Asian Pacific Journal of Tropical Disease

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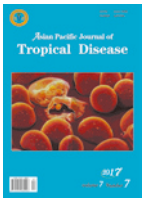

Original article https://doi.org/10.12980/apjtd.7.2017D7-14

Detection of parvovirus B19 IgM in patients with sickle cell disease in Lagos, Nigeria

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ARTICLE INFO

Article history:
Received 23 Jan 2017
Received in revised form 11 May 2017
Accepted 12 Jun 2017
Available online 2 Jul 2017

Keywords:
Parvovirus B19
Sickle cell disease
Infection
Lagos

ABSTRACT

Objective: To determine anti-B19V IgM seroprevalence among sickle cell disease (SCD) patients and patients with other blood genotypes.
Methods: In this study, a commercially available ELISA kit (Parvovirus B19 RIDASCREEN Biopharma, Germany) was used to analyze 93 serum samples, comprising 68 SCD patients and 25 control samples from individuals with other blood genotypes aged between 1 and 35 years.
Results: Anti-B19V IgM was detected in 10 (~15%) samples of the SCD patients, in which amongst the SC, 1 (25%) positive was recorded, CC had 2 (~67%) positive cases and SS had 7 (~11%) positives. Among the study control group, a 16% seropositivity was observed, in which among those with genotype AA, 1 (20%) was positive, for those with AS, 1 (~7%) positive was recorded, for AC, 3 (50%) were positive. The age range of 11–20 years showed a positivity of 3 (~4%), those of 21–30 years showed 12 (~24%) positivity and both 1–10 years and > 30 years had no positive case recorded.
Conclusions: This study showed that B19V is a significant blood borne pathogen in our environment and concerted effort is needed to advance strategies to curb this infection and diminish the common severe complications usually associated with this virus infection among SCD patients.

1. Introduction

The form of clinical disease associated with human parvovirus B19 (B19V) varies and depends on both the haematological and immunological status of the infected individual[1]. B19V infections are frequently associated with mild disease, but in immunocompromised and anemic patients, complications can arise[2]. B19V was discovered accidentally by Yvonne Cossart's group in 1974[3]. It is a small (22–24 nm) non-enveloped virus containing a single stranded DNA of 5 600 nucleotides and composed of two capsid proteins, VP1 (84 kDa) and VP2 (58 kDa), and a non-structural protein, NS1 (77 kDa). The two capsid proteins have an overlapping open reading frame and VP1 is identical to VP2 except for an additional 227 amino acids at the N–terminus. Above 95% of capsid proteins are VP2, whereas VP1 accounts for less than 5%. These B19V structural proteins are known to determine the virus tropism and stimulate neutralizing antibody response[4-6]. Many strains with substantial sequence diversity have been studied, resulting in the identification of three different genetic clusters designated as genotypes 1, 2, and 3, and these are responsible for most human infections worldwide[7,8].

Parvovirus B19 is associated with multiple conditions and Nigeria is among the highest ranked in the worldwide occurrence of sickle cell disease (SCD) with several etiologies and associated complications[9-11]. Rajput *et al.*[12] suggest that parvovirus B19 can have significant marrow aplastic effects even in immunocompetent individuals. There are currently no approved vaccines for the prevention of B19 virus in Nigeria. However, virus like particles-based parvovirus B19 vaccine candidates have been produced by co-expressing VP2 and either wild-type VP1 or phospholipase-negative VP1 in a regulated ratio from a plasmid in *Saccharomyces cerevisiae*[13]. Although the outcome of transient red cell aplasia occurrences in children with SCD is mostly non-threatening, many are treated with red cell transfusions to minimize the threat of circulatory collapse due to severe anaemia[14]. Hydroxyurea may reduce the requirements for blood transfusion and may attenuate symptoms during transient aplastic crisis episodes caused by parvovirus B19[15]. In Nigeria, this virus is not routinely screened

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Ethical approvals were obtained from the University of Lagos Medical Centre, Lagos, Nigeria. Verbal consent was obtained from each of the participating patients and the experiment was performed accordance to Helsinki declaration.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

for during blood transfusion, and there is paucity of data on the burden of B19V in different blood genotypes and people with SCD. Accurate and current epidemiologic data on the prevalence of B19V and its associated complications in SCD and other haemoglobinopathies are critical for evaluating the prospective impact of viral prevention programmes in these populations in Nigeria. This study shows the current seroprevalence IgM antibodies among patients with SCD and individuals with other blood genotypes.

2. Materials and methods

2.1. Study subjects

A total of 93 individuals aged between 1 and 35 years were recruited for this study between June and August, 2015. These consisted of 68 SCD patients and 25 individuals of other genotypes. Blood samples (5 mL) were collected from study subjects, spun at 3000 r/min for 5 min, separated with micropipettes and stored at –20 ˚C until further analysis. Ethical approvals were obtained from the University of Lagos Medical Centre, Lagos, and the General Hospital, Randle, Surulere, Lagos where samples were obtained. Verbal consent was obtained from each of the participating patients and the experiment was performed accordance to Helsinki declaration.

2.2. Detection of anti-B19V antibodies

Immunoglobulin (Ig) M antibodies against B19V was detected using the commercially available ELISA kit (Parvovirus B19 RIDASCREEN Biopharma, Germany). This assay, which uses the VP1 and VP2 recombinant proteins to capture IgM, was performed according to the manufacturer's instructions using peroxidase-labeled rabbit anti-human IgM as the secondary antibody, tetra methyl benzene as a substrate and 1 mol/L H_2SO_4 as a stop solution. Briefly, 100 μL standard controls were added into the first three wells, then 100 μL of diluted sample (ratio 1:10) was dispensed into subsequent wells and incubated at 37 ˚C for 30 min. At the end of incubation time, the wells were washed four times with wash buffer to remove unbound antibodies after which 100 μL of conjugate was added to each well and incubated at 37 ˚C for 30 min. The washing process was also carried out four times before adding 100 μL of substrate. A 50 μL of the stop solution was added to the wells and incubated for 15 min after which the result was read macroscopically and absorbance read spectrophotometrically at a wavelength of 450 nm.

