirus B19 rises with age, where it is detected among 40% of children, 60% of adults, and 75% of adults older than 40[9]. The establishment of parvovirusB19 persistence is of critical importance in high-risk patients and blood transfusion[1].

Aim of the work:

This study aimed to detect the presence of B19 DNA and its IgG antibodies in the sera of children with chronic hemolytic anemia and in apparently healthy children in Menoufia University Hospitals and to detect the prevalence of chronic parvovirus B19 infection by PCR among positive anti-parvo virus B19 IgG in different types of anemia.

METHODOLOGY

Study population and selection of patients

This study was conducted at the Microbiology and Immunology Department, Faculty of Medicine, Menoufia University in collaboration with Pediatric Department, Faculty of Medicine, Menoufia University during 2015. It involved three groups: group I included 40 children with chronic hemolytic anemia without a history of aplastic crisis, group II included 20 children with chronic hemolytic anemia with a history of aplastic crisis and group III included 20 age- and sex-matched apparently healthy children. Age of patients and controls ranged from 1-15 years. All the studied patients received multiple blood transfusions. An informed consent was obtained from the studied subjects and the study was approved by the local ethics committee.

The patients were subjected to the following:

1. History taking; including history of the present illness, jaundice, blood transfusion, drug intake and past history of first blood transfusion and frequency of blood transfusion.
2. Full clinical and anthropometric measurements.
3. Laboratory investigations including complete blood count (CBC) by using AC 920 auto-counter (SWELAB, France), G6PD activity, osmotic fragility test for diagnosis of spherocytosis and hemoglobin electrophoresis to confirm the presence of HbS in sickle cell anemia and for diagnosis of thalassemia.

Serum samples were obtained from all the studied groups and stored at -20°C for quantification of anti-parvovirus B19 IgG antibodies and then seropositive IgG samples were processed for detection of B19 viral DNA by nested PCR.

Quantification of anti-parvovirus B19 IgG antibodies serum levels:

Anti-parvovirus B19 IgG antibodies serum levels were quantified by using EUROIMMUN human anti-parvovirus B19 IgG ELISA Kit according to the Manufacturer's instructions.

Detection of parvovirus B19-DNA by nested PCR:

DNA was extracted from seropositive samples by using a DNA-PCR template preparation kit (Thermo Fisher Scientific 81, Wyman Street, Waltham, MA, USA) and RNA Purification Kit; Lethwania) as directed by the Manufacturer. PCR was performed according to Zerbini et al., [10]. Nearly, 0.4 µl of extracted DNA was added to the PCR mix for a total volume of 25 µl of DreamTaq Green PCR MasterMix (2´) (Thermoscientific (Fisher Biotec, 198 Cambridge St, Wembley, WA 6014, Australia)), 200 µmol/l deoxynucleotide triphosphate (Stratagen (4040 Lake Washington Blvd NE #201, Kirkland, WA 98033, United States)) and 300 ng of each primer. The first round of PCR amplification was performed after the denaturation step (5 min at 95°C), about 3 µl of the product of this round was transferred to a second 50 µl PCR mix. The second-round reaction mix contained the same constituents as the first-round mix, but 300 ng of each second primer was substituted for each first primer. The primer used in the first round of amplification was 5′-CTTTAGGTATAGCCAAC TGG-3′(Biosearch Technologies, USA) and 5′-ACACTGAGTTTACTAGTGGC-3′, to yield a product of 1112 bp. The primer of second-round PCR was 5′-CAAAAGCATGTGGAGTGAGG3′ and 5′-CC TTATAATGGTGCTCTGGG 3′ to give a product of 104 bp. Thirty-five cycles of both the first and second-rounds amplification were performed under the following conditions after one cycle of heating at 95°C for 5 min, at 95°C for 1 min, at 55°C for 1.5 min, and at 72°C for 1 min, followed by a final extension at 72°C for 7 min for one cycle. About ten microliters of second-cycle PCR products were analyzed by 2% agarose gel electrophoresis and the bands were visualized after ethidium bromide staining.

