RESEARCH BRIEF

Prevalence of Parvovirus B 19 Infection in Children with Aplastic Anemia

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We studied the prevalence of parvovirus B19 infection in pediatric patients with acquired aplastic anemia. Detection of parvovirus B19 DNA by PCR and IgM antibodies by ELISA was carried out in 66 pediatric patients with acquired aplastic anemia. 45 healthy children acted as controls. Parvovirus B19 DNA was detected in significantly higher number of patients in comparison to controls (27% *vs* 2%, $P = 0.001$). Similarly, parvovirus B19 IgM antibodies were detected in 17 (25.8%) patients as against one control (2.2%) ($P<0.05$). Clinical and hematological profile of the patients with or without parvovirus infection was comparable.

Key words: Aplastic anemia, Etiology, Children, Parvovirus B19.

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Parvovirus B19 is a well-recognized cause of marrow aplasia in children with hemoglobinopathies causing transient aplastic crisis in sickle cell disease and hereditary spherocytosis [1,2]. It is also associated with a wide range of hematological disorders such as chronic anemia, red cell aplasia, neutropenia and thrombocytopenia in immunodeficient hosts. However, there are reports of marrow aplasia in previously healthy immunocompetent hosts and it has been postulated as one of the causes of acquired aplastic anemia [3,4]. A study of adult patients suggested significant association of parvovirus with aplastic anemia [5]. No such study has been carried out in Indian children with aplastic anemia. We conducted this study to document the prevalence of parvovirus B19 infection in children with acquired aplastic anemia.

METHODS

Study included patients with aplastic anemia in the age group of 4-14 years admitted from July 2009 to June 2011 in the Department of Pediatrics, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Detailed history of drug intake, area of origin, occupation of father, and socioeconomic status was taken in each case. Complete blood count, examination of peripheral smear, bone marrow aspiration and trephine biopsy was carried out in all the cases. Inclusion criteria were: hypocellular bone marrow on trephine biopsy in the absence of fibrosis or neoplastic infiltration with at least two of the following: (*a*) hemoglobin <10g/dL; (*b*) platelet count ≤50 $\times 10^9$/L; (*c*) granulocyte count <1.5 $\times$ 10^9/L based on diagnostic criteria by International Agranulocytosis and Aplastic Anemia Study [6]. Neutrophil count of <0.2 $\times$ 10^9/l was classified as very severe and <0.5 $\times$ 10^9/l as severe aplastic anemia [7]. Patients with inherited bone marrow failure syndrome (IBMFS) were excluded based on family history, complete physical examination, ultrasonographic examination of abdomen, and chromosomal fragility test with mitomycin C. Study was approved by Ethics Committee of the hospital. Informed consent was taken from parents/guardians for collection of samples. Patients were given immunosuppressive treatment (IST) according to published guidelines [8].

Venous blood (2 mL) was collected in plain sterile vials. Samples for parvovirus studies were collected at least 3-4 weeks after last blood transfusion. 45 healthy, age and sex matched siblings of patients were selected as controls. Samples were tested for parvovirus B19 DNA by nested PCR (polymerase chain reaction) and IgM antibody using ELISA with commercially available kit. All samples were tested for HBsAg, anti HCV and HIV.

DNA for PCR amplification was extracted from whole blood samples by standard techniques [9]. The extracted DNA from whole blood was subjected to nested PCR assay which was carried out using in-house primers designed for the study.

Primary PCR amplification yielded a 1480-bp product and nested PCR amplification produced two bands measuring 390-bp and 600-bp. Amplicons of first and second round PCR products were analysed by electrophoresis on 2% agarose gel. Bands were visualized by ethidium bromide staining using gel documentation system.

Statistical analysis was done using SPSS (Statistical Package for Social Sciences) software version 16.0. Chi-square test was applied to compare differences between categorical variables. Comparison between means was done by Student's t-test /Mann-Whitney U test as per requirement. *P* value <0.05 was considered as significant.

RESULTS

66 patients with aplastic anemia (mean age 9.2 ± 2.4 years, male: female ratio of 2.7:1) were included in the study. Mean hemoglobin (g/dL), absolute neutrophil count ($\times 10^9$/L) and platelet count ($\times 10^9$/L) of study group was 3.9 ± 1.86, 0.69 ± 0.44 and 19.17 ± 15.6, respectively. 71% of the patients were classified as severe, 20% as very severe and rest as non-severe aplastic anemia. Pallor was the commonest presenting complaint followed by bleeding manifestations and fever. No patient had history of typical facial erythema. Three patients had history of arthralgia in the preceding month without receiving any specific treatment. Bone marrow biopsy samples were markedly hypocellular in all the patients. Mean cellularity was less than 25% and was comparable in the two groups.

