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Research Paper

Seroprevalence of parvovirus B19 antibodies and evidence of viremia among Nigerian patients with sickle cell anemia

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Abstract

Clinical, biochemical and molecular evidence for the sickle cell anemia (SCA) crisis in Nigerian patients arising from parvovirus b19 infection remains inadequate. This study determined the prevalence and correlates of anti-parvovirus b19 antibodies in a population of SCA patients and non-SCA healthy controls in Lagos, Nigeria. In this prospective cross-sectional study, we enrolled 73 confirmed SCA patients from 5 district hospitals in Lagos and 81 sex and age-matched non-SCA healthy controls. Serum sample from each study participant was screened for anti-parvovirus b19 by ELISA and PCR techniques. Standard biomedical assays were also done. Anti-parvovirus b19 IgM and IgG antibodies were detected in 22 (14.3%) and 97 (62.9%) of the 154 sera screened, 13 (17.8%) and 45 (61.6%) in SCA patients; 9 (11.1%) and 52 (64.2%) in non-SCA controls. The overall seronegativity rate was 19.5%. Parvovirus B19 DNA was found in 2 (11.1%) of the 18 IgM seropositive SCA serum samples screened. On the whole, parvovirus b19 infection was more commonly asymptomatic in non-SCA controls but caused significant elevation in liver enzymes in infected SCA patients ($P < 0.05$). The risk of acute parvovirus b19 infection increased 65 times during unsteady state among the SCA patients. Although no deaths of infected patients were recorded during the study, age below 12 years, hospitalization and overcrowded environment were risk factors for infection. We conclude that parvovirus b19 is common in SCA patients, incurring greater susceptibility to infections.

Keywords: parvovirus b19 infection, seroprevalence, sickle cell anemia

INTRODUCTION

Parvovirus B19, a non-enveloped 5.5 kb single-stranded DNA virus discovered in 1975, is now a global health problem with variations in prevalence and incidence rates, clinical manifestations and sequelae in afflicted human populations within and between countries[1-4]. Epidemiologically, parvovirus b19 infection, primarily transmitted via the respiratory route, has been reported to elicit cyclic outbreaks at 3-5-year intervals, display endemicity and occur sporadically in different populations with children, patients with hemolytic disorders, immunocompromised patients, and pregnant women being most affected[5]. Several

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seroprevalence studies have shown that the prevalence rates of anti-parvovirus b19 IgG antibody range from 1 to 15% in children below 5 years, 15 to 30% in children between 5 to 19 years, 30 to 60 % in adults and 80% and above in the elderly population 6. In pregnant women, seroconversion rates of 1.5% and 13% have been reported in endemic and epidemic situations[7].

Nigeria ranks among the highest in the global burden of sickle cell anemia (SCA) with crisis in patients arising from multiple etiologies. Despite being in the tropics and a country that is highly burdened by SCA to which infections with parvovirus b19 are synonymous with, data on parvovirus b19 in this target population are scanty. Parvovirus b19 infections cause aplastic anemia and may severely affect hematological parameters[5]. The occurrence of these manifestations is of grave concern in SCA patients as it increases the risks of recurrent crisis, hospitalization and early deaths[3,4]. Therefore, early detection of parvovirus b19 infection and better understanding of the pathologic course of infection is critical to reducing the burden of disease in SCA patients in every affected country of the world.

Parvovirus b19 infection is serologically diagnosed in humans as significant anti-parvovirus IgM positivity and genotypically by the detection of viral DNA in the serum using PCR[1,2]. The presence of anti-parvovirus b19 IgG in the serum is indicative of an immune response to previous viral challenge[1-3]. In Nigeria, data defining the burden of parvovirus b19 infections in the general population and SCA sub-population are scanty. The few prevalence data in sickle cell patients to date were from Jos in north central Nigeria. In the first study, anti-parvovirus b19 IgG rate of 39.5% was reported, revealing 21.7% and 92.8% levels of exposure in patients between 1 to 5 years and 15 years and above. In the same region for the second study, Emiasegen et al.[8] reported the proportions of pregnant women with acute parvovirus b19 infection, protective immunity and immuno-compromised status to be 13.2%, 27.5% and 40.7%, respectively. The occurrence of parvovirus b19 infection among hepatitis patients has also been recently reported from the southwestern part of the country. In that study, anti-parvovirus b19 IgM and IgG antibodies were detected in 24/72 (32%) and 25/76 (33%) in hepatitis B infected patients; in 6/17 (35%) and 8/17 (47%) among hepatitis C patients and in 14/44 (32%) and 12/44 (12%) non-hepatitis healthy control[9].