Statistical analysis

The collected data were tabulated and analyzed by SPSS (statistical package for social science) version 22.0 on IBM compatible computer. Two types of statistics were done: descriptive statistics: e.g. percentage (%), mean and standard deviation (SD) and analytical statistics: e.g. Chi-square, Fisher's exact, Student t, Mann-Whitney, ANOVA (F), Kruskal-Wallis and Post Hoc tests. P value <0.05 was considered to be significant. Spearman correlation coefficient (r) was used to measure the association between two quantitative variables not normally distributed.

- Positive predictive value (PPV) is the proportion of patients who are test positive and in whom the disease is present.

$$\frac{\text{True positive x 100}}{\text{True positive (TP) +False positive (FP)}}$$

True positive by PCR / all positive cases of IgG

Transfusion index: -

$$\frac{\text{Age of cases (month) - age of the start of blood transfusion (month)}}{\text{Frequency of blood transfusion (month)}}$$

RESULTS

Group I patients without a history of aplastic crisis included 35 (87.5%) with β-thalassemia major, 4 (10%) with thalassemia intermedia and 1 (2.5%) with hereditary spherocytosis. Group II included 20 children with a history of aplastic crisis included 15 (75%) with β thalassemia major and 5 (25%) with sickle-cell anemia as shown in figure 1. There was significant difference between both group I & group II and group II & control regarding the age (pvalue=0.002 for each). Highly significant differences were detected between group I & control and group II & control with p value <0.001 regarding status of spleen as shown in table 1.

Anti-parvovirus B19 IgG antibodies were detected in 62.5% and 100% of group I and group II patients respectively. Only 20% of the control group had a detectable level of anti-parvovirus B19 IgG. There were significant differences between group I & group II; group I & controls and between group II & controls.

Thirty-nine children with β-thalassemia major out of 50 (78%) had detectable levels of anti-parvovirus B19 IgG. Anti-parvovirus B19 IgG antibodies were detected among 25%, 0% and 100% of children with thalassemia intermedia, hereditary spherocytosis and sickle-cell anemia respectively. There were significant differences between β thalassemia major and thalassemia intermedia, thalassemia intermedia and sickle-cell anemia, hereditary spherocytosis and sickle-cell anemia as shown in table 2.

There was significant positive correlations between the level of anti-parvovirus B19 IgG and age of the patients (r=0.424), frequency of transfusion (r=0.282), and transfusion index (r=0.414), in both groups and as shown in table 3.

Seven B19 IgG seropositive cases had B19 DNA (14.3%) as shown in table 4. Two cases (8%) out of 25 cases of group I and five cases (25%) out of 20 cases of group II had B19 DNA by PCR with no significant difference. As regard the control group; none of them had B19 DNA by PCR as shown in table 5.

There was no significant difference between the different types of anemia regarding B19 DNA among anti-parvo virus B19 IgG positive cases,. Six cases (15.4%) out of 39 cases of β Thalassemia major and one case (20%) out of 5 cases of sickle-cell had positive B19 DNA as shown in table 5.

Positive anti-parvo virus B19 IgG cases had a significant higher transfusion index than negative anti-parvo virus B19 IgG cases (137.8 ± 57.7 and 77.5 ± 50.8, respectively) ($p \leq 0.001$). Also, positive B19 DNA cases had a higher transfusion index than negative PCR cases but with no significant difference (167.9 ± 32.9 and 132.3 ± 59.8, respectively) as shown in table 6.

