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Original Article

Microbiology Section

Seroprevalence of Human Parvovirus B19 in Haematological and Extra-haematological Disorders: A Retrospective Observational Study

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ABSTRACT

Introduction: Human parvovirus B19 (B19V) has an affinity for multiple organs and causes a myriad of clinical diseases depending on the host's immunological and haematological status. The seroprevalence of human parvovirus B19 has mostly been studied in haematological disorders, but there is still a lack of data on B19V seroprevalence in extra-haematological disorders.

Aim: To study the seroprevalence of B19V in haematological and extra-haematological disorders.

Materials and Methods: This retrospective observational study was conducted at the Microbiology Laboratory of Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, Uttar Pradesh, India. Data was collected from September 2017 to September 2020, and data analysis was done from October 2020 to January 2021. A total of 702 serum samples from patients suspected of B19V infection were received over a three-year duration for parvovirus B19 antibody testing. Of these, 674 serum samples were included in the study as per the inclusion and exclusion criteria. The prevalence of B19V antibodies in different clinical disorders was investigated by collecting patient details like age, gender, underlying clinical disorder, and B19V-specific Immunoglobulin M (IgM) and IgG antibodies detected by quantitative enzyme immunoassay on all serum samples suspected of B19V infection using Statistical Package for Social Sciences (SPSS) version 25.0 software. The Chi-square test was used to analyse statistically significant variables.

Results: B19V-specific IgM and IgG antibodies were detected in 35.7% (241/674) of the serum samples received over a three-year duration. The positivity rate was 94 (13.9%) for IgG, 108 (16%) for IgM, and 39 (5.8%) for both IgG and IgM. The positivity in adults aged 18 years and over (39.6% or 160/404) was statistically significantly higher compared to children aged 17 years and younger (30% or 81/270) (p=0.0109). Among the 241 B19V-positive patients, 126 (52.3%) had haematological disorders, and 115 (47.7%) had extra-haematological disorders. The total positivity of IgG plus IgM antibodies was highest in musculoskeletal and connective tissue disorders (33 (54.1%) and haematological disorders 126 (48.3%).

Conclusion: The B19V seroprevalence was relatively low in the present study compared to most serological studies conducted in other regions. The present study provides information on the seroprevalence of B19V in both haematological and extra-haematological disorders simultaneously.

Keywords: Coronavirus disease-2019, Enzyme immunoassays, Immunoglobulins

INTRODUCTION

Parvovirus B19 (B19V) belongs to the genus Erythrovirus in the family Parvoviridae and is a small, non enveloped, icosahedral, single-stranded Deoxyribonucleic Acid (ssDNA) virus that infects only humans [1]. B19V infection is common worldwide and shows a wide array of clinical manifestations affecting different body systems. Erythema infectiosum, haematological disorders, chronic arthritis, spontaneous abortion, myocarditis, encephalitis, and glomerulonephritis are just some of these [2-4]. B19V spreads from person to person through infected respiratory secretions, infected blood and blood-product transfusions, and vertical transmission from mother to foetus [5,6].

About 10-12 days after exposure to the virus, B19-specific IgM antibodies appear and can be detected in serum for 3-6 months. IgG antibodies appear after approximately two weeks and persist for life [7]. The prevalence of B19-specific IgG antibodies varies worldwide, ranging from 2% to 15% in children aged 1-5 years and from 30% to 60% in adults [8].

Since the discovery of B19V, several studies have been conducted to clarify its relationship with various diseases. Although B19V is recognised as a pathogen associated with many haematological and extra-haematological disorders, there is no comprehensive study on its seroprevalence in both haematological and extra-haematological disorders together from India and around the world. Thus, to fill this gap in the literature, the present study evaluated the seroprevalence of B19 antibodies in both haematological and extra-haematological disorders.

MATERIALS AND METHODS

This was a retrospective observational study conducted at the microbiology laboratory of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India. Data were collected from medical records of the previous three consecutive years, from September 2017 to September 2020, and data analysis was carried out from October 2020 to January 2021. The study protocol was approved by the Institutional Ethics Committee (IEC Code: PGI/BE/1303/2020), and individual consent was not obtained from each case as this was a retrospective study and the tests were performed as part of routine procedures in the microbiology laboratory.

