



Published in final edited form as:

Arch Basic Appl Med. 2018 February ; 6(1): 113–117.

Prevalence and Genotypes of Parvovirus B19 Among HIV Positive Children in Ibadan, Oyo State, Nigeria

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Abstract

Human Parvovirus B19 (B19V) is a global infection with over 50% of infected children residing in sub-Saharan Africa. It causes persistent anaemia under immuno-compromised states such as HIV infection, thereby complicating the course of HIV infection. This study was therefore designed to determine the prevalence and genotypes of B19V among HIV positive children. Blood specimens were collected from HIV positive children and genomic DNA extracted and assayed for the presence of Parvovirus B19 DNA using polymerase chain reaction and the product detected by gel electrophoresis. Amplicons for positive PCR were purified and sequenced for genotype analysis. For the purpose of comparison (differences in the sequences of the NS1/VP1u region), nine HIV negative children were enrolled in this study. Two (1.3%) of the 158 HIV infected children were positive for Parvovirus B19 DNA. Analysis of the results showed a low prevalence of Parvovirus B19 among HIV positive children but a significant relationship was established between Parvovirus B19 infection and the severity of anaemia ($p=0.015$). Phylogenetic analysis of the sequence data showed that all the B19 virus isolates detected in this study were genotype 1. This study therefore has been able to give an insight to the prevalence and circulating genotypes of Parvovirus B19 among HIV infected children and also establishing a relationship between anaemia and parvovirus B19 infection.

Keywords

Parvovirus B19; HIV; Anaemia; Genotype

INTRODUCTION

Human Parvovirus B19 (B19) is a global infection affecting both healthy and immuno-compromised individuals. It has become a public health challenge in recent time with over 50% of infection in children residing in sub-Saharan Africa (Toan *et al.*, 2013). Infection with the Parvovirus B19 seropositivity rate is about 5–10% in children 2–5 years, 50% in teenagers about 15 years and 60% in adults 30 years and above. Although antibody to the virus is prevalent in the general population, viremia/presence of viral DNA is thought to be rare (Gallinella *et al.*, 2003). In immuno-competent individuals, B19 causes subclinical infections while in immuno-compromised individuals such as those infected with HIV, it causes persistent anaemia (Azadmanesh *et al.*, 2015).

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A World Health Organization report in 2013 showed that an estimated 3.2 million children below the age of 15 years were living with HIV/AIDS. The 2013 UNAID gap report also showed that more than 90% of paediatric HIV infection occurred in sub Saharan Africa and that 75% of these children die before their fifth birthday. In 2010, WHO recommended initiation of Antiretroviral Therapy (ART) from age six weeks in all HIV-exposed children (Dahourou *et al.*, 2016) but this recommendation has not been strictly adhered to in most sub Saharan countries including Nigeria. Most HIV infected children start treatment late, after six weeks thereby complicating HIV infection by co-morbidities such as malnutrition, anaemia, opportunistic and endemic infections such as helminths, malaria (Jesson and Leroy, 2015).

Anaemia is a common condition among HIV-infected children this complicating the course of HIV infection and contributing to disease progression and mortality (Mwiru *et al.*, 2015). It is therefore important to prevent and treat these co-morbidities so as to greatly reduce pediatric HIV-related morbidity and mortality. Generally, anaemia is attributed to different causes ranging from deficiency in certain nutrients that are important in the production of red blood cells to chronic infections such as HIV/AIDS, cancer etc.

There is limited information on the burden of anaemia as a result of Parvovirus B19 infection in children that are HIV infected. Hence, this study was designed to determine the prevalence of Parvovirus B19 among anaemic HIV positive children.

MATERIALS AND METHODS

Ethics Statement

Ethical approval for this study was obtained from the UI/UCH IRB committee.

Study Location

This study was carried out in Ibadan, a large metropolitan city that lies in the geographical coordinates of 10°23' 0"N, 12° 5' 0" E. It is the third most populous city in Nigeria and located in the southwestern region of Nigeria with about 5 million people and a land area of 3,080km².

Study Design

This was a cross sectional study aimed at detecting the prevalence of Human Parvovirus B19 among anaemic HIV positive children as well as analysing the circulating genotypes found in Ibadan.