In epidemiological context, SCA is estimated on the average to affect 2% of the Nigerian population, which translates to about 30 million people in the country[10]. The risk factors for hospitalization and deaths that have been consistently reported in this category of people include SCA and there have been parvovirus b19-associated complications such as severe anemia, hemolytic crisis, bone infarction, osteomyelitis and myocarditis[10,11]. However, the role of parvovirus b19 in the pathogenesis of these complications among Nigerian SCA patients remains unclear. There is currently no substantial evidence to justify the use of intravenous human immunoglobulin as a component of standard of care for SCA patients in Nigeria. As a component of the holistic approach to care of SCA, a better understanding of etiologies of complications and crisis is highly essential as it would enable the deployment of appropriate and cost-effective prevention and therapeutic interventions. As part of the ongoing research efforts in the country, aimed at ascertaining risk factors of sickle cell crisis in this environment, we have determined the seroprevalence of parvovirus b19 and associated risk factors in a cohort of SCA patients in Lagos, Nigeria. A population of non-SCA healthy controls in the same environment was also surveyed for comparison and contact prediction.

SUBJECTS AND METHODS

Study design

This was a prospective cross-sectional pilot study that was ancillary to the previously reported leptin study[12] in which 55 SCA patients were enrolled consecutively as they came for care at 5 health facilities in Lagos State, Nigeria. However, this study extended to September 2010, translating to 4 additional months after the leptin study during which 18 new patients were enrolled. Blood samples were collected at enrollment for the parvovirus B19 seroprevalence study.

Study population

The study population consisted of 73 SCA patients aged 5 to 37 years (mean age, 16.9 ± 0.7 years) with males accounting for 54.8% (40/73) of the patient population. A total of 81 age and sex-matched apparently healthy volunteers of genotype AA (HbAA) or AS (HbAS) at the health facilities of the patients were also enrolled as non-SCA controls. Informed consent was obtained from each patient or guardian/parent of a minor prior to enrollment. SCA status was confirmed by a positive sickling test[13] and cellulose acetate electrophoresis of β^s-hemoglobin at pH 8.6 compared to the controls: HBAA, HBAS, HBSS and HBSC[13]. Each presenting SCA patient was clinically examined by the attending physician coupled

with laboratory evidence to ascertain the state of the disease. Patients in a steady state were defined as those without any of the following clinical conditions 4 weeks prior to or at enrollment. They include painful bone crisis, severe anemia, laboratory diagnosis of bacteremia, acute chest syndrome, aplastic anemia, splenic sequestration, systemic inflammatory response syndrome (SIRS) and behaviors such as anxiety and hallucination. Patients with any one or a combination of these clinical and behavioral presentations were said to be in an unsteady state or crisis[14,15]. Fever was defined as axillary temperature > 37.4°C, while absence of symptoms (i.e. asymptomatic condition) in the presence of anti-parvovirus IgM antibodies was indicated by the absence of fever, rash erythema, and swollen joints in seropositive SCA patients and non-SCA controls[2,5,6]. The study was approved by the Ethical Committee of the Hospital Management Board, Lagos State, Nigeria. The medical record of each SCA patient was reviewed and a questionnaire was administered to capture clinicopathological and demographic parameters such as age, sex, number of children per household, frequency of outpatient attendance and number of hospitalization or crisis episodes in the last 12 months. Excluded from this study were patients with sickle cell diseases other than SCA, history of alcohol abuse, HIV seropositive subjects and those who declined consent.

Sample preparation and hematology

At enrollment, 3 mL of venous blood aliquoted into EDTA (2.0 mL) and plain tubes (1.0 mL) was collected by venipuncture from each study participant. Capillary blood (~10 uL) was also obtained by finger pricking for blood film examination using Leishman´s stain. The EDTA blood samples and blood films were used for full blood count using standard hematological methods[16,17]. Hemoglobin was determined spectrophotometrically using Drabkin´s reagent[18]. After clotting, blood samples in the plain tubes were centrifuged at 3,000 rpm for 10 minutes and the resulting sera were collected into separate cryo tubes for biochemistry and serological analysis of parvovirus b19 infection. The serum samples were used immediately or stored at -20°C prior to use.

Biochemistry

Serum glutamate-oxaloacetate transaminase (SGOT) and glutamate-pyruvate transaminase (SGPT) activity was determined according to Reitman and Frankel[19], while serum albumin was assayed using the bromoscresol green (BCG) method described by Doumas et al.[20].

Serological assays

The detection and quantitation of parvovirus B19 IgM or IgG was done using a solid phase enzyme-linked immunosorbent (ELISA) technique that was based on the sandwich principle using the purified recombinant parvovirus B19 VP2 protein as an antigen to coat the IgM and IgG 96-well microtitre plates (Biotrin International, Dublin, Ireland). Each sample was diluted at a ratio of 1:101 (v/v) with a diluent buffer and the influence of interfering substances was annulled by mixing the diluted serum (200 µL) with RF-absorbent solution (10 µL) provided by the manufacturer. Assay was carried out according to the manufacturer´s instruction using peroxidase-labeled rabbit anti-human IgM as the secondary antibody, tetramethyl benzidine as a substrate and 1 M H_2SO_4 as a stop solution. Absorbance was read at 450 nm using an ELISA reader within 15 minutes of color development. Assays were standardized using serial dilutions of parvovirus B19 IgM or IgG standard solutions (10-50 U/mL) and run twice per sample. The absorbance of anti-parvovirus B19 IgM/IgG standard level of 20 U/mL was taken as the cutoff value. Index value was calculated as the ratio of absorbance of sample to the cutoff value. Index value between 0.8-1.2 was taken as an equivocal result. Samples below this range were taken as negative, while samples above this range were considered as positive for IgG or IgM depending on the assay plate used.