Patient details of all consecutive non duplicate serum samples suspected of B19V infection sent to the microbiology laboratory of this hospital during the study period were included in the study.

Inclusion criteria: Data on demographic information, clinical details, and serological analysis of all consecutive patient serum samples suspected of B19V infection sent to the microbiology laboratory of this hospital during the study period were collected retrospectively and included in the study.

Exclusion criteria: Patients whose antibody results did not cover the planned years were excluded from the study. Duplicate or erroneous reports were also excluded.

Study Procedure

A total of 702 serum samples from patients suspected of B19V infection were received over a three-year duration for Parvovirus B19 antibody testing, of which 674 serum samples were included in the study according to the aforementioned inclusion and exclusion criteria. Febrile patients (both outpatient department and inpatient department) presenting after February 2020 were also tested for Coronavirus Disease-2019 (COVID-19).

Demographic and clinical details, as well as B19V-specific IgM and IgG antibody status of all patients, were recorded on a predesigned proforma.

Serological analysis: B19V-specific IgM and IgG antibodies in serum samples were determined using Parvovirus B19 quantitative IgM and IgG Enzyme-linked Immuno Sorbent Assay (ELISA) kits (DRG Diagnostics, Germany). Serum was diluted one in 100, and ELISA tests were performed according to the manufacturer's instructions using a substrate blank, a negative control, a positive control, and two cut-off controls. All serum samples were tested in duplicate. Absorbance values (Optical Density; OD) were read against the blank at 450 nm in an ELISA reader (Tecan Austria GmbH, Austria; model Sunrise). Interpretation of the results was done according to the manufacturer's instructions [9].

Molecular testing for Severe Acute Respiratory Syndrome-Coronovirus-2 (SARS-CoV-2): Two swabs, oropharyngeal and deep-nasal, were collected from the suspected patients and transported in Viral Transport Media (VTM). Viral Ribonucleic Acid (RNA) extraction was performed using the Qiagen Viral RNA kit (QIAamp, USA) according to the manufacturer's instructions. Real-time Polymerase Chain Reaction (RT-PCR) was conducted using the DiagSure TM nCoV-19 detection assay (Multiplex, TaqMan-based) kit manufactured by GCC BIOTECH [10].

STATISTICAL ANALYSIS

The data were analysed using the Statistical Package for the Social Sciences, version 25.0 software (SPSS Inc., Chicago, IL, USA). The significance among percentages was calculated using the Chi-square test, and a p-value of <0.05 was considered statistically significant.

RESULTS

B19 serology according to patient characteristics: A total of 674 serum samples from patients suspected of B19V infection were received over a three-year duration for Parvovirus B19 antibody testing. The results of B19V serology in 674 patients, categorised by sex and age groups, are summarised in [Table/Fig-1]. B19V antibodies were detected in 241 out of 674 patients (35.7%). Ninety-four patients (13.9%) had only IgG antibodies, 108 (16%) had only IgM antibodies, and 39 (5.8%) had both types of antibodies. When patients positive for both IgG and IgM antibodies were included, the seropositivity rate increased to 19.7% for IgG and 21.8% for IgM. IgG and/or IgM antibodies were positive in 110 out of 349 females (31.5%) and in 131 out of 325 males (40.3%). The seroprevalence among males was significantly higher than in females (p=0.0173). The prevalence of IgG antibodies showed a tendency to increase after the age of five years. When the positivity of this antibody in adults aged 18 and over (160/404, 39.6%) was compared with the positivity in children aged 17 and younger (81/270, 30%), the results were statistically significant (p=0.0109).

Distribution of haematological and extra-haematological disorders in B19 positive patients: Out of 241 B19V positive patients, 126 (52.3%) had haematological disorders, and 115 (47.7%) had extra-haematological disorders. The most common haematological disorders were aplastic anaemia (33, 26.2%) and pure red cell aplasia (25, 19.8%). The distribution of all haematological disorders in B19V positive patients is depicted in [Table/Fig-2].

