African Scientist Vol. 26, No. 1 March 31, 2025
Printed in Nigeria

1595-6881/2025 $80.00 + 0.00
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https://africansciientistjournal.org

afs2025011/26112

Human Parvovirus B19-Induced Anaemia among Febrile In-Patient Paediatrics in Benin Metropolis, Edo State, Nigeria

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(Received March 3, 2025; Accepted in revised form March 21, 2025)

ABSTRACT: Human Parvovirus B19 (B19V) is a highly contagious pathogen that causes amongst others transient aplastic anaemia in infected individuals. This study investigated febrile inpatient paediatrics for the presence of human parvovirus B19 infection. In this cross-sectional study, 150 consenting febrile paediatrics (90 males and 60 females) admitted into the paediatric hospital wards were recruited. The sera obtained from the participants were screened for B19V IgM antibodies. The model (forest plot) explained a 9% prevalence among the study participants. The results of the multivariate logistic regression analysis predicted seropositivity. For gender, (OR=1.74, 95% CI: 0.52–5.84, p=0.370) indicated no significant association between gender and seropositivity. The participants in the "Above 4" age group were 1.134 times more likely to be infected (OR: 1.13, 95% CI: 0.05–24.25, p = 0.936). At the kindergarten level, (OR=1.41, 95% CI: 0.07–30.29, p = 0.826), suggesting no significant impact on seropositivity. Severe anaemic status (OR=1.41, 95% CI: 0.07–30.29, p = 0.826), showing insignificant likelihood of seropositivity. Overall, all p-values exceeded 0.05, and CI for all OR included 1. This study's results highlight the importance of B19V as a potential causative agent of anaemia and help to understand the epidemiology of B19V among children.

Keywords: Anaemia, Febrile Paediatrics, B19 IgM antibodies, Risk factors, In-patients

Introduction

Parvovirus B19, being highly contagious, can easily spread in environments where children gather such as schools and day-care centres. Children have less-developed immune systems compared to adults, which makes them more susceptible to viral infections, including Parvovirus B19. Children also have more frequent physical contact and are less aware of personal hygiene habits. This raises the possibility of infection spreading through respiratory droplets or close contact with sick people (Lefrere *et al.*, 2005; Hunter and Ayala, 2021).

Infection with human Parvovirus B19 (also called B19 virus) causes severe complications especially in individuals with less developed immune systems such as children or in people with underlying medical conditions such as HIV/AIDS patients, cancer patients undergoing chemotherapy, thalassemia, pregnant women and people with autoimmune diseases. The virus infects the bone marrow and interferes with RBC production, leading to transient aplastic crisis, and chronic or recurrent anemia with reticulocytopenia (Heegaard and Brown, 2002; Landry, 2016). Thus, resulting in a drastic decline in RBC count and worsening of anaemic symptoms. A low packed cell volume (PCV) in febrile children is an indication of anaemia, which is a common complication of Parvovirus B19 infection. Children with underlying causes of anaemia are more likely to experience increased mortality and morbidity, which may be caused by parvovirus B19 infection (Wildig *et al*., 2006).

Seroprevalence studies typically employ serological tests such as Enzyme-Linked Immunosorbent assays (ELISA) to detect Parvovirus B19-specific antibodies in blood samples. These tests detect immunoglobulin M

(IgM) and immunoglobulin G (IgG) antibodies against Parvovirus B19. By conducting this study, researchers can gain a better understanding of the relationship between Parvovirus B19 infection and febrile illness in this specific population. This knowledge can contribute to improved diagnostic approaches, treatment strategies and preventive measures for managing anaemia associated with Parvovirus B19 infection.