Detection of parvovirus B19 DNA in the serum

Serum samples from 18 SCA patients that were seropositive for anti-parvovirus B19 IgM or both IgG and IgM and 9 IgM (±ve) non-SCA controls were further subjected to DNA extraction using the QIAamp DNA blood kit (QIAamp, Germany) as directed by the manufacturer. The extracted DNA was subsequently used as a template for the detection of parvovirus B19 DNA by nested PCR using two primer pairs that targeted the minor (VP1) and major (VP2) capsid protein genes according to Yamakawa et al.[21]. The outer primers were: forward, 5´-CAAAAGCATGTGGAGTGAGG-3´ (nt 3187-3206); reverse, 5´-CTACTAACATGCATAGGCGC-3´(nt3584-3565). The inner primers were; forward, 5´-CCCAGAGCACCATTATAAGG-3´(nt 3271-3290); reverse, 5´-GTGCTGTCAGTAACCTG-TAC-3´ (nt 3558-3539). Here, each PCR assay was a 25 µL reaction mixture containing 2.0 µL of extracted DNA sample or 1 µL of the primary PCR product, 1.5 mM $MgCl_2$ (Promega, Madison, WI, USA), 10 pico-

mole each or 20 picomole each of the outer or inner primers ((Biomers, Germany) and 1 U of *Taq* DNA polymerase (Promega) in 1 x PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3) and ultrapure DNAse and RNAse free water (GIBCO, USA). Each PCR run included a blank control containing ultrapure water instead of target DNA and negative control containing leukocyte DNA extracted from a seronegative blood sample of an apparently healthy human volunteer. Amplification was performed in a thermocycler (ATC 200, BIORAD, USA) with a program, consisting of 1 cycle of denaturation at 94°C for 5 minutes, 30 cycles denaturation at 94°C for 1 minute, annealing at 55°C with outer primers or 57°C with inner primers for 2 minutes and extension at 72°C for 3 minutes and 1 cycle of a final extension step at 72°C for 5 minutes. Both primary and secondary PCR products were separately electrophoresed on 2% agarose gel pre-stained with ethidium bromide (0.5 µg/mL) to obtained bands of sizes 398 bp and 288 bp after extrapolation for mobility with 100 bp ladder DNA markers. The absence of these bands in the blank and negative control was taken as an indication of specificity of the PCR and the absence of cross-contamination.

Statistical analysis

Data are reported as mean ± SEM (for continuous variables), numbers and percentages (for categorical variables). Continuous variables were compared between steady and unsteady SCA patients and between SCA patients and non-SCA patients using Student´s *t* test, while categorical variables were compared using chi-square test that was corrected for continuity. They were also submitted to univariate and multivariate logistic regression analyses to compute relative risk (RR) and odd ratio (OR) at 95% confidence interval (95%CI) between cases (Steady *vs*. unsteady SCA patients) and between cases and controls. The various tests were carried out as two-tailed and outcomes with probability value below 0.05 were considered to be significant.

RESULTS

The baseline characteristics of the 73 SCA patients compared with those of 81 non-SCA controls in this study are presented in Table 1. Both the SCA patients and non-SCA controls were comparable in mean age and gender distribution ($P > 0.05$). Of the 73 SCA patients enrolled, 25 (34.2%) were clinically unsteady with a significantly lower mean age (14 ± 0.7 years) compared to those at steady state of the disease (18.5±0.9 years; $P = 0.002$). Significant ($P < 0.05$) disparity was also observed between SCA and non-SCA patients in the frequency of hospitalization and out-patient visits in the preceding 12 months, in BMI distribution and in all the hematological/biochemical parameters measured (***Table 1***).

Anti-parvovirus b19 IgM and IgG antibodies were detected in 22 (14.3%) and 97 (62.9%) of the 154 sera screened, 13 (17.8%) and 45 (61.6%) in SCA patients; 9 (11.1%) and 52 (64.2%) in non-SCA control. The overall seronegativity rate was found to be 19.5% (***Table 2***).

Occurrence of parvovirus B19 DNA was found in 2 (25%) of the 18 SCA serum samples screened. The two positive samples (1 of 3 IgM + IgG and 1 of 6 IgM seropositive serum samples) were from SCA patients in the unsteady state. This represents 22.2 % and 11.1%, respectively, of the 9 and 18 IgM seropositive unsteady state (2 of 9) and overall (2 of 18) and SCA serum samples screened. None of the 9 IgM seropositive sera of non-SCA control was B19 DNA positive (***Table 3***).