2.3. Statistical analysis

The data obtained were subjected to descriptive statistical analysis using SPSS Version 20. *Chi*-squared test was used to determine association between gender, age and genotype with B19V infection and the differences were considered to be statistically significant when the *P*-value obtained was less than 0.05

3. Results

A total of 93 samples were analyzed consisting of 68 SCD patients and 25 controls from individuals with other blood genotypes including AA, AS, and AC. Seropositivity to anti-B19V IgM among SCD patients was 10 (~15%) whereas the normal and carrier individuals of other genotypes used as control showed a seropositivity of 5 (~16%) (Tables 1 and 2). Table 1 shows the distribution of B19V infection among the specific SCD genotypes in which CC showed a prevalence of ~67%, whereas SC and SS were 25% and ~11% respectively. Among the study control with genotype AA, 1 (20%) was positive, for those with AS, 1 (~7%) positive was recorded, for AC, 3 (50%) were positive, as illustrated in Table 2. The age distribution of anti-B19V IgM positivity is given in Table 3 in which the age range of 11–20 years showed a positivity of 3 (~4%), those of 21–30 years showed 12 (~24%) positivity and both 1–10 and > 30 years had no positive samples. Table 4 shows the sex distribution of anti-B19V IgM seropositivity in which 6 (~14%) males and 9 (~18%) females were positive. There was no significant association between gender and B19V infection $P < 0.05$.

Table 1
Distribution of anti-B19V IgM positivity among specific SCD genotypes.

Test results	CC	SC	SS	Column total
Negative	1 (33.3%)	3 (75.0%)	54 (88.5%)	58 (85.3%)
Positive	2 (66.7%)	1 (25.0%)	7 (11.5%)	10 (14.7%)
Row total	3 (4.4%)	4 (5.9%)	61 (89.7%)	68 (100.0%)

Table 2
Distribution of B19V IgM among the control group genotypes.

Test results	Blood Group			Column total
	AA	AC	AS	
Negative	4 (80.0%)	3 (50.0%)	13 (92.9%)	20 (83.9%)
Positive	1 (20.0%)	3 (50.0%)	1 (7.1%)	5 (16.1%)
Row total	5 (20.0%)	6 (24.0%)	14 (56.0%)	25 (100.0%)

Table 3
Age distribution of anti-B19V IgM positivity.

Test results	Age group (year)				Row total
	(1–10)	(11–20)	(21–30)	(> 30)	
Negative	5 (100.0%)	19 (86.4%)	40 (76.9%)	14 (100.0%)	78 (83.9%)
Positive	0 (0.0%)	3 (3.6%)	12 (23.1%)	0 (0.0%)	15 (16.1%)
Column total	5 (5.4%)	22 (23.7%)	52 (55.9%)	14 (15.1%)	93 (100.0%)

Table 4
Sex distribution of anti-B19V IgM positivity.

Test results	Male	Female	Column Total
Negative	38 (86.4%)	40 (81.6%)	78 (83.9%)
Positive	6 (13.6%)	9 (18.4%)	15 (16.3%)
Row total	44 (47.3%)	49 (53.3%)	93 (100.0%)

4. Discussion

This study revealed a 15% sero-positivity of B19V IgM among SCD patients in Lagos and this is significantly higher than the 5.3% previously reported in Lagos[16]. There has also been a lower 4.1% recorded in a recent study conducted among Israeli population[17]. The prevalence of 4% and 13.2% were observed among pregnant women in Oyo State[18] and Nasarawa State[19], respectively. Though this study showed a high prevalence of anti-B19V IgM among SCD patients, it was conversely lower than the findings of few other Nigerian studies, including Iwalokun *et al.*[9] in which 17.8% was recorded; Opaleye *et al.*[20] also recorded 32% among a non-hepatitis control group. These variations in the prevalence of anti-B19V IgM may not be unconnected with the characteristics of the populations studied, the sample sizes used in these studies, and the assay kits' sensitivities and specificities.

Among the SCD patients recruited in this study, individuals with genotype CC showed the highest anti-B19V IgM seropositivity of 67%, compared to SS in which 12% was observed, and SC in which 25% was recorded. The authors are not aware of any studies in Nigeria that have determined the prevalence of anti-B19V IgM in specific genotypes of SCD patients. However, the distribution of the SCD genotypes follows the pattern of some studies that have defined the prevalence of the different haemoglobin genotypes in Nigeria[21-23].

The age distribution of anti-B19V showed the highest positivity among the 21–30 age group in which 23% was recorded. This was different from the findings of Iwalokun *et al*.[9] who recorded the highest prevalence among the ⩽ 12 year patients; and Alao *et al*.[24] observed the highest seroprevalence among the > 15 year age group. Although, these studies investigated children population.

There was a higher B19V IgM (16%) positivity observed among the control group which is made up of individuals with genotypes AA, AS, and AC. This suggests that there is also active virus infection among non-SCD individuals in our environment. Elsewhere, Zhou *et al*.[25] had found 9.3% anti-B19V IgM positivity among pregnant women with first-trimester causing spontaneous abortions.

Conclusively, this study showed that B19V is a significant blood borne pathogen in our environment and also justifies the need for development of B19V prevention policies to diminish the frequent and severe complications as a result of B19V infections among SCD patients in Nigeria.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors thank the management and staff of Medical Center Laboratory, University of Lagos, Nigeria, for giving us access to their patients.

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