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Association of Viruses with Aplastic Anemia: A Case Control Study

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Abstract

Several viruses are often believed to be associated with acquired aplastic anemia. There is paucity of literature proving the association between viruses and aplastic anemia. We aimed to study the association of aplastic anemia with Human Parvovirus B19 (B19V), Epstein Barr virus (EBV), Cytomegalovirus (CMV), Hepatitis viruses, Measles virus (MV), Varicella Zoster virus (VZV) and Adenovirus (AdV). Between January 2020 to December 2020, confirmed cases of aplastic anemia and age and sex matched controls of iron deficiency anemia were enrolled in the study. They were tested for the above- mentioned viruses for antigen and/or IgM antibody by ELISA and/or nucleic acid by Real Time PCR in serum samples. Relevant history was collected. Cases were followed up at 3, 6 and 12 months after enrollment for recording the outcome. Total 68 cases and 34 controls were included in the study of which 61(89.70%) cases and 12(38.23%) controls were positive for markers of at-least one of the 10 viruses studied. B19V, EBV, CMV and Hepatitis B virus were found to be significantly associated with aplastic anemia. Five patients died within 12 months. Mortality was not associated with viral infections. Viral infections may play a role in pathogenesis of acquired aplastic anemia.

Keywords: CMV, EBV, B19V, Aplastic Anemia, Viral Infections

Highlights

- Viruses were found to be significantly associated with aplastic anemia of the ten viruses included in the study, human parvovirus B19, Epstein bar virus, cytomegalovirus and hepatitis B virus were found to be significantly associated with aplastic anemia.

- Mortality among the patients were not associated with presence of viral infection.

Introduction

Aplastic anemia (AA) is defined as peripheral blood pancytopenia with hypo cellular bone marrow [1]. While the incidence of aplastic anemia in Europe and North America has been reported as 2 per million populations per year, In India and other Asian countries like Thailand and China, it could be as high as 6 to 8 per million populations per year [1,2].

Acquired aplastic anemia can be associated with several infectious and non-infectious causes. Non-infectious causes which have been documented are exposure to toxins like benzene, arsenic, quinine and chloramphenicol and pesticides, radiation and chemotherapy for cancer, treatment for autoimmune diseases and metastasis of cancer from other parts of the body. Infectious agents known to be associated with aplastic anemia are viruses such as hepatitis viruses, Epstein Barr Virus, Cytomegalovirus, HIV etc. [3]. In most of the cases etiology remains unknown as testing for viral infections is not routinely done in diagnostic work up of aplastic anemia. As definite treatment based on diagnostic tests is not given, the condition of the patient may deteriorate [4]. There is paucity of data from Indian literature regarding the association of viruses with aplastic anemia.

Therefore, the present case-control study was done to establish the association of viruses including EBV, CMV, Parvovirus B19, hepatitis virus (A, B, C, E), measles virus, varicella zoster virus and adenovirus with aplastic anemia.

Materials and Methods

This case-control study was conducted from January 2020 to December 2020 in the Departments of Microbiology, Clinical hematology, Pediatrics and Pathology, King George's Medical University, Lucknow. Ethical clearance for the study was obtained from the Institutional Ethics committee (Ref. code: 101st ECM II B-Thesis/P32).

Newly diagnosed cases of aplastic anemia aged 1 month to 60 years who have never received any immunosuppressive therapy were enrolled as cases. Patients with documented inherited bone marrow failure syndromes or with clonal disease or those in whom the cause of aplastic anemia other than viral infection is known and those who were not willing to consent were excluded from the study. Age and sex matched patients of Iron deficiency anemia (IDA) were enrolled as controls. Iron deficiency anemia (IDA) patients were selected as controls because the etiology in most of these cases is either blood loss or dietary deficiency [5,6]. To the best of our knowledge, there is no evidence in the literature which states that IDA might be caused by a virus. Therefore, to minimize selection bias, patients of IDA were included. Moreover, collection of blood samples from IDA patients was easy as they were already referred to the Department of Pathology as a part of their management and no extra sample was required from them. After obtaining written informed consent from the patients or their guardians/parents in case of children, blood samples were collected from cases and controls.