Variables	Total tested n*(%)	IgG (+) n*(%)	IgM (+) n*(%)	IgM (+) IgG (+) n*(%)	Total seropositive n*(%)	p-value
Gender						
Male	325 (48.2%)	55 (16.9%)	56 (17.2%)	20 (6.2%)	131 (40.3%)	**0.0173**
Female	349 (51.8%)	39 (11.2%)	52 (14.9%)	19 (5.4%)	110 (31.5%)	
Age groups						
<5 years	88 (13%)	4 (4.5%)	12 (13.7%)	4 (4.5%)	20 (22.7%)	**0.0109**
5-17 years	182 (27%)	16 (8.8%)	37 (20.3%)	8 (4.4%)	61 (33.5%)	
18-30 years	157 (23.3%)	24 (15.3%)	27 (17.2%)	10 (6.3%)	61 (38.8%)	
31-50 years	150 (22.3%)	30 (20%)	18 (12%)	10 (6.7%)	58 (38.7%)	
>50 years	97 (14.4%)	20 (20.7%)	14 (14.4%)	7 (7.2%)	41 (42.3%)	

[Table/Fig-1]: Results of B19 serology in 674 patients according to sex and age. Data presented as n*(%) of patients; †Chi-square test was used; p<0.05 is significant

Haematological disorders	Number of patients n*(%)
Aplastic anaemia	33 (26.2%)
Pure red cell aplasia	25 (19.8%)
Paroxysmal nocturnal haemoglobinuria	15 (11.9%)
Acute lymphoblastic leukaemia	11 (8.7%)
Acute myeloid leukaemia	10 (7.9%)
Thalassemia major	9 (7.2%)
Hodgkin's lymphoma	7 (5.5%)
Myelodysplastic syndrome	3 (2.4%)
Autoimmune haemolytic anaemia	3 (2.4%)
Idiopathic thrombocytopenic purpura	3 (2.4%)
Sideroblastic anaemia	2 (1.6%)
Amegakaryocytic thrombocytopenia	2 (1.6%)
Haemophagocytic lymphohistiocytosis	2 (1.6%)
Hereditary spherocytosis	1 (0.8%)
Total number of patients	126 (100%)

[Table/Fig-2]: Distribution of haematological disorders of B19V positive patients. Data presented as n*(%) of patients

Among the extra-haematological disorders, the most common were musculoskeletal and connective tissue disorders (33, 28.6%), liver disorders (21, 18.3%), neurological disorders (18, 15.6%), and renal disorders (16, 13.9%), followed by others as shown in [Table/Fig-3].

B19 serology in haematological and extra-haematological disorders in B19V positive patients: The total positivity of IgG plus IgM antibodies was highest in musculoskeletal and connective tissue disorders (33/61, 54.1%) and haematological disorders (126/261, 48.3%). Neurological disorders, liver disorders, and renal disorders had a total positivity of 18/62 (29%), 21/82 (25.6%), and 16/85 (18.8%), respectively. The results of B19 serology in various clinical disorders in B19V positive patients are shown in [Table/Fig-4].

During the COVID-19 pandemic in the year 2020, the authors also analysed the samples received after February 2020 for COVID-19 infection in febrile patients. Out of 176 samples received during this defined time period, only seven samples (3.9%) were positive for COVID-19 by RT-PCR. Interestingly, one case infected with COVID-19 was also seropositive for B19V.

