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. Epidemiologically, parvovirus b19 infection, primarily transmitted via the respiratory route, has been reported to elicit cyclic outbreaks at 3-5-year intervals, display endemcity and occur sporadically in different populations with children, patients with hemolytic disorders, immunocompromised patients, and pregnant women being most affected. Several

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Parvovirus B19 antibodies and viremia in Nigerian sickle cell anaemia patients

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. 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 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 and cellulose acetate electrophoresis of β²-hemoglobin at pH 8.6 compared to the controls: HBAA, HBAS, HBSS and HBSC. 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 pain-
ful 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[22,5,6]. 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 at-
tendance and number of hospitalization or crisis epi-
isodes in the last 12 months. Excluded from this study
were patients with sickle cell diseases other than
SCA, history of alcohol abuse, HIV seropositive sub-
jects 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 col-
lected by venipuncture from each study participant.
Capillary blood (~10 µL) 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 hematologi-
ical methods[16,17]. Hemoglobin was determined spec-
trophotometrically using Drabkin’s reagent[18]. After
clotting, blood samples in the plain tubes were cen-
trifuged at 3,000 rpm for 10 minutes and the resulting
sera were collected into separate cryo tubes for bio-
chemistry 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 anti-
gen to coat the IgM and IgG 96-well microtitre plates
(Biotrin International, Dublin, Ireland). Each sam-
ples was diluted at a ratio of 1:101 (v/v) with a dilu-
ent 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, te-
ramethyl benzidine as a substrate and 1 M H2SO4 as
a stop solution. Absorbance was read at 450 nm using
an ELISA reader within 15 minutes of color develop-
ment. 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 se-
rum

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 detect-
tion of parvovirus B19 DNA by nested PCR using
two primer pairs that targeted the minor (VP1) and
major (VP2) capsid protein genes according to Ya-
makawa et al.[21]. The outer primers were: forward,
5’-CAAGACATGAGTGGAGTGGG-3’ (nt 3187-
3206); reverse, 5’-CTACTACATGCATAGG-3’ (nt3584-3565). The inner primers were: forward,
5’-CAAGACATGAGTGGAGTGGG-3’ (nt 3271-
3290); reverse, 5’-CTACTACATGCATAGG-3’ (nt3584-3565). 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 MgCl2 (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 DNAs and RNAsse 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/bio-chemical 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 (53.6%) 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 (N = 81) | SCA Patients | OR (95% CI) |
|----------------------------------|------------------|--------------|-------------|
| Number of subjects, n (%)        | 81               | 73 (9.1)     | 66 (72.9)   |
| Sex (M/F)                        | 43/38            | 40/33        | 43/30       |
| Age (year, mean ± SD)            | 16.3 ± 0.7       | 16.9 ± 0.7   | 0.800 ± 0.55|
| Hospitalization in the preceding 12 months, n (%) | 0.00001 < 0.001 | 4.4 (3.6-11.6)|
| 0                               | 58 (71.6)        | 10 (13.7)    | 16.7 (2.8-1.2) |
| 1                               | 23 (28.4)        | 51 (69.9)    | 35 (72.9)   |
| 2                               | 0 (0)            | 0 (0)        | 0 (0)       |
| 3                               | 0 (0)            | 0 (0)        | 4 (5.4)     |
| Outpatient visit in the preceding 12 months, n (%) | 0.00001 < 0.001 | 5.85 (2.8-12.5)|
| 0                               | 35 (43.2)        | 0 (0)        | 0 (0)       |
| 1                               | 34 (42)          | 18 (24.7)    | 10 (20.8)   |
| 2                               | 11 (13.6)        | 22 (30.1)    | 17 (35.4)   |
| 3                               | 1 (1.2)          | 33 (45.2)    | 21 (43.9)   |
| Haematological parameters       |                 |              |            |
| Reticulocyte count (%)           | 1.5 ± 0.05       | 4.6 ± 0.3    | 4.9 ± 0.3   |
| Platelet count (cells/μL)        | 421.9 ± 5.0      | 401.6 ± 7.0  | 412.3 ± 7.2 |
| Hb (g/dL)                        | 11.8 ± 0.3       | 8.4 ± 0.1    | 8.7 ± 0.1   |
| WBC (cells/μL, × 10^3)           | 5.7 ± 0.1        | 10.9 ± 0.2   | 10.8 ± 0.3  |
| Biochemical Parameters           |                 |              |            |
| SGPT (UL)                        | 16.9 ± 0.6       | 36.9 ± 2.1   | 27.1 ± 0.9  |
| SGOT (UL)                        | 19.4 ± 0.7       | 45.5 ± 2.3   | 34.7 ± 1.3  |
| Albumin (g/dL)                   | 3.9 ± 0.03       | 3.5 ± 0.03   | 3.6 ± 0.03  |
| Body mass index [kg/m², n (%)]   |                 |              |            |
| < 20                             | 12 (14.8)        | 31 (42.5)    | 11 (22.9)   |
| 20-24                            | 64 (79)          | 42 (57.5)    | 37 (77.1)   |
| > 25                             | 5 (6.2)          | 0 (0)        | 0 (0)       |
| Children/Household, n (%)        |                 |              |            |
| 1                               | 3 (3.7)          | 3 (4.1)      | 2 (4.2)     |
| 2                               | 21 (25.9)        | 19 (26)      | 14 (29.2)   |
| 3                               | 37 (45.7)        | 37 (50.7)    | 25 (52.1)   |
| 4+                              | 20 (24.7)        | 14 (19.2)    | 7 (14.5)    |