Study population and recruitment

Blood samples were collected from HIV positive children attending the HIV clinic and those with red blood cells lower than 50g/dL were particularly recruited for this study.

Laboratory Procedure

DNA extraction and Polymerase chain reaction—Genomic DNA was extracted from whole blood samples of the 158 children included using an in-house protocol for genomic

DNA extraction. 600µl of erythrocyte lysis buffer was added to 200µl of whole blood in an Eppendorf tube. Each tube containing the mixture was inverted gently and then incubated for 5 minutes at room temperature after which the mixture was centrifuged at 3000 rpm for 2 minutes. The supernatant was discarded and 100µl of erythrocyte lysis buffer and 300µl of cell lysis buffer were added. The mixture was incubated for 20 minutes at room temperature again after which 400µl of isopropanol was added and the mixture centrifuged at 14000 rpm for 15 minutes. The supernatant was discarded and 1ml of ethanol was added and the mixture was centrifuged for 5 minutes at 14000rpm. The supernatant was discarded and each tube left to dry after which 50µl of nuclease free water was added and the tube was vortexed for 10 seconds to resuspend the DNA pellets.

The extracted DNA was subsequently used as template for the detection of Parvovirus B19 DNA in a nested-PCR procedure at an amplicon size of about 1170-bp of the region spanning the NS1-VP1u junction. The primers used have been previously described by Hubschen *et al.* (2009). The first PCR round was a 25-µl reaction mixture containing 5µl of DNA, 0.5µl of each primer (e1855f and B19R1), 5µl of a 5x concentration PCR mix (Jena ready to load Taq master) and 14µl of PCR water which amplified the NS1/VP1u region of the genome (1241bp). 1µl of the product from the first PCR was used as DNA template for the second round PCR. A 25µl reaction containing 1µl of the first round product, 0.5µl each of the second round primers (e1863f and B19R2), 5µl of a 5x concentration PCR mix (Jena ready to load Taq polymerase) and 18µl of PCR grade water. The cycling condition for both rounds included an initial denaturation of the DNA at 94°C for 2 minutes, followed by 40 cycles of (94°C for 30 seconds, 58°C for 30 seconds, 72°C for 90 seconds) and a final extension step at 72°C for 8 minutes.

Gel Electrophoresis—After the second round of PCR, 5µl each of the amplicons was carefully loaded into a well of 2% agarose gel while 5µl of the molecular ladder was loaded into first well of the agarose gel and run horizontally at 120volts for 35 minutes.

DNA purification, genotyping and phylogenetic analysis—Purification of the second round PCR products that were positive after the gel electrophoresis procedure was done using the Wizard Purification Kit Protocol after which the purified product was sequenced.

The sequences were read by CLC main workbench 5.5 program. The consensus sequences were aligned with MEGA version 5.2 using the Clustal W algorithm against Parvovirus B19 reference sequences obtained from GenBank with the following accession numbers and countries of origin South Africa (FJ904121, FJ904120, GQ337720, GQ337719), Nigeria (FN295710), Serbia (FN295732, FN295733), United Kingdom (M24682, M13178), USA (NC000883). Nucleotide and amino acid sequence pair distances were calculated and phylogenetic tree was constructed by the neighbour joining method with 1000 bootstrap resampling using the MEGA 5.2 software program.

Statistical Data Analysis

Analysis was carried out using descriptive statistics with mean and standard deviations (SD). Chi square test was used to assess difference in proportion (association) between categorical

variables while independent T-test was used to assess the difference in means for quantitative data. Data analysis was performed using the Statistical Package for Social Sciences (SPSS) programme version 20 (Chicago, IL, USA) at 5% significant level.

RESULTS

A total of 158 HIV infected children with a mean age of 9.5 ± 3.9 years were enrolled in this study. 93 (58.86%) were males and 65(41.14%) were females. Parvovirus B19 DNA was detected in two (1.3%) of the children. As shown in the table 1, Parvovirus B19 was detected in children with moderate and severe anaemia thereby establishing a relationship between Parvovirus B19 virus infection and severity of anaemia ($p=0.015$). There was no significant relationship between age of the children and Parvovirus B19 virus infection.