Univariate analysis of risk factors revealed significant association of age below 12 years, 2 or more hospitalizations in the last 12 months and habitation in a households 4 or more children with acute parvovirus b19 infection in the SCA patients ($P < 0.05$) (***Table 4***). Of these associated risk factors, only 2 hospitalizations in the last 12 months lost significance as a correlate of acute parvovirus b19 infection in multivariate logistic regression analysis in which being in an unsteady state of SCA attained significance ($P < 0.05$) with an OR of 6.15 after adjustment for sex and co-expression of anti-parvovirus b19 IgG and IgM antibodies (***Table 5***).

Of the 18 SCA patients that were seropositive for anti-parvovirus b19 antibodies, 2 (11.1%) were asymptomatic, 13 (72.2%) had fever and 12 (66.7%) had swollen joints. However, higher rate of asymptomatic infection (55.6%) but lower rate of fever (11.1%) were seen in the non-SCA controls $P < 0.05$). None of the non-SCA control subjects seropositive for anti-parvovirus b19 IgM antibodies had swollen joints, while non-significant disparity was found in the occurrence of rash between IgM seropositive SCA patients and the non-SCA controls (33.3 vs. 61.1%; $P > 0.05$) and between IgM seronegative SCA and IgM seropositive SCA patients (62.5 vs. 66.7%; $P > 0.05$).

However, when IgM seropositivity SCA patients were compared with their IgM seronegative counterparts, significant ($P < 0.05$) disparity was found in the occurrence of asymptomatic infection, fever, rash and acute bone pain that also involved the 2 B19 DNA positive cases (***Table 6***). Generally, no deaths of infected SCA patients were observed during the study. The results presented in ***Table 7*** show that anti-parvo–

Table 1 **Baseline characteristics of SCA patients and controls**

Parameter	Control	SCA Patients			*P*-value*	*P*-value**	OR (95% CI)^
		Total	Steady	Unsteady			
Number of subjects, *n* (%)	81	73	48 (65.8)	25 (34.2)	0.41	-	
Sex (*n*, M/F)	43/38	40/33	30/18	10/15	0.27	0.83	
Age (year, mean±SD)	16.3±0.7	16.9±0.7	18.5±0.9	14±0.7	0.002	0.55	
Hospitalization in the preceding 12 months, *n* (%)							
0	58 (71.6)	10 (13.7)	8 (16.7)	2 (8)	0.31	< 0.001	6.44 (3.6-11.6)
1	23 (28.4)	51 (69.9)	35 (72.9)	16 (64)	0.43	< 0.001	5.85 (2.8-12.5)
2	0 (0)	8 (11.0)	5 (10.4)	3 (12)	0.83	0.002	
3	0 (0)	4 (5.4)	0 (0)	4 (16)	0.01	0.048	
Outpatient visit in the preceding 12 months, *n* (%)							
0	35 (43.2)	0 (0)	0 (0)	0 (0)			
1	34 (42)	18 (24.7)	10 (20.8)	8 (32)	0.44	0.04	0.5 (0.2-1)
2	11 (13.6)	22 (30.1)	17 (35.4)	5 (20)	0.27	0.02	2.8 (1.1-6.7)
3	1 (1.2)	33 (45.2)	21 (43.8)	12 (48)	0.73	< 0.0001	66 (9.1-1343.8)
Haematological parameters							
Reticulocyte count (%)	1.5 ± 0.05	4.6±0.3	4.9±0.3	4.1±0.5	0.13	< 0.0001	
Platelet count (cells/uL)	221.9±5.0	401.6±7.0	412.3± 7.2	368.2±18.5	0.03	< 0.0001	
Hb (g/dL)	11.8±0.3	8.4±0.1	8.7±0.1	7.6±0.2	< 0.0001	< 0.0001	
WBC (cells/uL × 10^3)	5.7±0.1	10.9±0.2	10.8±0.3	11.2±0.3	0.23	< 0.0001	
Biochemical Parameters							
SGPT (U/L)	16.9±0.6	36.9±2.1	27.1±0.9	55.7±3.6	< 0.00001	< 0.0001	
SGOT (UL)	19.4±0.7	45.5±2.3	34.7±1.3	65.9±3.2	< 0.00001	< 0.0001	
Albumin (g/dL)	3.9±0.03	3.5±0.03	3.6 ±0.03	3.3±0.04	< 0.001	< 0.001	
Body mass index [kg/m^2, *n* (%)]							
< 20	12 (14.8)	31 (42.5)	11 (22.9)	20 (80)	< 0.0001	< 0.0001	4.4 (1.9-10.2)
20-24	64 (79)	42 (57.5)	37 (77.1)	5 (20)	< 0.0001	0.09	0.5 (0.2-1.2)
25	5 (6.2)	0 (0)	0 (0)	0 (0)	-	0.06	-
Children/Household, *n* (%)							
1	3 (3.7)	3 (4.1)	2 (4.2)	1 (4)	0.56	0.77	1.1 (0.2-7.2)
2	21 (25.9)	19 (26)	14 (29.2)	5 (20)	0.57	0.86	1.0 (0.5-2.2)
3	37 (45.7)	37 (50.7)	25 (52.1)	12 (48)	0.93	0.54	1.3 (0.7-2.5)
4+	20 (24.7)	14 (19.2)	7 (14.5)	7 (28)	0.29	0.58	0.8 (0.3-1.7)