Each sample was tested for 10 viruses namely Human Parvovirus B19 (B19V), Epstein Barr virus (EBV), Cytomegalovirus (CMV), Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis E virus (HEV), Varicella-Zoster virus (VZV), Measles virus (MV) and Adenovirus (AdV) for antigen/ IgM antibody by Enzyme Linked Immunosorbent Assay (ELISA) and for nucleic acid by Real Time PCR. For ELISA commercially available kits (Table 1) were used as per the manufacturer's instructions. A sample was considered positive when the cycle threshold (Ct value) was less than or equal to 35 and negative when it was more than 35. For quality control, positive and negative controls were run with each batch of tests.

S. No	Virus Tested	ELISA for detection of anti-viral IgM/ viral antigen	Real Time PCRfor detection of viral nucleic acid	Reference	
1	Human Parvovirus B19	Anti-Parvovirus B19 IgM ELISA (NovaLisa Parvovirus B19 IgM ELISA, NovaTech Immunodiagnostica GMBH , CA, USA)	B19V Fwd	GACAGTTATCT GACCACCCCCA	In-house
			B19V Rvs	GCTAACTTGC CCAGGCTTGT	
			B19V Probe	CCAGTAGCAGTCATGCA GAACCTAGAGGAGA	
2	Cytome galovirus	Anti CMV IgM ELISA (CALBIOTECH Inc, El Cajon, CA)	CMV Fwd	GCTGACGCGTTTGGTCATC	[38]
			CMV Rvs	ACGATTCACGGAGCACCAG	
			CMV Probe	TCGGCGGATCACCACGTTCG	
3	Epstein Barr Virus	Anti EBV-VCA IgM ELISA (EUROIMMUN Anti-EBV-VCA-IgM ELISA, PerkinElmer, Germany)	EBV Fwd	GGAACCTGGTCATCCTTGC	[39]
			EBV Rvs	ACGTGCATGGACCGGTTAAT	
			EBV Probe	CGCAGGCACTCG TACTGCTCGCT	
4	Varicella Zoster Virus	Anti-VZV IgM ELISA (CALBIOTECH Inc, El Cajon, CA)	VZV Fwd	CAAAGGCAACCAT GCAGGACACTT	In-house
			VZV Rvs	TACGCGCACGCA GTGTGTCTAATA	
			VZV Probe	TGTGGATCATCCAACGT TTCGTCGCA (5HEX/3IABkFQ)	
5	Hepatitis B virus	HBsAg ELISA (HEPALISA, J.Mitra & Co, New Delhi, India)	HBV Fwd	GTGTCTGCGGCGTTTTATCA	In-house
			HBV Rvs	GACAMACGGGCAACATACCTT	
			HBV Probe	/5Cy5/CCTCKCATCCKGCT GCTATGCCTYMWC	