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; SGPT: serum glutamate-oxaloacetate transaminase; SGOT: 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* | Steady(N = 48) | Unsteady (N = 25) | Total (N = 73) | RR (95% CI) | OR (95% CI) | P-value | Overall population (N = 154) |
|-------------------------------------|----------------|-------------------|---------------|-------------|-------------|---------|-----------------------------|
| IgM                                 | 14.6 (4.6-24.6)| 24 (7.3-40.7)     | 27.8 (9.26)   | 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 (55.7-81.8)| 48 (26.4-67.6)    | 116 (72.8)   | 0.70 (0.45-1.10) | 64.2 (51.8-74.6) | 0.90 (0.44-1.82) | 0.74 | 62 (53-70.5) |
| IgM+IgG                             | 4.2 (1.5-9.9) | 12 (0.7-24.7)     | 16.4 (12.6)  | 2.70 (1.08-15.1) | 0 (0)       | ND     | 3.2 (0.4-6) |
| Seronegative                        | 12.4 (3.1-21.9)| 16 (1.6-36.4)     | 28.8 (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. *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)+ test. P < 0.05 was considered to be significant.
remains a public health concern in Nigeria\textsuperscript{[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\textsuperscript{[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\textsuperscript{[2,6]}. Therefore, the presence and absence B19 DNA positivity

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

| 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.

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

| Parameter                                      | N   | IgM(%) | RR (95% CI) | \(\chi^2\) | \(P\) |
|------------------------------------------------|-----|--------|-------------|-------------|-------|
| Sex                                            |     |        |             |             |       |
| Male                                           | 40  | 15     | 1           |             |       |
| Female                                         | 33  | 21.2   | 1.41        | 0.47        | 0.5   |
| Age group (year)                               |     |        |             |             |       |
| \(\leq 12\)                                    | 18  | 44.4   | 10.22 (1.4-74.4) | 7.3         | 0.007 |
| 13-18                                         | 27  | 14.8   | 3.41 (0.4-23.4) | 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  | 55     | 4.75 (1.2-19.5) | 0.02 |       |

SCA: sickle cell anemia; RR: relative risk; CI: confidence interval.
in the anti-parvovirus B19 IgM seropositive samples screened are indicative of recent and old infections in afflicted patients and controls. This observation is also indicative of a persistent infection characterized by sequestration of the virus in P antigen rich tissues/organisms 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. 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 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. 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. 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 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 (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 overcrowded environment as risk factors for parvovirus B19 infection. Multivariate logistic regression analysis of risk factors associated with acute parvovirus B19 infection among the sickle cell anemia (SCA) patients is recommended.