Extra-haematological disorders (*n=115)			
Musculoskeletal and connective tissue disorders	**n=33 (28.6%)**	**Neoplasms**	**n=6 (5.2%)**
Rheumatoid arthritis	10 (8.7%)	Breast carcinoma	2 (1.7%)
Arthropathy	16 (13.9%)	Colon carcinoma	1 (0.9%)
Systemic lupus erythromatosis	5 (4.3%)	Thyroid carcinoma	1 (0.9%)
Myositis	2 (1.7%)	Hepatoblastoma	1 (0.9%)
Liver disorder	**n=21 (18.3%)**	Gallbladder carcinoma	1 (0.9%)
Acute viral hepatitis	8 (7%)	Metabolic disorders	n=4 (3.5%)
Autoimmune hepatitis	3 (2.6%)	Wilson disease	3 (2.6%)
Chronic liver disease	10 (8.7%)	Glycogen storage disease	1 (0.9%)
Neurological disorders	**n=18 (15.6%)**	**Infections**	**n=4 (3.5%)**
Acute encephalitis	3 (2.6%)	Malaria	1 (0.9%)
Multiple sclerosis	2 (1.7%)	Tuberculosis	2 (1.7%)
Mononeuropathy multiplex	1 (0.9%)	Enteric fever	1 (0.9%)
Myelitis	3 (2.6%)	**Others**	**n=13 (11.3%)**
Optic neuritis	2 (1.7%)	Hydrops foetalis	2 (1.7%)
Guillain-Barré syndrome	3 (2.6%)	Congenital heart disease	1 (0.9%)
Cerebellar ataxia	1 (0.9%)	Pyrexia of unknown origin	2 (1.7%)
Seizures	2 (1.7%)	Polyposis coli	1 (0.9%)
Stroke	1 (0.9%)	Graves disease	1 (0.9%)
Renal disorder	**n=16 (13.9%)**	Mucocele of gall bladder	1 (0.9%)
Chronic Kidney Disease (CKD) on dialysis	7 (6 %)	Acute tonsillitis	1 (0.9%)
Postrenal transplant	6 (5.2%)	Erythema infectiosum	2 (1.7%)
Endocapillary proliferative GN	1 (0.9%)	Myocarditis	2 (1.7%)
Focal segmental GN	1 (0.9%)	**Where *n=number of patients**	
Membranoproliferative GN	1 (0.9%)		

[Table/Fig-3]: Distribution of extra-haematological disorders of B19V positive patients.

Clinical disorders	Total patient sample tested	IgG (+)	IgM only/ or together with IgG (+)	Total seropositive patients
Haematological disorders	261	47 (18%)	79 (30.3%)	126 (48.3%)
Musculoskeletal and connective tissue disorders	61	15 (24.6%)	18 (29.5%)	33 (54.1%)
Liver disorders	82	3 (3.6%)	18 (22%)	21 (25.6%)
Neurological disorders	62	8 (12.9%)	10 (16.1%)	18 (29%)
Renal disorders	85	6 (7 %)	10 (11.8%)	16 (18.8%)
Neoplasms	23	4 (17.4%)	2 (8.7%)	6 (26.1%)
Infections	13	2 (15.4%)	2 (15.4%)	4 (30.8%)
Metabolic disorders	16	2 (12.5%)	2 (12.5%)	4 (25%)
Others	71	7 (9.8%)	6 (8.4%)	13 (18.3%)
Total	674	94	147	241

[Table/Fig-4]: Results of B19 serology in clinical disorders in B19 positive patients. Data presented as number (%) of patients

DISCUSSION

The present novel study from India aims to determine the prevalence of B19V antibodies in both haematological and extra-haematological disorders. Detection of B19 IgM and IgG antibodies in a serum sample can provide valuable information about the course of parvoviral diseases. A positive IgM result, with or without IgG antibodies, indicates an acute infection, while a positive IgG result in the absence of IgM antibodies suggests a past B19 infection [11].

The total seroprevalence of B19V in the present study was found to be 35.7%, with a seropositivity of 19.7% for IgG and 21.8% for IgM. There are no population-based seroprevalence studies on parvovirus B19 from India, although studies conducted among blood donors and hospital-based studies have been reported. Kishore J et al., reported a B19V seroprevalence of 39.9% among blood donors [12], while Kumar S et al., found a seroprevalence of 27.9% [13]. A hospital-based study by Abraham et al., from South India reported a high B19V seroprevalence of 50% [14]. The lower seroprevalence of IgG antibodies in the present study may be attributed to differences in patient numbers, health status, sex, and age. The high seroprevalence of B19V IgM in the patients of the present study indicates recent infections, suggesting that their underlying clinical conditions made them more susceptible to B19V infection. However, to prove this hypothesis, a case-control study would need to be conducted.

In the present study, the seroprevalence of B19V among males was significantly higher than in females (p=0.0173), which is consistent with a previous study conducted at the same centre [12]. Age has consistently been shown to be a major predictor of antiparvovirus B19 IgG seropositivity [15-17]. The present study also demonstrates that IgG seropositivity significantly increases with age, with higher seropositivity observed in adults aged 18 years and over compared to children aged 17 years and younger (p=0.0109).