Materials and methods

Study area: The study focused on febrile paediatrics who were admitted between May 2023 through August 2023 in the various paediatric hospital wards in a secondary health facility located within the Benin metropolis
Study design: A cross-sectional single-centre study was used to access study participants in a secondary health facility located within Benin Metropolis, Benin City, Edo State, Nigeria.
Ethical considerations: Ethical approval for this research was obtained from the Ethics and Research Committee, Ministry of Health, Edo State, Nigeria with reference number HA/737/23/D/071100142. Participants were adequately briefed on the research protocols, while informed consents were obtained from the parents of the participants before sample collection with adequate explanations of the possible risks that might be involved.
Study population: The participants were a combination of neonates and young children, between 6 months to 14 years of age and were admitted to the in-patient paediatrics hospital wards of the secondary health centre. Clinical information was obtained from the participants through the administration of prepared and validated questionnaires. Verbal and informed consent was obtained from each participant's parent/guardian before samples were collected.
Procedure for blood sample collection and analysis
ELISA assay: Approximately 4 millilitres of venous blood were aseptically collected into appropriately labelled sterile plain containers by venipuncture using disposable syringes and needles. Serum samples were obtained by centrifugation at room temperature at 3,000 revolutions per minute for 5 minutes. The sera obtained were appropriately labelled and stored at -20 °C until use. The sera were processed in the Molecular Diagnostic and Virology Laboratory, a unit of the Medical Microbiology Laboratory of the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria. Laboratory analysis was done using the Sunlong Biotech Human Parvovirus B19 ELISA test kit according to the manufacturer's instructions. A negative reaction indicates the absence of significant Human Parvovirus B19 IgM antibodies while a positive reaction indicates either an acute or current infection.
Full blood count (FBC) analysis: The full blood count of each participant was analyzed using a Sysmex KX-2IN haematology analyzer (Sysmex Cooperation, Japan) using whole blood specimens dispensed in EDTA containers. The Sysmex KX-2IN haematology auto-analyzer is an eighteen (18) analyzer in whole blood mode operation and eighteen (18) parameters in pre-dilute throughput rate of 60 samples per hour. All samples were placed on an electronic rotor before analysis. The samples were well mixed by gentle inversion before the test. The automated analysis was done following the manufacturer's operational guidelines. All samples were analyzed within 30 minutes of collection. Results were displayed on an LCD screen and printed on a thermal printer. Anaemia was defined using the WHO criteria as hacmoglobin concentration 1 3g/dl for males and 12g/dl for females
Statistical analysis: The data collected from laboratory experiments were organized into tables, processed and statistically assessed utilizing the IBM Statistical Package for Social Sciences (SPSS) version 28.0 for Windows. The Pearson Chi-square test, Odds ratio (O.R) with a confidence interval of 95 %, was employed to determine the associations between demographic data and prevalence rates. Values of $p < 0.05$ were adopted to ascertain statistical significance.

Results

Sociodemographic characteristics of participants: The study involved 150 participants, comprising 40.0 % females and 60.0 % males. The prevalence of seropositivity was 9 %, with 91 % testing negative, as shown in Figure 1. Regarding anaemic status, 45.3 % of participants had mild anaemia, 31.3 % were non-anaemic, and 23.3 % had severe anaemia. Educationally, 46.0 % attended kindergarten, 33.3 % primary school and 20.7 % secondary school. In terms of age distribution, 46.7 % were aged 0 – 4 years, 31.3 % were 5 – 9 years, and 22.0 % were 10 – 14 years. Clinically, 6.7 % had blood disorders, 8.0 % had cancer, 40.0 % had fever, 28.0 % had infectious diseases, 14.7 % were grouped into the "other" category and 2.7 % had respiratory issues (Table 1).

Table 1: Sociodemographic characteristics of participants

Variable	Category	Frequency	Percentage (%)
Gender	Female	60	40.0%
	Male	90	60.0%
Anaemic Status	Mild Anaemia	68	45.3%
	Non-Anaemic	47	31.3%
	Severe Anaemia	35	23.3%
Education	Kindergarten	69	46.0%
	Primary	50	33.3%
	Secondary	31	20.7%
Age	10-14	33	22.0%
	5-9	47	31.3%
	0-4	70	46.7%
Clinical Information	Blood Disorders	10	6.7%
	Cancer	12	8.0%
	Fever	60	40.0%
	Infectious	42	28.0%
	Other	22	14.7%
	Respiratory Distress	4	2.7%

Mean haematological parameters of participants: From the results presented in Table 2, mean haematological parameters for PCV, MCV, MCH and MCHC were similar between seropositive and seronegative participants, with no statistically significant differences ($p > 0.05$).

Table 2: Mean comparison of haematological parameters based on seropositivity

	Negative	Positive	*T*	*P*
PCV	26.91±0.58	26.58±1.37	0.175	0.861
MCV	75.57±0.98	72.90±2.50	0.845	0.400
MCH	25.78±0.37	24.25±0.94	1.286	0.201
MCHC	33.16±0.17	32.31±0.48	1.553	0.123

Prevalence of seropositivity among study participants: The Pie chart (Figure 1) illustrates the prevalence of seropositivity among study participants. Out of 150 participants, 136 (91 %) tested negative for seropositivity, while 14 (9 %) tested positive. This indicates that majority of the participants are seronegative, with only a small proportion being seropositive.