S. No	Virus Tested	ELISA for detection of anti-viral IgM/ viral antigen	Real Time PCRfor detection of viral nucleic acid	Reference	
6	Hepatitis C virus	Ani HCV Total antibodies ELISA (HCV Microlisa, J.Mitra & Co, New Delhi, India)	HCV Fwd	AAGGCCTTGTGG TACTGCCTGATA	In-house
			HCV Rvs	CGACGGTTGGTGT TWCKTTTGGTT	
			HCV Probe	556'-5'-FAM/ ACCGTGCAMCATGAGCA CRMWTCCTAAA-3'	
7	Hepatitis A virus	Anti HAV IgM ELISA (Dia.pro Diagnostic Bioprobes srl, Milan Italy)	HAV Fwd	TCACCGCCGTTTGCCTAG	[40]
			HAV Rvs	GGAGAGCCCTGGAAGAAAG	
			HAV Probe	TTAATTCCTGCAGGTTCAGG	
8	Hepatitis E virus	Anti HEV IgM ELISA (Dia.pro Diagnostic Bioprobes srl, Milan Italy)	HEV Fwd	CAAGGHTGGCGYT CKGTTGAGAC	[41]
			HEV Rvs	CCCTTRTCCTGCT GAGCRTTCTC	
			HEV Probe	G YTCKGTTGAGA CCWCBGGBGT	
9	Measles virus	Anti-Measles IgM ELISA (CALBIO TECH Inc, El Cajon, CA)	MV Fwd	CCCTGAGGGATTC AACATGATTCT	In-house
			MV Rvs	ATCCACCTTCTTA GCTCCGAATC	
			MV Probe	FAM/TCTTGCTC GCAAAGGCGG TTACGG/BHQ_1	
10	Adenovirus	Not done	ADV Fwd	GGACGCCTCG GAGTACCTGAG	In-house
			ADV Rvs	GTGGGGTTTCTGAACTTGTT	
			ADV Probe	FAM/CTGGTGCAGTTCGCCC GTGCCA/3BHQ_1	

Table 1: List of viruses tested and the test modality used for diagnosing each virus

Detailed history was taken from the cases and controls according to the predesigned questionnaire. The patients were followed up at 3, 6 and 12 months telephonically to know their outcome.

Odd's Ratio (OR) and 95% Confidence intervals (95% CI) were calculated to know the possible association between total virus positivity and single virus positivity with aplastic anemia. All statistical analysis was done using SPSS software version 21 (IBN Corporation, N.Y., USA). Mean and standard deviation was calculated for age. Statistical analysis for non-parametric variables among cases and controls were tested by Chi square test and Fischer's exact test. For continuous variables, Mann Whitney U test was used. 95% Confidence intervals (95% CI) and Odd's Ratio (OR) was calculated among cases and controls to know the possible association between a variable and an outcome.

Results

Total 68 cases and 34 controls were included in the study (Ratio 2:1). The age range of cases was 1-46 years (median = 12.5 years) and of controls was 4-45 years (median = 13 years). Amongst the cases, 39 (57.4%) were of pediatric age group and 29(42.6%) were of adult age group. Amongst the controls, 19 (55.9%) were of pediatric age group and 15 (44.2%) were of adult age group. The ratio of children to adults was 1.3:1 in both cases and controls. The Male: Female ratio of cases and controls were 1.72:1 and 1.83:1 respectively. Hence it can be seen that the cases and controls were age and sex matched.

According to the Camitta classification, of the 68 cases, 42 patients were classified as Severe Aplastic Anemia, 6 patients were classified as Very Severe Aplastic Anemia, 20 patients were classified as Moderate Severe Aplastic Anemia.

Fever was observed in most number of patients (54, 79.41%) followed by pallor (50, 73.52%), nausea (42, 61.76%), fatigue (41, 60.29%) and rash (38, 55.88%). Breathlessness and neurological features were not found in any case and diarrhea was seen in only one patient. History of blood transfusion was documented in 27 (39.70%) cases.

The absolute cell count of all the three lineages of cells was significantly lower in cases than controls (Red blood cell count: p-value = <0.00001, total leucocyte count: 0.0005, platelet count: <0.0001). Of the total RBCs visible, the values of MCV, MCH and MCHC were significantly higher in controls than cases.

Of the 68 cases, 61 (89.70%) were positive for markers of at-least one of the 10 viruses included in the study. Only 7 (10.29%) cases did not show any evidence of positivity for viruses under study. In contrast, of the 34 controls only 12 (38.23%) individuals were positive for at-least one virus under study and 22 (61.76%) did not show any evidence of the virus positivity. Therefore, the overall virus positivity was significantly higher amongst cases as compared to controls (OR = 14.07, 95% CI = 4.95 to 39.99).