Continuous variables are expressed as mean ± SEM and categorical variables as number (percentage), *n*(%). Comparisons were done using Chi square or Fischer exact test and Student´s *t*-test **P*-value of statistical test (Steady *vs.* Unsteady); ***P*-value of statistical test (SCA patients vs. non-SCA Control); ^Odd ratio statistics between SCA patients (Case) and apparently healthy population (Control). SCA: sickle cell anemia; SGOT:serum glutamate–oxaloacetate transaminase; SGPT: serum glutamate–pyruvate transaminase; WBC: white blood cell.

virus B19 IgM seropositivity with B19 DNA detected was associated with further significant reduction ($P < 0.05$) in anti-parvovirus B19 IgG titers, platelet and total leukocyte counts, serum liver enzyme activity (sGPT and sGOT) and albumin levels when compared with B19 IgM seropositivity without B19 DNA detected among the SCA patients.

DISCUSSION

Sickle cell anemia, a disease characterized by high erythrocyte turnover to compensate for red cell lysis and chronic hemolytic anemia in afflicted patients[22],

Table 2 **Prevalence and distribution of serological markers of Parvovirus B19 among sickle cell anemia (SCA) patients and controls**

Parvovirus B19 Serological markers^	SCA Patients [% (95% CI)]			Non-SCA Control % (95% CI)				Overall population
	Steady(N = 48)	Unsteady (N = 25)	Total (N = 73)	RR (95% CI)^	(N = 81)	OR (95% CI)	*P*-value*	(N = 154)
IgM	14.6 (4.6-24.6)	24 (7.3-40.7)[b]	17.8 (9-26.6)	1.65 (0.62-4.37)	11.1 (4.3-17.9)	1.73 (0.64-4.77)	0.24	14.3 (8.8-19.8)
IgG	68.8 (55.7-81.9)	48 (26.4-67.6)[b]	61.6 (50.4-72.8)	0.70 (0.45-1.10)	64.2 (53.8-74.6)	0.90 (0.44-1.82)	0.74	62.9 (55.3-70.5)
IgM+IgG	4.2 (1.5-9.9)	12 (0.7-24.7)[b]	6.8 (1-12.6)	2.70 (0.48-15.1)	0 (0)	ND	0.02	3.2 (0.4-6)
Seronegative	12.4 (3.1-21.9)	16 (1.6-30.4)[b]	13.8 (5.8-21.6)	1.28 (0.40-4.12)	24.7 (17.7-37.1)	0.48 (0.19-1.2)	0.09	19.5 (13.2-25.8)

Data are percentage (95% Confidence interval, CI) of cases with parvovirus B19 serological markers. [b]$P > 0.05$ (Steady vs. Unsteady SCA patients); ^Relative risk (95% CI) values of steady state SCA patients compared to their unsteady state counterparts. **P*-value (chi-square or Fischer exact test) of comparison between the SCA patients (Total) and non-SCA control. ^$P < 0.05$ (SCA *vs*. non-SCA for total frequency of IgM)-χ^2 test. $P < 0.05$ was considered to be significant.

Table 3 **Parvovirus B19 DNA status in a subset of sickle cell anemia (SCA) patients with anti-parvovirus B19 IgM seropositivity^**

Parvovirus B19 IgM	Serological status in SCA patients			Parvovirus B19 DNA (+ve) in SCA patients		
Serological markers	Steady	Unsteady	Total	Steady	Unsteady	Total
IgM (+)	7	6	13	0	1	1
IgM (+) and IgG (+)	2	3	5	0	1	1
Total	9	9	18	0	2 (22.2)	2 (11.1)

Data are presented as numbers with percentages in parentheses. ^None of the 9 IgM seropositive non-SCA serum samples screened was B19 DNA positive. + or ve means positive.

remains a public health concern in Nigeria[10]. Defining the risk of crisis and poor life expectancy in these patients is now a topmost priority in the research agenda of the country[23]. In this study, we found equal occurrence of IgM seropositivity for parvovirus B19 between our steady and unsteady state SCA patients. However, when compared with our non-SCA group, we found the disparity in IgM seropositivity to be non-significant when analyzed alone, but it became significant when we took into account cases with dual anti-parvovirus IgM and IgG seropositivity that were absent in our control subjects. We also found no significant difference in the occurrence of lack of exposure (i.e. seronegativity) to parvovirus B19 virus between the patients and controls. Furthermore, parvovirus B19 DNA was detected in two unsteady state SCA patients who were also anti-parvovirus IgM seropositive, while no B19 DNA positivity was found among the IgM seropositive controls. Based on our findings, it can be deduced that, unlike non-SCA individuals, SCA patients in this environment are prone to parvovirus B19 infection even after previous exposure (as evidenced by cases of dual IgM and IgG seropositivity), with viremia (as evidenced by B19 DNA positivity) promoting crisis. In diagnosing parvovirus B19 infection in children and adults, the occurrence of viremia indicated by B19 DNA seropositivity has been found to precede production of anti-parvovirus b19 IgM and IgG antibodies[2,6]. Therefore, the presence and absence B19 DNA positivity