The present study demonstrates that B19V infection is associated with both haematological and extra-haematological disorders, which is consistent with earlier literature [7,18]. This study further supports the expanding clinical spectrum of B19V infection. The highest positivity for B19 antibodies was observed in diseases of the musculoskeletal and connective tissues (54.1%) and haematological disorders (48.3%). However, the seropositivity of parvoviral antibodies can vary considerably in the same or different diseases within the human body system.

In a study by Albayrak HT et al., from Turkey, the positivity rates of parvovirus B19 IgG and IgM in patients with a pre diagnosis of arthritis/arthralgia were 65.6% and 3.3%, respectively [19]. Türk Dağı H et al., detected B19 IgG in 85 out of 114 patients with rheumatoid arthritis and 29 out of 46 healthy individuals, and they reported that the difference in B19 IgG frequencies between these groups was not statistically significant [20]. Zaki MES et al., found that the incidence of B19 infection is significantly higher among children with haematological disorders, including haemolytic anaemias, lymphomas, and leukaemias undergoing chemotherapy [21]. The seroprevalence of B19V antibodies varies depending on the types of haematological disorders and the study population recruited.

In a study conducted by Jain P et al., in India, they enrolled 238 patients (103 with leukaemia, 77 with aplastic anaemia, and 58 with chronic haematological disorders) and found a positivity of anti-B19V IgM and anti-B19V IgG in 16 (6.7%) and 127 (53.4%) patients, respectively [22]. In another study from India, Kishore J et al., found that the prevalence of B19 IgG antibodies was 34.3% in children with hematologic malignancies such as leukemia and lymphoma [23].

In a multicenter study conducted by Alves ADR et al., B19V DNA was detected in 65% (145/221) of Chronic Kidney Disease (CKD) patients, which was significantly higher (p<0.001) than in blood donors (6.3%) [24]. Detection of B19V IgG and viremia was seen in 40.3% of CKD patients, indicating the presence of persistent B19V infection. CKD patients showed an increased risk of developing B19V infection (OR=28.1, CI=13.5-58.5, p=0.001).

Parvovirus B19-associated hepatitis and aplastic anaemia and its co-infection with other hepatotropic viruses, are underrecognised. There is sufficient evidence suggesting that B19 infections can cause

a spectrum of liver diseases, ranging from elevated transaminases to acute hepatitis, fulminant liver failure, and even chronic hepatitis. According to a study by Mihály I et al., parvovirus B19-related hepatitis may occur in 4.1% of patients infected with this virus [25]. The spectrum of liver diseases has been reported in all age groups, from neonates to the elderly.

The present study also aimed to assess the presence of COVID-19 in B19V seropositive patients, as the COVID-19 pandemic has affected millions of people worldwide and has proven to be more dangerous than MERS and Severe Acute Respiratory Syndrome (SARS) coronaviruses. The most common symptoms observed in COVID-19 patients were respiratory symptoms such as cough, sputum, shortness of breath, and fever, as well as musculoskeletal symptoms including myalgia, joint pain, headache, and fatigue, and enteric symptoms like abdominal pain, vomiting, and diarrhoea. These findings are consistent with previous studies [26,27].

The positivity rate of COVID-19 in the study was 3.9%, with one patient presenting with unexplained anaemia receiving treatment for COVID-19 secondary to reactivation of parvovirus. This patient was also seropositive for B19V. This finding highlights the importance of assessing for parvovirus infections in COVID-19 patients with otherwise unexplained anaemia.

Limitation(s)

Since the present study is a single hospital-based study, the findings cannot be generalised to the entire population.

CONCLUSION(S)

The B19V seroprevalence was relatively low in the present study. However, there are limited studies in the literature on the seroepidemiology of B19 in relation to extra-haematological disorders, and most of these studies are case reports or focus on a single organ type. Therefore, authors believe that the current study contributes to the literature by providing information on the seroprevalence of B19 in both haematological and extra-haematological disorders, as well as its association with uncommon clinical disorders like B19V hepatitis associated aplastic anaemia and its co-infection with other hepatotropic viruses and renal disorders. B19V infection often goes unrecognised due to a lack of awareness about its association with numerous clinical manifestations, and this can lead to complications in high-risk groups such as pregnant women and patients with haematological disorders, neoplasms, chronic diseases, and immunodeficiency.

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