Positivity of B19V was seen in highest number of cases followed by CMV, EBV, HBV, VZV and AdV. MV, HCV, HAV and HEV each were positive in less than 10% cases. In contrast, in the control population, positivity was highest for HAV followed by VZV and AdV. Viruses such as EBV, CMV, HCV, HEV, MV each were positive in only 1 control. It is important to note that none of the controls were positive for B19V or HBV. The total positivity included mono positives (positive for that virus only) and co-positives (positive for that particular virus along with some other virus). On statistical analysis of total positives, it was found that B19V, EBV, and CMV were found to be significantly associated with Aplastic anemia (Table 2).

Amongst the 61 cases positive for viral markers, 33 (48.5%) patients were positive for only 1 virus and 28 (41.1%) patients showed co-positivity for two or more viruses. Of these 28 patients, 16 (23.5%) patients showed co-positivity for two viruses and 12 (17.6%) patients were positive for three viruses (Table 2). Co-positivity of B19V occurred along with all the other viruses in the study and was seen in 18 patients. EBV was co-positive with B19V, HBV, ADV, CMV, VZV and HEV. Co-positivity of CMV was seen with B19V, EBV, VZV, HAV, HBV and ADV. In four cases, positivity of VZV was seen along with HCV, HBV, MV or ADV but none

of the viruses with significant association with aplastic anemia i.e. B19V, EBV and CMV tested positive. HAV, HEV and Measles virus were seen only as co-infections and did not occur individually. In contrast, no co-positivity was seen among controls testing positive.

From Table 2, it can also be seen that when mono-positives were only considered, HBV was also found to have statistically significant association with aplastic anemia.

Virus under study	Total positives			Co positives	Monopositivity		
	Cases positive (%)	Controls positive (%)	Statistical significance: OR (95% CI, p-value)		Cases positive (%)	Controls positive (%)	Statistical significance: OR (95% CI, p-value)
B19V	31 (45.58)	0	57.96 (3.41 to 983.90, 0.005)	18	13	0	124.20 (6.624 to 2328.52: 0.0013)
EBV	14 (20.58)	1 (2.94)	8.55 (1.07 to 68.10, 0.04)	10	4	1	18.85 (1.81 to 195.40: 0.01)
CMV	15 (22.05)	1 (2.94)	9.33 (1.17 to 74.04, 0.03)	11	4	1	18.85 (1.81 to 195.40: 0.01)
HBV	10 (14.70)	0	12.38 (0.08 to 218.01, 0.08)	3	7	0	69.00 (3.5 to 1344.50: 0.005)
HCV	4 (5.88)	1 (2.94)	2.06 (0.22 to 19.20, 0.52)	3	2	1	9.42 (0.74 to 118.98: 40.08)
HAV	2 (2.94)	3 (8.82)	0.31 (0.04 to 1.97, 0.21)	2	0	3	0.60 (0.02 to 12.90: 0.74)
HEV	2 (2.94)	1 (2.94)	1.00 (0.08 to 11.43, 1.00)	4	0	1	1.488 (0.05 to 40.25: 0.81)
VZV	9 (13.23)	2 (5.88)	2.44 (0.49 to 11.98, 0.27)	9	2	2	4.57 (0.54 to 38.22: 0.16)
ADV	7 (10.29)	2 (5.88)	1.83 (0.36 to 9.35, 0.46)	6	1	1	4.71 (0.26 to 84.77: 0.29)
MV	6 (8.82)	1 (2.94)	3.19 (0.36 to 27.65, 0.29)	6	0	2	0.866 (0.03 to 19.99: 0.92)

Table 2: Virus positivity in cases and controls and their association with aplastic anemia

The maximum number of cases positive for B19V, CMV, VZV, EBV, HCV and HEV were positive only by ELISA and only few were positive for PCR or both the tests. On the contrary for measles virus and HBV, maximum cases were positive by PCR or both ELISA and PCR but only few were positive for only ELISA (Table 3).