Table 4 **Evaluation of risk factors associated with acute parvovirus B19 infection among the SCA patients**

Parameter	N	IgM(%)	RR (95% CI)	χ^2	*P*
Sex					
Male	40	15	1		
Female	33	21.2	1.41	0.47	0.5
Age group (year)					
⩽ 12	18	44.4	10.22 (1.4 -74.4)	7.3	0.007
13-18	27	14.8	3.41 (0.4 - 28.4)	1.5	0.2
19-25	23	4.3	1		
26+	5	0	-		
Sickle cell status					
Steady	48	14.6	1		
Unsteady					
Hospitalization in the preceding 12 months, *n* (%)					
0	10	0	-		
1	51	11.8	1		
2	8	50	4.25 (1.5-11.3)		0.02
3+	4	75	6.38 (2.5-16.3)		0.01
Outpatient visit in the preceding 12 months, *n*(%)					
0	0	0	-		
1	18	16.7	1		
2	22	27.3	1.64		0.5
3+	33	12.1	0.73		0.7
Body mass Index, kg/m^2, *n* (%)					
< 20	31	16.1	1		
20-24	42	19.0	1.18 (0.4-3.3)	0.1	0.3
25+	0	0			
Children/Household, *n* (%)					
1	3	0	-		
2	19	10.5	1		
3	37	10.8	1.03 (0.2-5.1)		1.0
4+	14	50	4.75 (1.2-19.5)		0.02

SCA: sickle cell anemia; RR: relative risk; CI: confidence interval.

Table 5 **Multivariate logistic regression analysis of risk factors associated with acute parvovirus B19 infection among the sickle cell anemia (SCA) patients**

Risk factor	OR (95% CI)
Age below 12 years	9.28 (2.15-42.3)*
Frequency of hospitalization in the preceding 12 months	
2	2.5 (0.27-19.2)
3+	15 (0.75-501)*
Unsteady SCA	6.15 (0.78-55.9)*
4 or more children per household	4.5 (0.81-22.2)*

OR: odd ratio. *$P < 0.05$.

in the anti-parvovirus B19 IgM seropositive samples screened are indicative of recent and old infections in afflicted patients and controls[1,2,4]. This observation is also indicative of a persistent infection characterized by sequestration of the virus in P antigen rich tissues/organs such as the liver, myocardium, bone marrow and endothelium coupled with a possibility of a viremia below the detection threshold of the PCR method used[1,2,4,24,25]. These scenarios have also been reported in similar studies from other SCA endemic countries. They thus represent important challenges to be addressed if national surveillance of parvovirus b19 infection is to be undertaken in Nigeria. Since parvovirus B19 DNA detection from biopsy samples is practically not feasible in this setting, the use of Q-PCR to detect copies of parvovirus B19 DNA as low as 1-5 is recommended.

Furthermore, the evidence of viremia that suggests acute infection due to parvovirus B19 DNA positivity provided by this study is weakened by the small sample size screened and the prospective cross-sectional study design. Therefore, to strengthen viremia evidence, future studies will be made prospective and longitudinal to monitor the occurrence of viremia via multiple time point serological and genotypic screening for parvovirus B19 infection in the study cohorts. Nevertheless, in terms of parvovirus b19 burden, the anti-parvovirus b19 IgM prevalence rates of 14.3% and 17.8% observed in our study population of 154 subjects as a whole and in the 73 SCA patients screened are higher than the rates of 3.5% and 13.2% found recently among SCA patients and pregnant women in Jos, north-central Nigeria[26,8]. Our rates are, however, lower than the 32% and 32-35% found in non-hepatitis controls and hepatitis B and C patients in Oshun, another southwestern region of the country[9]. Therefore, our data and the three recent epidemiological data reported in Nigeria suggest a possible variation in parvovirus b19 activity in different parts of the country. Similar scenarios with anti-parvovirus b19 IgM prevalence rates ranging from 3.9% to 11.1% have been reported in Saudi-Arabia, USA and Tunisia[24,25,27] in the setting of SCA. Furthermore, in other target populations such as blood donors, healthy adults, patients with other hemolytic disorders, cancer patients and HIV patients, variations in the prevalence and incidence of parvovirus b19 infections have also been reported in many countries of the world[28-38] (***Table 8***). Generally, these findings indicate that immuno-compromised patients, including SCA patients that we investigated, are more susceptible to-parvovirus b19 infections with pregnant women and blood donors as potential reservoirs of the virus for sporadic, endemic and epidemic transmission. Therefore, variations in factors driving the transmission of parvovirus b19 including the level of infectious disease control are likely to be key correlates of parvovirus b19 epidemiology in these countries. In this study, we found age below 12 years, hospitalization on more than 2 occasions and living in an overcrowd-