Table (1): Demographic and clinical characters of the studied groups:

The studied parameter	*Group I (No.=40)*		*Group II (No.=20)*		*Controls (No.=20)*		*Test of significance*	*p value*
	Mean±SD		**Mean±SD**		**Mean±SD**			
Age (years)	9.15±3.55		11.95±3.02		8.65±2.52		**F** Test=6.64 P =0.002*	**Post hoc** P1=0.002* P2=0.57 P3=0.002*
	No.	**%**	**No.**	**%**	**No.**	**%**	**X^2 test**	**p value**
Gender							**X^2** 1=0.57	P1=0.45
Male	24	60.0	14	70.0	8	40.0	**X^2** 2=2.14	P2=0.14
Female	16	40.0	6	30.0	12	60.0	**X^2** 3=3.64	P3=0.06
Liver							**X^2** 1=0.36	P1=0.55
Normal	29	72.5	13	65.0	20	100.	**X^2** 2=6.74	P2=0.009*
Enlarged	11	27.5	7	35.0	0	0.0	**X^2** 3=8.49	P3=0.004*
Spleen								
Normal	0	0.0	0	0.0	20	100.	**X^2** 1=6.07	P1=0.01*
Splenomegaly	19	47.5	3	15.0	0	0.0	**X^2**2=60.00	P2=<0.001**
Splenectomy	21	52.5	17	85.0	0	0.0	**X^2**3=40.00	P3=<0.001**

* = significant (pvalue ≤ 0.05) **= highly significant (pvalue ≤ 0.001).
Non-significant (pvalue > 0.05)
P1: Comparison between group I and group II.
P2: Comparison between group I and control.
P3: Comparison between group II and control.

Table (2): Prevalence of anti-parvovirus B19 IgG (IU/ml) among the studied groups and different types of anemia.

Anti-parvo virus B19 IgG	*Studied groups*						*Test*	*P value*	
	Group I (No.=40)		**Group II (No.=20)**		**Controls (No.=20)**				
	No.	**%**	**No.**	**%**	**No.**	**%**			
Positive	25	62.5	20	100	4	20.0	$\mathbf{X^2}1=10.0$ $\mathbf{X^2}2=9.64$	I versus II =0.002* I versus control =0.002*	
Negative	15	37.5	0	0.0	16	80.0	$\mathbf{X^2}3=26.67$	II versus control =<0.001**	
Anti-parvo virus B19 IgG (<4IU/ml) **Mean ± SD**	35.91±34.32		56.29±29.32		16.33±29.98		**Kruskal Wallis Test** =6.64 **P value**= <0.001**	**Post Hoc test** I versus II =0.02* I versus control =0.04* II versus control =<0.001**	
Anti-parvo virus B19 IgG	**Different types of anemia among cases**							**Fisher's Exact test**	**P value**
	β-thalassemia major (No.=50)		**Thalassemia intermedia (No.=4)**		**Hereditary spherocytosis (No.=1)**		**Sickle-cell (No.=5)**		
Positive	39	78.0	1	25.0	0	0.0	5	100	**1** = 5.42 **2** = 3.32 **3** = 1.38 **4** = 0.31 **5** = 5.63 **6** = 6.00	P1=0.02* P2=0.07 P3=0.24 P4=0.58 P5=0.02* P6=0.01*
Negative	11	22.0	3	75.0	1	100	0	0.0		

Non-significant (P-value > 0.05)
* = significant (P-value ≤ 0.05) **= highly significant (P-value ≤ 0.001).
P1: comparison between β thalassemia major and thalassemia intermedia
P2: comparison between β thalassemia major and hereditary spherocytosis
P3: comparison between β thalassemia major and sickle-cell anaemia
P4: comparison between thalassemia intermedia and hereditary spherocytosis
P5: comparison between thalassemia intermedia and sickle-cell anaemia
P6: comparison between hereditary spherocytosis and sickle-cell anaemia

Table (3): Spearman correlation between level of anti-parvovirus B19 IgG and age, age at starting blood transfusion, frequency of transfusion, transfusion index and different CBC parameters among cases (group I and group II).