Virus	Cases				Controls			
	Positives (%)	Only positive by ELISA	Only positive by PCR	Positive by both ELISA and PCR	**Positives (%)**	Only positive by ELISA	Only positive by PCR	Positive by both ELISA and PCR
B19V	**31 (45.5)**	24	3	4	**0**	0	0	0
EBV	**14 (20.5)**	9	0	5	**1 (2.9)**	1	0	0
CMV	**15 (22.0)**	10	1	4	**1 (2.9)**	1	0	0
HBV	**10 (14.70)**	1*	0	9	**0**	0	0	0
HCV	**5 (7.35)**	4	0	1	**1 (2.9)**	1	0	0
HAV	**2 (2.94)**	0	0	2	**3 (8.8)**	3	0	0
HEV	**4 (5.88)**	3	0	1	**1 (2.9)**	0	1	0
VZV	**11 (16.17)**	7	3	1	**2 (5.8)**	2	0	0
ADV	**7 (10.29)**	ND	7	ND	**2 (5.8)**	ND	2	ND
MV	**6 (8.8)**	1	3	2	**1 (2.9)**	1	0	0

Table 3: Virus positivity by different techniques (ELISA and/or PCR) in cases and controls

*ELISA was done to detect IgM antibodies of B19V, EBV, CMV, HCV, HAV, HEV, VZV and MV except HBV where antigen was detected

The patients were followed up telephonically at 3 months, 6 months and 12 months and outcome was noted. Of the total 68 cases, mortality occurred in 5 and remaining 63 was alive and still on treatment for aplastic anemia. The time duration between mortality and date of diagnosis ranged from 16 days to 6 months. Of the deceased cases, only B19V was detected in two cases, B19V along with HAV was detected in one case and no virus was detected in the other two cases. Mortality was not significantly associated with B19V positivity (OR= 0.53, 95% CI = 0.08-3.41).

Discussion

On the basis of gross blood picture, complete blood count and bone marrow analysis, the clinician made the diagnosis of aplastic anemia. A detailed history of patients was taken to know the possible cause of aplastic Anemia. Once all these causes were excluded, the patients were enrolled in the study to find the viral association. Cases and control were enrolled in a ratio of 2:1. IDA cases were selected as controls to avoid selection bias and to minimize ethical issues.

The age range of cases was 1-46 years and of controls was 4-45 years. Some studies have been done only on children [7,8] and some only on adults [9,10]. Since, in this study, focus was given on acquired aplastic anemia, it was important to enroll patients of all age groups.

In this study, the Male: Female ratio of cases and controls were 1.72:1 and 1.83:1 respectively. The male: female ratio of aplastic anemia cases in various studies were 2.2:1 [11], 2.78:1 [12]. From most of the studies documented in literature, it can be seen that males outnumbered females which may be due to hospital based nature of all these studies.

The modified Camitta criteria are used to assess severity of Aplastic Anemia: Severe AA (SAA): Marrow cellularity of <25%, with at least 2 of the following: (i) neutrophil count of <500/μL, (ii) platelets count of <20.000/μL, (iii) reticulocyte count of <20.000/μL. Very Severe AA (VSAA): Criteria is same as that for SAA but neutrophils count of <200/μL [5,8,10].

As per the current study, fever (79.41%) and pallor (73.52%) were the most common clinical symptoms followed by nausea (61.76%) and fatigue (60.29%). However, pallor was the most common clinical manifestation seen in aplastic anemia patients followed by bleeding and fever in previous studies [10,11,13]. Presence of fever in most cases may point towards an infectious etiology of aplastic anemia.