Table 6 **Clinical manifestation of parvovirus B19 infection among the sickle cell anemia (SCA) patients and the controls**

Clinical manifestation	Control	SCA Patient^		*P*-value*	*P*-value**
	IgM+ ($n = 9$)	IgM+ ($n = 18$)	IgM-ve ($n = 55$)		
Asymptomatic	5 (55.6)	2 (11.1)	22 (40)	0.01	0.02
Fever	1 (11.1)	13 (72.2)	14 (25.4)	0.003	0.004
Rash⊥	3 (33.3)	11 (61.1)	9 (16.4)	0.2	0.0002
Swollen joints⊥	0 (0)	12 (66.7)	10 (18.2)	ND	0.0009
Splenic sequestration	0 (0)	2 (11.1)	5 (9.1)	ND	0.8
Poliomyelitis	0 (0)	1 (5.6)	0 (0)	ND	ND
Leg ulcer	0 (0)	2 (16.7)	3 (5.5)	ND	0.4
Acute chest syndrome⊥	0 (0)	3 (16.7)	0 (0)	ND	ND
Severe haemolytic anaemia	0 (0)	1 (16.7)	4 (7.3)	ND	0.8
Sepsis	0 (0)	1 (5.6)	3 (5.5)	ND	0.9
Acute Bone pain⊥	0 (0)	4 (22.2)	2 (3.6)	ND	0.01

^Inclusion of SCA patients (N = 9) with sera containing anti-parvovirus B19 IgG and IgM antibodies; ⊥Included the two B19 DNA positive cases. *P*-values were obtained from Fischer exact test; **P*-value (IgM + Control vs IgM + SCA); ***P*-value (IgM + SCA *vs.* IgM -ve SCA). ND: Not determined; $P < 0.05$ was considered to be significant.

Table 7 **Effects of acute parvovirus B19 infection on certain haematobiochemical parameters in the sickle cell anemia (SCA) patients**

Parameter	IgM+ve (N = 18)*	IgM-ve (N = 55)	*P*-value
Age (year)	11.5±1.0	18.1±1.0	< 0.05
BMI (kg/m^2)	20±0.08	20.3±0.1	> 0.05
Anti-parvovirus B19 IgG titre (OD)	0.9±0.1	2.3±0.1	< 0.05
Hb (g/dL)	8.0±0.3	8.5±0.1	> 0.05
WBC count (cells /μL × 10^3)	11.6±0.1	10.8±0.2	> 0.05
Platelet count (cells/μL × 10^3)	318±9.7	416.7±4.2	< 0.05
Reticulocyte count (%)	1.7±0.2	5.1±0.2	< 0.05
SGPT (U/L)	23.9±0.8	21.8±0.7	< 0.05
SGOT (U/L)	32.3±0.7	25.1±0.7	< 0.05
Albumin (g/dL)	3.6±0.05	3.5±0.02	> 0.05

Data are mean + SEM. Disparity between mean values was analyzed using Student´s t-test. *Two of the 18 IgM+ve samples contained parvovirus B19 DNA, *P*-value < 0.05 was considered to be significant.

ed environment as independent predictors of parvovirus b19 infection in our SCA patients. Our findings strongly suggest nosocomial and person-to-person transmission as vehicles of propagation of parvovirus b19 in Lagos. This suggestion aligns with parvovirus b19 epidemiological reports from other countries[5]. Studies have estimated parvovirus b19 transmission rates to be 50% in household contacts and to vary between 10-60% in school and day care exposure[7,39]. It is also important to note that in recent studies from a few malarious countries, higher prevalence rate of parvovirus b19 infection have been reported[40]. This suggests that malaria co-infection may also play an important role in the propagation of parvovirus b19 in afflicted populations.

In terms of dual anti-parvovirus B19 IgG and IgM response observed in this study, which implies a stronger immunocompromised state or new infection/viral reactivation despite previous exposure, similar findings were also reported by Opaleye et al.[9]. The workers showed anti-parvovirus b19 IgG and IgM dual seropositivity rate of 2.9% among Nigerian hepatitis B and C infected patients (***Table 8***). However, these workers found higher parvovirus B19 DNA rates of 9 -19% in their cohort. The difference in our results may be attributed to sample size and sample type differences. Opaleye et al.[9] screened for parvovirus B19 DNA in 93 sera that included 44 IgG (-) IgM (-) samples, while 27 IgM seropositive samples were screened in this study. However, it is noteworthy that anti-parvovirus b19 IgM and IgG antibodies in chronically ill patients have been scantily reported[41,42]. It was not documented in the recent sickle cell study in Nigeria[8] or in the largest surveillance study among sickle cell disease patients in the USA[27]. This is also the first report of the occurrence of parvovirus B19 DNA in SCA patients from Nigeria. It is now recognized that patients with dual seropositivity pose