	Level of Anti-parvo virus B19 IgG	
	r	**P value**
Age (year)	0.424	0.001**
Age at starting blood transfusion/month	-0.351	0.006*
Frequency of transfusion/week	0.282	0.03*
Transfusion index	0.414	0.001**
Hb (g/dl)	0.028	0.83
MCV (mm^3)	0.130	0.32
MCH	0.060	0.65
MCHC (hg/dl)	-0.009	0.95
RDW (h%)	-0.069	0.60
WBCs ($x10^3/mm^3$)	0.065	0.62
Platelets ($x10^3/mm^3$)	0.072	0.58

r: Spearman correlation coefficient Non-significant (P-value > 0.05)
* = significant (P-value ≤ 0.05) **= highly significant (P-value ≤ 0.001).
There was a positive correlation between level of anti-parvo virus B19 IgG and transfusion index among cases (r=0414)

Table (4): PCR of anti-parvo virus B19 IgG positive cases and positive predictive value (PPV) of anti-parvo virus B19 IgG positive cases versus PCR.

PCR	*Anti-parvo virus B19 IgG positive cases (No.=49)*	
	No.	**%**
Positive	7	14.3
Negative	42	85.7
PPV of Anti-parvo virus B19 IgG	14.3%	

Table (5): Prevalence of parvovirus B19 infection through detection of PCR among positive anti-parvo virus B19 IgG cases of studied groups and different types of anemia.

PCR	*Anti-parvo virus B19 IgG positive cases among studied groups (No.=49)*						*X^2 test*	*P value*
	Group I (No.=25)		**Group II (No.=20)**		**Control (No.=4)**			
	No.	**%**	**No.**	**%**	**No.**	**%**		
Positive	2	8.0	5	25.0	0	0.0		
Negative	23	92.0	15	75.0	4	100	3.35	0.19
PCR	**Anti-parvo virus B19 IgG positive cases among different types of anemia (No.=45)**						**X^2 test**	**P value**
	β-thalassemia major (No.=39)		**β-thalassemia intermedia (No.=1)**		**Sickle-cell (No.=5)**			
Positive	6	15.4	0	0.0	1	20.0	0.26	0.88
Negative	33	84.6	1	100	4	80.0		

Table (6): Comparison between positive and negative anti-parvo virus B19 IgG and PCR cases regarding transfusion index.

Parameter	*Positive anti-parvo virus B19 IgG cases (No.=45)*	*Negative anti-parvo virus B19 IgG cases (No.=15)*	*Mann Whitney test*	*P value*
	Mean ± SD	**Mean ± SD**		
Transfusion index	137.8 ± 57.7	77.5 ± 50.8	3.29	0.001*
Parameter	**Positive PCR cases (No.=7) Mean ± SD**	**Negative PCR cases (No.=38) Mean ± SD**	**Mann Whitney test**	**P value**
Transfusion index	167.9 ± 32.9	132.3 ± 59.8	1.82	0.07

*highly significant difference

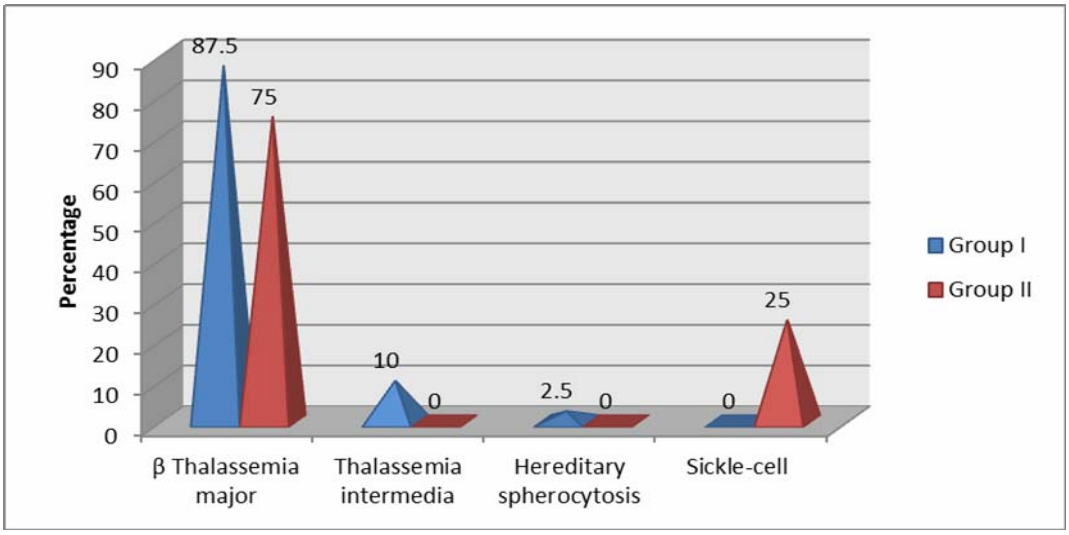

Fig. 1: Types of chronic hemolytic anemia among group I and group II.