In the present study 10 viruses were selected for understanding their association with aplastic anemia. To our knowledge, there are only few studies on Human parvovirus B19 [9,10,14] and a single study on Epstein Barr virus [7] showing association with aplastic anemia. For the remaining viruses such as cytomegalovirus [15], Hepatitis A virus [16], Hepatitis B virus [7,16], Hepatitis C virus [17], Hepatitis E virus [17], Varicella Zoster virus [18,19], Adenovirus [20] and measles virus [21] only case reports exist. Since we could not find any study that has systematically observed the association of all these viruses with aplastic anemia but were reported in the literature as possible causes, these viruses were included in the study wherein 61 (89.70%) of the 68 cases and 12 (38.23%) of the 34 controls were positive for markers of at-least one virus. Previously, in a study done to find the risk factors and outcome of aplastic anemia in 32 children, 9 (28.12%) were found to have a viral infection preceding the onset of aplastic anemia (HBV, VZV, B19V, EBV, HAV) [16]. Likewise, in an epidemiological study on 185 children aged <15 years with aplastic anemia, it was found that B19 virus, EBV and hepatitis virus were positive in 25.8%, 20% and 6.7% cases respectively [7].

B19V has been described in literature as a possible cause of aplastic anemia. Many case reports show that B19V can cause aplastic anemia in previously healthy individuals [22,23]. Few descriptive studies have also been done on the role of B19V in aplastic anemia (Table 4) wherein its positivity ranged from 20% to 66.7% of the study population. This is in concordance with the present study where B19V was present in maximum number of cases (45.5%) of which 41.93% was positive for only B19V. In the present study, among the positive cases only IgM positivity was seen in higher number of patients. Previous studies have obtained varying results with some predominantly positive for DNA and others for IgM antibodies (Table 4). The observed difference may be due to the different stages at which patients were enrolled in the study. Hence it becomes imperative to test a patient of aplastic anemia for both IgM and DNA otherwise the diagnosis may be missed. It is important to diagnose B19V among such patients as administration of pooled Intravenous Immunoglobulins (IVIG) along with the standard treatment for aplastic anemia (prednisolone and acyclovir) has been shown to give better results [24,25].

Sl.No	Authors	Journal	Place of study	Year of publi shing	Period of study	Age group	Total no. of patients enrolled	Total number of patients positive for Parvovirus B19 (%)	Patients positive by ELISA only (%)	Patients positive by PCR only (%)	Patients positive by both ELISA and PCR (%)	References
1	Qian XH et al	Archives of disease in childhood	China	##	1.5 years	1-14 years	30	6 (20)	-	2 (33.3)	4 (66.7)	[14]
2	Mishra B et al	American Journal of Hematology	Chan digarh, India	##	1 year	13-70 years	27	18 (66.7)	8 (44.4)	7 (38.9)	3 (16.7)	[9]
3	Gupta V et al	Indian Journal of pediatrics	Uttar Pradesh	##	5 years	<15 years	185	48 (25.8)	48 (25.8)	-	-	[7]
4	Gupta V et al	Indian Journal of pediatrics	Uttar Pradesh	##	2 years	4-14 years	66	18 (27.3)	-	1 (5.5)	17 (94.4)	[13]
5	Tizeba YA et al	Journal of tropical Pediatrics	Tanzania	##	5 months	6-59 months	265	70 (26.4)	24 (34.2)	-	-	[42]
6	Kalita BC et al	Paipex-Indian Journal of Research	Assam, India	##	1 year	15-47 years	22	9 (40.9)	5 (55.5)	4 (44.4)	-	[10]
	Present study		Uttar Pradesh	##	1 year	1 month-60 years	68	31 (45.6)	24 (77.4)	3 (9.6)	4 (12.9)	

Table 4: Comparison of present study with previous studies showing association of B19V with aplastic anemiad MV except HBV where antigen was detected

In the current study, EBV was found to be significantly higher among cases as compared to controls thereby proving its association with aplastic anemia. Among the cases, only anti-EBV VCA IgM was positive in 64.28% cases pointing towards acute stage of the disease (table 2 and 3). To the best of our knowledge, only few studies have been done till date showing the association of EBV with aplastic anemia [7]. Few case reports have been documented showing EBV infection as a cause of aplastic anemia [26], or as an effect of treatment of aplastic anemia [27].