### 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 | 2.5 (0.27-19.2), 3+ (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.

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

| Clinical manifestation | Control IgM+ (n = 9) | Control IgM- (n = 16) | SCA Patient IgM+ (n = 15) | SCA Patient IgM- (n = 55) | P-value | P-value * |
|------------------------|----------------------|-----------------------|--------------------------|--------------------------|---------|---------|
| Asymptomatic           | 5 (55.6)             | 2 (11.1)              | 22 (40)                  | 0.01                     | 0.02    | 0.03    |
| Fever *                | 1 (11.1)             | 13 (72.2)             | 14 (25.4)                | 0.005                    | 0.004   | 0.0002  |
| Rash *                 | 3 (33.3)             | 11 (61.1)             | 9 (16.4)                 | 0.2                      | 0.0002  | 0.0009  |
| Swollen joints *       | 0 (0)                | 12 (66.7)             | 10 (18.2)                | ND                       | ND      | ND      |
| Splenic sequestration  | 0 (0)                | 1 (6.3)               | 3 (5.5)                  | ND                       | ND      | ND      |
| Polymyelitis           | 0 (0)                | 0 (0)                 | 0 (0)                    | ND                       | ND      | ND      |
| Leg ulcer              | 0 (0)                | 1 (6.3)               | 3 (5.5)                  | ND                       | ND      | ND      |
| Acute chest syndrome   | 0 (0)                | 1 (6.3)               | 3 (5.5)                  | ND                       | ND      | ND      |
| Severe haemolytic anaemia | 0 (0)            | 0 (0)                 | 4 (7.3)                  | ND                       | ND      | ND      |
| Sepsis                 | 0 (0)                | 1 (6.3)               | 3 (5.5)                  | ND                       | ND      | ND      |
| Acute Bone pain        | 0 (0)                | 1 (6.3)               | 2 (3.6)                  | ND                       | 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.
ed environment as independent predictors of parvo-
virus 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
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[8]. 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 hep-
atitis 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 notewor-
thy that anti-parvovirus b19 IgM and IgG antibodies
in chronically ill patients have been scantily report-
ed[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 7 Effects of acute parvovirus B19 infection on certain haematobiochemical parameters in the sickle cell anaemia (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/m²)                | 20±0.8          | 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 × 10⁹) | 11.6±0.1        | 10.8±0.2        | > 0.05  |
| Platelet count (cells/µL × 10⁹) | 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±0.3        | 21.8±0.7        | < 0.05  |
| SGOT (U/L)                 | 32.3±0.7        | 25.3±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.

### 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         | 2006       | 13.2    | 27.5    | 40.7     | 18.7     |                 |           |
| HBV/HCV patients         | Nigeria         | 137         | 2010       | 32/32   | 33/47   | 2.9      | 27.7     |                 | [8]       |
| HCV                      | Nigeria         | 200         | 2010       | 3.5     | 39.5    | -        | -        |                 | [9]       |
| Control                  | USA             | 102         | 2003       | -       | 53      | -        | -        |                 |           |
| 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.
greater susceptibility to infections that may deteriorate genotyping in this region of the country. Type 1 parvovirus B19 accounts for 70% of isolates 11. This indicates the importance of clonal spread. The constraint has limited our ability to genotype the parvovirus B19 in our study population.

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[42,43,44,45,46] 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 aflected 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, Opaleyeye 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 parvovirus B19 using both serological and molecular methods (Q-PCR and sequencing) to gain more insight into the epidemiologic and clinicopathologic 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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