DISCUSSION

Parvovirus is a common pathogen worldwide. Serological studies have indicated that more than 50% of people had infection during childhood, however, higher prevalence rates have been observed among children with hematological disorders. Moreover, B19V infection may have a different outcome in patients with hemolytic anemias[1].

In this study,60% of patients with chronic hemolytic anemia without history of aplastic crisis and 70% of those with a history of aplastic crisis were males. This agrees with that reported by Palit et al.,[11] in Bangladesh. The highest mean age in this study was found among children with chronic anemia with a history of aplastic crisis (11.95 ±3.02). Bukar et al.,[12] and Borsato et al.,[13] supported this result where they demonstrated that the incidence of aplastic crisis increases with age because of the need of multiple blood transfusion.

Children with splenectomy in this study were more than those with splenomegaly. This result was in accordance with that reported by Porecha et al.,[14] in India. In chronic hemolytic anemia, the patient's hemoglobin levels may drop causing the body to try to create more red blood cells in the bone marrow and some other organs as spleen (extramedullary erythropoiesis) leading to hypersplenism which is one of the major indications of splenectomy[14].

Traditionally, the diagnosis of chronic or old B19 virus infection depends on IgG persistence[15]. Major advances in diagnosis of B19 infection have taken place including standardization of serological and DNA based detection methodology[16]. As there is no reliable immunological method for antigen detection, polymerase chain reaction (PCR) is needed for detecting viremia[17]. Makhlouf et al.,[18] classified B19 infections according to the previous fact into acute (by presence of IgM & B19 DNA), chronic (by presence of B19 DNA and anti-B19 IgG in patients' sera in the absence of anti-B19 IgM) and old (by presence of anti-parvovirus B19 IgG only).

According to the current study, the prevalence of anti-parvovirus B19 IgG in children with group I was 62.5%. This finding is similar to that reported by many authors. Azzazy et al.,[19] in Egypt, Badr,[20] in Saudi Arabia and Regaya et al.,[21] in Tunis demonstrated that the prevalence of anti-parvovirus B19 IgG in this group of patients was 52% ,56.5% and 61% respectively. Anti-parvovirus B19 IgG antibodies were detected in 100% among children of group II in this study. On the other hand, Zaki et al.,[22] and Azzazy et al.,[19] found that parvovirus B19 IgG positivity was lower (50% and 34% respectively). The highest prevalence in the present study may due to the presence of transient aplastic crisis that is caused by parvovirus B19[11].

There was significant positive correlations between the level of anti-parvovirus B19 IgG and patient's age, frequency of transfusion, and transfusion index. This result agrees with that reported by Cennimo,[23] in New Jersey; Eid et al.,[24] and Green & Fraire,[25] who found that seropositivity rates were5-10% among young children, increasing to 50% by age 15 years and 60% by age 30 years. Higher seroprevalence of anti-B-19 IgG in patients with hematological disorders including thalassemia and sickle cell disease were reported in Nigeria, Taiwanand Sweden[26, 27, 28].

In a study by Regaya et al.,[21] in Tunisia, the prevalence of anti-B19 IgG was 56.5% in patients with chronic blood disorders, while viral DNA was reported in only 8.7% of the patients, which is lower than that reported in our study. We found that seven B19 IgG seropositive cases had B19 DNA (14.3%); Two cases (8%) out of 25 cases of group I and five cases (25%) out of 20 cases of group II had B19 DNA. The high prevalence in the present study may be attributed to high frequency of multiple blood transfusion in all patients. In another study in Turkey that was done by Us et al.,[29]; the prevalence of viral DNA was 29.1%.