In the current study, CMV was significantly higher among cases as compared to controls. Of the CMV positives, 66.7% cases were positive for CMV IgM only and 26.7% cases were positive for both CMV IgM and CMV DNA. It is known that CMV IgM is detectable from first week of infection to four months and DNA can be detected in blood even before manifestation of symptoms up to four months of infection. These evidences prove the association of CMV infection with aplastic anemia. Though CMV is often listed as a cause of aplastic anemia, to the best of our knowledge, there are only few case reports documented in literature that show this association [28,29] of the hepatitis viruses, only HBV was found to be significantly associated with aplastic anemia when mono-positives for HBV was considered. Studies show that prevalence of Hepatitis associated aplastic anemia ranges from 5-9% [30,31] and up to 33% in people who underwent liver transplantation for fulminant hepatitis [32]. Some patients of AA have also been noticed to be positive for HAV or HCV, but causality between such infections and AA could not be established [16,33]. In contrast, studies also exist that refute the hypothesis that hepatitis is a direct cause of aplastic anemia, rather they suggest that infection with other viruses [17] may be the cause of aplastic anemia in patients with hepatitis. This is in concordance with the present study as co-infection of hepatitis virus infection was seen with other viruses. However, in any case scenario, it becomes empirical to know the status of hepatitis viruses in cases of aplastic anemia since, as treatment with Anti-thymocyte globulin (ATG) has been found to be more effective than prednisolone [34].

In the current study, Measles virus, Adenovirus and VZV were not found to be significantly associated with aplastic anemia. Though these viruses have previously been thought to be linked with acquired aplastic anemia [28], there is paucity of literature proving the association. VZV has however been reported as a cause of aplastic anemia shortly after its infection [16,19] or following varicella vaccination [35]. Conversely, measles virus and adenovirus infections have been reported in patients of aplastic anemia following bone marrow transplantation (BMT) [21,36], proving that they are the consequence of treatment of aplastic anemia rather than the cause. In this study, three cases were PCR-Positive-IgM-negative for Measles virus. Similar findings were found in a study where 27 of the 165 patients were PCR-Positive-IgM-negative for Measles virus. This may be due to early sampling in the cases studied when viremia is present, but IgM antibodies have yet not developed. This may also be due to reinfection after natural immunity or prior vaccination failures when IgM may not develop [37]. In such patient's serological confirmation in a convalescent-phase serum for IgG must be done to rule out reinfection.

The case fatality rate in our study was 7.3% of which three patients were infected with a virus and two were not found to have any viral infection. Therefore, mortality did not correlate with presence of any viral infection. Similarly, in a previous study of the 30 patients tested for parvovirus, 2 (6.7%) patients who did not have any viral infection died due to hemorrhage [14].

It can be speculated that though all the viruses in the present study did not show association with aplastic anemia it is essential to test such patients for these viruses so that management protocol may be patient tailored, which may impact the outcome. The limitation of this study was that the patients were followed up only telephonically and their samples could not be collected for follow-up. Therefore, the natural history of disease in these patients could not be studied. Also, immunoassay for ADV could not be done, so it's IgM and PCR results could not be correlated.

Therefore, to conclude B19V, EBV, CMV and HBV were found to be significantly associated with aplastic anemia but not HAV, HEV, HCV, Measles virus, Adenovirus and VZV. Mortality was not found to be significantly associated with any viral infection in cases with aplastic anemia.

Author statements

All authors reviewed and approved the final version. AJ helped in conceptualizing and planning PJ and AJ consequently contributed to drafting and editing. GV contributed to methodology and result of scientific work. HM helped GV in performing real-time PCR. SPV, RM and NV helped in procuring the samples.

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Conflict of interest

None

Ethical Approval

Ethical approval was obtained from the Institutional Ethics Committee (Ref. code: 101st ECM II B-Thesis/P32).

Consent for Publication

Written informed consent was obtained from cases and controls or their parents/ guardians in case the study subject is a minor.

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