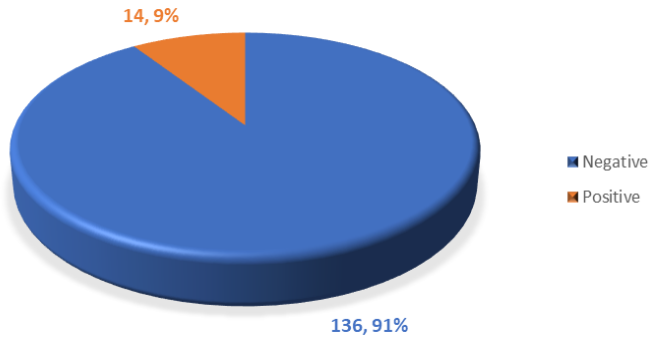

Figure 1: Prevalence of seropositivity

Also, there were no significant associations between sociodemographic characteristics and seropositivity ($p > 0.05$; Table 3). For gender, 6.7 % of females and 11.1 % of males were seropositive. Anaemic status shows that those with mild anaemia were 8.8 % seropositive, followed by non-anaemic participants (8.5%) while severe anaemia had 11.4% seropositivity. Regarding education, seropositivity was observed in 10.1 % of kindergarten participants, 8.0 % of primary-level participants and 9.7 % of those with secondary education. By age, seropositivity rates were 9.1 % for ages 10 – 14, 8.5 % for ages 5 – 9 and 10.0 % for ages 0 – 4. For clinical information, no significant differences were noted, with 13.6 % of infectious cases and 15.4 % of "other" cases showing slightly higher seropositivity compared to other categories.

Table 3: Association between sociodemographic characteristics with seropositivity

	Negative	Positive	χ^2	*P*-value
Gender				
Female	56 (93.3)	4 (6.7)	0.840	0.359
Male	80 (88.9)	10 (11.1)		
Anaemic status				
Mild anaemia	62 (91.2)	6 (8.8)	0.240	0.887
Non-anaemic	43 (91.5)	4 (8.5)		
Severe anaemia	31 (88.6)	4 (11.4)		
Education				
Kindergarten	62 (89.9)	7 (10.1)	0.163	0.922
Primary	46 (92.0)	4 (8.0)		
Secondary	28 (90.3)	3 (9.7)		
Age				
10-14	30 (90.9)	3 (9.1)	0.077	0.962
5-9	43 (91.5)	4 (8.5)		
0-4	63 (90.0)	7 (10.0)		
Clinical information				
Blood disorders	10 (100.0)	0 (0.0)	1.562	0.906
Cancer	12 (92.9)	1 (7.1)		
Fever	60 (90.0)	3 (10.0)		
Infectious	42 (86.4)	3 (13.6)		
Other	22 (84.6)	2 (15.4)		
Respiratory distress	4 (100.0)	0 (0.0)		

Table 4: Multivariate logistic regression associated sociodemographic characteristics with seropositivity

	P	OR	95% CI for OR
Gender			
Male	0.370	1.740	0.518-5.841
Female		1.000	
Anaemic status			
Mild		1.000	
Non	0.962	0.967	0.249-3.753
Severe	0.622	1.412	0.358-5.561
Age group			
0 – 4		1.000	
Above 4	0.936	1.134	0.053-24.253
Education			
Kindergarten	0.826	1.412	0.066-30.291
Others		1.000	
Constant	0.066	0.053	

The forest plot presents the results of a multivariate logistic regression analysis predicting seropositivity. The odds ratio for males was 1.74, with a 95 % confidence interval of 0.52 –5.84 and a *p* value of 0.370, indicating no statistically significant association between gender and seropositivity. For education at the kindergarten level, the odds ratio was 1.41 (95 % CI: 0.07 – 30.29; $p = 0.826$), suggesting no significant impact on seropositivity due to the wide confidence interval and lack of significance. Severe anaemic status had an odds ratio of 1.41 (95 % CI: 0.36 – 5.56; $p = 0.622$), showing an increased but statistically insignificant likelihood of seropositivity. Individuals without anaemia had an odds ratio of 0.97 (95 % CI: 0.25 – 3.75; $p = 0.962$), implying no meaningful association. For individuals in the "Above 4" age group, the odds ratio was 1.13 (95 % CI: 0.05 – 24.25; $p = 0.936$), with no significant association identified (Table 4). Overall, none of the predictors were significantly associated with seropositivity, as all *p* values exceeded 0.05 and confidence intervals for all odds ratios include 1.