Parvovirus B19 DNA was detected in 18 (27.3%) patients as against one (2.2%) control. Parvovirus B19 IgM antibodies were detected in 17 (25.8%) patients and one control (2.2%), respectively. Occurrence of parvovirus B19 DNA and IgM antibody was significantly higher in patients than control group (P=0.001). 17 (25.8%) patients had both viral DNA and IgM antibody whereas one patient had viral DNA in the absence of IgM antibodies. In control group, one patient was positive for both parvovirus DNA and IgM antibody. Clinical and hematological profile of the patients with or without parvovirus infection was comparable (***Table* I**).

One patient in parvovirus negative group was positive for HBsAg. He was also positive for HBeAg and negative for anti-HBe. All patients and control were negative for anti-HCV antibodies and HIV.

20 patients (5 parvovirus positive and 15 negative) received immunosuppressive treatment. One patient in negative group died due to intracranial hemorrhage after therapy. In 19 patients, response was evaluated at 1, 3 and 6 months. 3 patients in parvovirus negative group had a response to IST at one month. 2 more patients responded to the treatment in next 2 months of which one was parvovirus negative and the other was parvovirus positive. Thus, 5 patients (26.3%) had response to IST at 3 months wherein 1/5 (20%) was in parvovirus positive and 4/14 (28.6%) in the parvovirus negative group. The response rate was same at 6 months.

DISCUSSION

In our study, parvovirus B19 DNA and IgM was detected in significantly higher number of patients compared to controls. Presence of DNA or IgM antibodies indicates acute infection. Presence of both DNA and IgM antibodies in 27.3% of patients suggests significant association of aplastic anemia with parvovirus infection. Patients with or without parvovirus infection were clinically and hematologically comparable. Signs of acute infection such as erythematous facial rash, and arthropathy were not observed at presentation. Possible explanation could be late presentation of patients to tertiary care center.

Little data is available in pediatric aplastic anemia patients. In a study of 30 pediatric patients, parvovirus DNA and IgM antibodies were detected in 6 (20%) and 4 (13.3%) patients respectively of which four achieved complete remission with IST [10]. In adult patients with aplastic anemia, parvovirus IgM and DNA were detected in 40.7% and 37% respectively [6].

There is no specific antiviral therapy for parvovirus infection. Intravenous immunoglobulins (IVIG) have been used with some success in immunocompromised patients but it provides only temporary remission and periodic re-infusions may be needed [11]. IVIG is not recommended for parvovirus induced arthropathy. As our patients presented with pancytopenia and hypocellular marrow, they were managed as aplastic anemia and none received IVIG. Only few patients received IST, therefore it is difficult to assess the response to therapy in presence of parvovirus infection. One third of patients had

TABLE I COMPARISON OF DIFFERENT VARIABLES BETWEEN PARVOVIRUS B19 PCR POSITIVE AND NEGATIVE CHILDREN WITH APLASTIC ANEMIA.

Variables	*Parvovirus B19 positive group (n=18)*		*Parvovirus B19 negative group (n=48)*	
	Mean ± SD	*Median*	*Mean ± SD*	*Median*
Age (years)	9.11 ± 2.62	8	9.17 ± 2.60	8
Mean duration (d)				
pallor	47.2 ± 52.88	25	50.2 ± 49.83	30
fever	43.8 ± 57.87	20	28.7 ± 33.55	15
bleeding	25.7 ± 43.41	15	31.9 ± 52.68	15
Hb (g/dL)	4.9 ± 1.94	3.9	3.6 ± 1.72	3.5
APC ($\times 10^9$/L)	24.2 ± 23.33	14.5	17.5 ± 11.70	14.0
ANC ($\times 10^9$/L)	0.67 ± 0.40	0.62	0.70 ± 0.46	0.66

Hb:hemoglobin, APC:absolute platelet count, ANC: absolute neutrophil count.

WHAT THIS STUDY ADDS?

- Significantly higher prevalence of parvovirus B19 infection was found in children with acquired aplastic anemia.

response which was equally distributed in the two groups. In the other study, 4/6 (66.7%) patients with parvovovirus infection had complete response whereas two died due to intracranial hemorrhage [10].

The exact mechanism by which parvovirus causes aplastic anemia is not very clear. A direct toxic effect of NS1 protein of parvovirus on the bone marrow has been suggested. Experimental studies have shown that infection with the virus in healthy volunteers can result in anemia and also granulocytopenia and thrombocytopenia [12]. Similar mechanism may operate in aplastic anemia also where all three cell lines become target of the virus. The second hypothesis is based on immune mediated damage to cell lines. Raised cytokines following an infection may result in hemophagocytic syndrome, pancytopenia and decreased hematopoesis [13]. Recovery following immunosuppressive therapy gives credence to this theory. More studies are needed in pediatric patients with acquired aplastic anemia to delineate the precise role of parvovirus infection.

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