Table 8 **Prevalence data by target populations of anti-parvovirus B19 antibodies from previous epidemiological studies within and outside Nigeria**

Target population	Country	sample size	Year	IgM (%)	IgG (%)	IgM	IgG (%)	Seronegative (%)	Reference
Pregnant women	Nigeria	273	2008	13.2	27.5		40.7	18.7	[8]
HBV/HCV patients	Nigeria	137	2010	32/32	33/47		2.9	27.7	[9]
HCV	Nigeria	200	2010	3.5	39.5		-	-	[9]
Control	USA	102	2003	-	53		-	-	[27]
SCA patients^	USA	633	1996-2001	11.3	30		-	70	[26]
SCA Patients	Saudi Arabia	138	2009-2010	2.9	37.6		-	-	[24]
SCA patient	Saudi Arabia	-	1993-1995	19	-		-	-	[29, 30]
SCA Patients	Mali	193	2011	-	64.8		-	-	[31]
SCA Patients	Brazil	165	1996	32.1	67.9		-	-	[32]
SCA patient	Libya	-	2009	5	61		-	-	[33]
Non-SCA control	Mexico	224	2002	36.1	45.9		-	-	[34]
Medical Students	Papua New Guinea	169	1996-2002	30.3	-		-	-	[35]
SCA Patients	Tunisia	46	2007	8.7	56.5		-	34.8	[25]
Cancer patients	Taiwan, China	127	1999-2000	-	61.8		-	38.2	[36]
Thalassemic patients	Thailand	60	2011-2002	4%	38		4	60	[37]
Sick patients	Hong Kong, China	276	1991-1996	2.5	19.6		-	-	[38]

^11.3% incidence of parvovirus B19 per year, diagnosed by IgM seropositivity was found among the SCA patients in whom 30% IgG seropositivity and 70% seronegativity were at the beginning of the study. SCA: sickle cell anaemia; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

a great threat in the dissemination of parvovirus b19 in their environment, making their surveillance and exclusion from non-infected patients very important in order to halt viral transmission and reduce the risk of an outbreak. Also in this study, we found a seronegativity rate of 19.5%. This represents the proportion of people in our study population that are unexposed but still susceptible to parvovirus b19 infection and thus need protection from the virus.

As a clino-pathogenic factor, parvovirus b19 has been found to cause red cell aplasia in the company of other clinical events such as pain, fever, splenic sequestration and acute chest syndrome with laboratory evidence of altered hematological parameters in SCA patients[43,44]. In this study, we also found the occurrence of fever, swollen joint, rash and acute bone pain to be associated with anti-parvovirus B19 IgM positivity with contribution from new viral infection. The clinical importance of parvovirus B19 in the pathogenesis of acute chest syndrome was also revealed in this study as two of the three cases detected were parvovirus B19 DNA positive. However, clinical grading of observed clinical manifestations was not done to be able to evaluate specific contributions of parvovirus B19 viremia. However, a clue to possible contribution of viremia to severe clinical manifestations in our SCA patients as reported elsewhere[2,27,43,44,45,46] was obtained from the biochemical profiles of the two parvovirus B19 DNA positive cases in which marked elevations in total leukocyte count, indicating enhanced leukocytosis, in sGOT and sGPT indicating enhanced liver pathology but reduction in Hb, suggesting enhanced risk of severe anemia, in platelet count, suggesting stronger coagulopathy and further risk of thrombocytopenia and in serum albumin, which is indicative of acute phase response. In our recent study, we have found parvovirus B19 viremia inducing stronger TNF-α and C-reactive protein response in afflicted SCA patients when compared with those without IgM and B19 DNA positivity (Iwalokun et-al, in-Press). Apart from the small sample size, the non-sequencing of the two parvovirus B19 DNA samples recovered is another limitation of this study. This constraint has limited our ability to genotype the parvovirus B19 strains and gain further evidence-based insight into their mode of transmission/clonal spread. Meanwhile, Opaleye et al.[9] recently reported genotype 1 parvovirus B19 accounts for 70% of 11 isolates genotyped in this region of the country.

We conclude that parvovirus b19 infection is common in this environment with SCA patients eliciting greater susceptibility to infections that may deteriorate hematobiochemical parameters during crisis and potentially be disseminated nosocomially and by person-to-person contacts.

Based on the outcomes of this study, we recommend a larger epidemiological study of parvovivrus b19 using both serological and molecular methods (Q-PCR and sequencing) to gain more insight into the epidemiologic and clino-pathologic importance of parvovirus b19 infection in SCA patients in this environment and Nigeria as a whole. Routine screening of SCA patients aged 12 years and below and the development of prevention, surveillance and treatment programs aimed at averting parvovirus b19 epidemics and improving the management of parvovirus b19 infections in SCA patients and non-SCA subjects are also recommended.

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