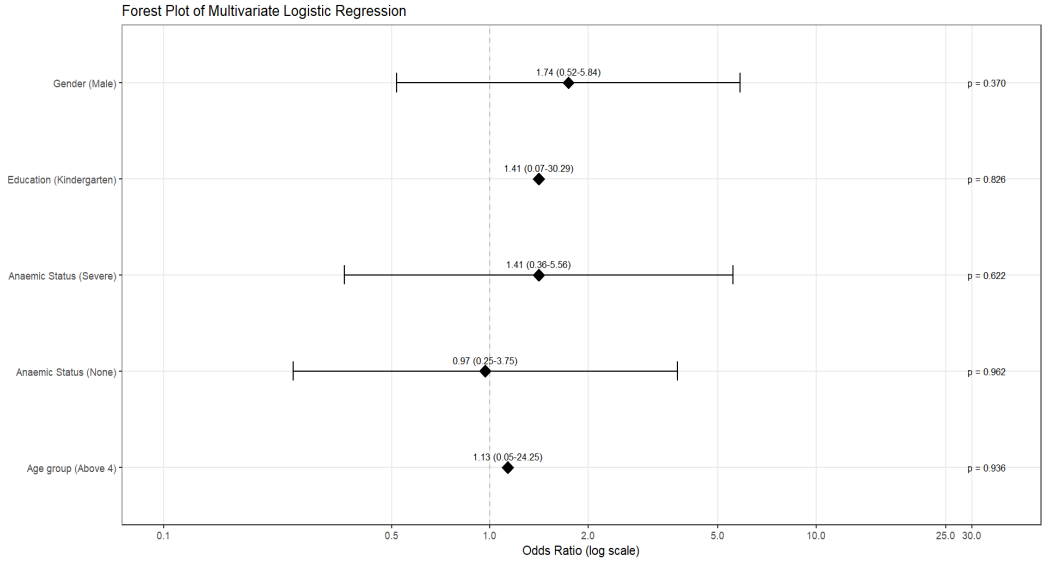

Figure 1: Forest plot of multivariate logistic regression

Discussion

The presence of serum IgM antibodies was found in 9.0 % (14/150) of these febrile children with various clinical conditions. Data on the seroprevalence of B19V in the paediatric population in Sub-Saharan Africa ranges between 14 % in Kenyan (in children less than 6 years) to 90 % in the Nigerian population (in children less than 2 years) (Wildig *et al.*, 2010; Duedu *et al*., 2013). Some studies have shown that parvovirus B19 infection plays an important role in the aetiology of severe anaemia (Regaya *et al*., 2007; Manning *et al*., 2012). The virus affects erythropoiesis by its replication in the erythroid progenitor cells, thereby leading to a suppression in erythropoiesis. Results from this study found that anaemic, mild anaemic and severe anaemic febrile paediatrics admitted into the paediatric hospital wards tested positive for human parvovirus B19 infection. Thus, severe anaemic status showed an increased likelihood of seropositivity among the study population. While early studies on B19V infection and anaemia showed no association with anaemia, more recent studies found the viral infection to be an important risk factor for transient aplastic anaemia, especially among pediatric population with a prevalence rate between 7% and 14% (Duedu *et al*., 2013; Ashaka *et al*., 2018).

The gender of participants in this study did not influence the prevalence of Human Parvovirus B19 infection among febrile paediatrics. In school-age children, the rate of exposure could differ between both genders depending on childhood behavioural activities, play and social patterns. The male child could engage in activities involving less hygienic practices that expose them to higher levels of the virus. Sample size variability could also be a factor significantly affecting seroprevalence in different studies. This attribute can be explained by the finding of Faddy *et al*. (2018) that females appear to have increased immunity to B19V compared to males.

In this study, the above 4 age group had an increased likelihood of higher, albeit non-significant, seropositivity compared to the other age groups. The prevalence of B19 virus infection has been shown to increase with the increase in age. In developed countries, the prevalence of parvovirus B19 in children under 5 years of age is 2 % - 10 %, 40 % - 60 % in adults older than 20 years and 85 % or more in people more than 70 years of age (Macri and Crane, 2023). Risk factors that predispose paediatrics to Parvovirus B19 include overcrowding in some day-care centres and preschools. This could also be as a result of an underdeveloped immune system in these age groups, that is, as people grow older, they become less susceptible to severe symptoms upon subsequent exposures.

In the present study, participants at the kindergarten level of education had a higher likelihood of seropositivity than the other levels of education, though not significant. This finding is in close agreement with a previous study that showed that outbreaks can arise especially in nurseries and schools (Enders *et al*., 2006). Parvovirus B19 main route of transmission is through respiratory secretions such as when an infected person coughs or sneezes, but can also be transmitted by blood and blood components. Cases are most infectious before symptoms develop and the incubation period is usually between four to twenty days. The transmission rate is about 50 per cent for those living with infected persons and about 20 to 30 per cent for susceptible teachers and

day-care workers who are exposed to infected children (Servey *et al*., 2007). Some studies have also documented case reports of nosocomial infection (Lui *et al*., 2001).

Conclusion

The seroprevalence of Human Parvovirus B19 IgM antibodies in febrile paediatrics was found to be 9.0 %. This study shows that the virus is relatively common and a good number of paediatrics have been exposed to the virus. Furthermore, male febrile paediatrics had a higher seroprevalence compared to their female counterparts.

Acknowledgments

The authors wish to thank Mr. Isaac O. Igbarumah and the laboratory staff of Molecular Diagnostic and Virology Laboratory, University of Benin Teaching Hospital, Benin City, for their assistance in the laboratory analysis

Competing interests

There are no competing interests.

Funding

Self-funded research by the researchers.

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