It is known that the B19 infection is either due to reactivation of a latent infection in a general immunosuppression situation or a result of a prolonged primary infection in an immunocompromised individual[30]. Therefore, cases of chronic hemolytic anemia should be evaluated for B19 positivity so that if parvovirus B19 is implicated, the patients should be diagnosed for optimal therapy[29].

In this study,50 out of 60 (83.3%) of all chronic hemolytic anemia children were categorized as β-thalassemia major, where 78% of them had a detectable level of anti-parvovirus B19 virus IgG antibodies. Parvovirus B19 DNA was found in only six out of 39 seropositive (15.4%) cases. This finding suggests that the possibility of chronic B19 carriage may exist in immunocompetent individuals, despite the presence of specific IgG antibodies[31]. A relatively higher prevalence was recorded by Nikoozad et al.,[32] where PCR analysis of 30 thalassemia major patients from Isfahan showed that parvovirus B19-specific nucleotide sequences were present in 20%, although the immunoglobulin G (IgG) concentration was high in the serological tests. Siritantikornetal,[33] noticed that parvovirus B19 DNA was found in 8 of 60 (13%) plasma samples and 3 (5%) were also anti-parvovirus B19 IgG positive.

In the present study, children with sickle cell anemia were present only in 5 (25%) of patients with aplastic crisis by percentage. The prevalence of anti-B19 IgG antibodies among them was 100%, however, only one child had both anti-B19 IgG antibodies and B 19 DNA (20%).A relatively similar result was observed by Makhlouf et al.,[18] where 24% of the studied patients with sickle cell disease have chronic infection (anti-B19

IgG antibodies and B 19 DNA). Also, similar rates were recorded by studies done in USA and Guinea on patients with sickle cell anemia[34, 35]. A lower prevalence was noticed in a study done by Obeid,[6] in Saudi Arabia; B19 DNA was demonstrated in 4 sickle cell disease patients out of 52 (7.7 %) B19 IgG seropositive. These results may be explained by the fact that there is a vicious circle; parvovirus B19 causes aplastic crisis which in turn causes increased transfusion need and blood transfusion increases risk of transfusion transmissible infection including parvovirus B19[12]. Moreover, Cennimo,[23] found that parvovirus B19 was the only infectious cause of transient aplastic crisis known that has been shown to be the cause of aplastic crisis in over 80% of patients with sickle cell disease.

All the apparently healthy children tested in this study were negative for B19-DNA by PCR analysis, although, the prevalence of anti-parvovirus B19 IgG antibodies were detected in 20%. The same result was observed by Kishore et al.,[7]. On the other hand, high anti-parvovirus B19 IgG antibodies in a control group was reported also by Obeid,[6] in Saudi Arabia (39%), Azzazy et al.,[19] in Egypt (40%) and Musa et al.,[36] in Nigeria (42%). This difference may be due to the younger age of apparently healthy children in this study as compared to the previous studies. An increased prevalence of anti-parvovirus B19 IgG with age was previously reported by Green & Fraire,[25].

A peculiar characteristic of B19V infection is that the virus is not always cleared and in many cases it can establish persistency in different body tissues and fluids[37]. This fact was verified in this study as patients with positive antiparvovirus IgG and had B19 DNA had higher transfusion index than others. Studies conducted in various countries showed that the prevalence of B19 infection in the patients suffered from hematologic disorders was very high[32].This may be due to repeated blood transfusions in those patients. The presence of persistent B19V infection in blood donors raises serious questions regarding transfusion safety of the obtained hemoderivatives, especially plasma products and packed erythrocytes. Moreover, B19V is highly resilient compared to almost all viruses[38].

CONCLUSIONS

B19 must be suspected and screened for in all children with hematological disorders. The direct detection of DNA by PCR in sera needs to be coupled with serology for a more reliable diagnosis of B-19 infections in these children. Further studies for genotyping and quantification of the virus will be more useful for diagnosis and staging of infection.

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