Phylogenetic analysis showed that the two samples sequenced from HIV positive samples and nine Parvovirus B19 isolates from HIV negative children belong to genotype 1. Further analysis to compare the divergence of the strains obtained from this study with genotype 1A from other countries revealed close relationship with the Serbian strain (Figure 2).

Nucleotide sequence changes among the HIV positive and negative children are shown in table 3 while the amino acid mutations observed in the sequences are shown in table 4

DISCUSSION

To date, few studies have been conducted globally on the prevalence of B19 DNA in HIV infected individuals especially among children. Most of the data on the epidemiology of Parvovirus B19 is on the sero-prevalence of the virus. The prevalence of Parvovirus B19 among HIV infected children in this study was 1.3%. The prevalence of Parvovirus B19 found in this study is similar to the result from a study in Brazil (Pereira *et al.*, 2014).

Anaemia is known to be a complication in HIV infection and one of such is the inability of seriously immune-compromised individuals to produce neutralizing antibodies against Parvovirus B19. Therefore, it can be inferred to some extent that Parvovirus B19 can be considered and included as an aetiology for anaemia in the diagnosis of the causes of anaemia in HIV infection. It is also important to note that the seemingly low prevalence of infection in this study might be as a result of antiretroviral treatment.

Some studies have observed low prevalence of Parvovirus B19 related anaemia in HIV infected individuals on highly active antiretroviral therapy (Pereira *et al.*, 2014, Aghakhani *et al.*, 2016). Genotype 1A was found in all the samples sequenced in this study and all the sequences were compared with some sequences from different countries in the Genbank. The sequences obtained in this study are closely related to that found to circulate in Serbia (Serbia 1102 with the ascension number FN295732 in the Genbank). The presence of genotype 1 in the study population supports reports from other studies (Hubschen *et al.*, 2009, Ivanova *et al.*, 2016) that genotype 1 is the predominant strain found globally.

On the whole, this study has given an insight into the prevalence of Parvovirus B19 virus among anaemic HIV infected children. The circulating genotype among the population studied has also been documented. However, some mutations were observed at some specific

regions on the NS1/VP1u region sequenced. Further studies need to be carried out to ascertain the importance of these mutations considering the fact that the proteins of this region play important roles in viral DNA replication, eliciting neutralizing of antibodies that are responsible for elimination of the virus from the peripheral blood as well as inducing lifelong immunity. Understanding these mutations therefore might help in drug design as well as development of an effective vaccine against Parvovirus B19 virus.

Acknowledgments

The authors are grateful to the staff and the Head of Department of the Department of Virology, University College Hospital, University of Ibadan, Ibadan, Nigeria for their technical and financial support of this study. Data analysis and writing of this paper was supported by the University of Ibadan Medical Education Partnership Initiative Junior Faculty Training Programme (UI-MEPI-J) project funded by Fogarty International Center, National Institute of Health under Award Number D43TW010140. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding organizations.

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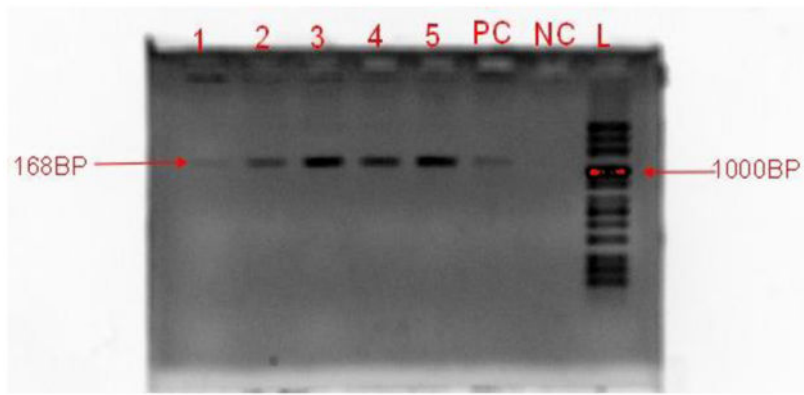


Figure 1. Gel Electrophoresis Image Showing Amplified Products of the Parvovirus B19 DNA (Band size =1168bp).

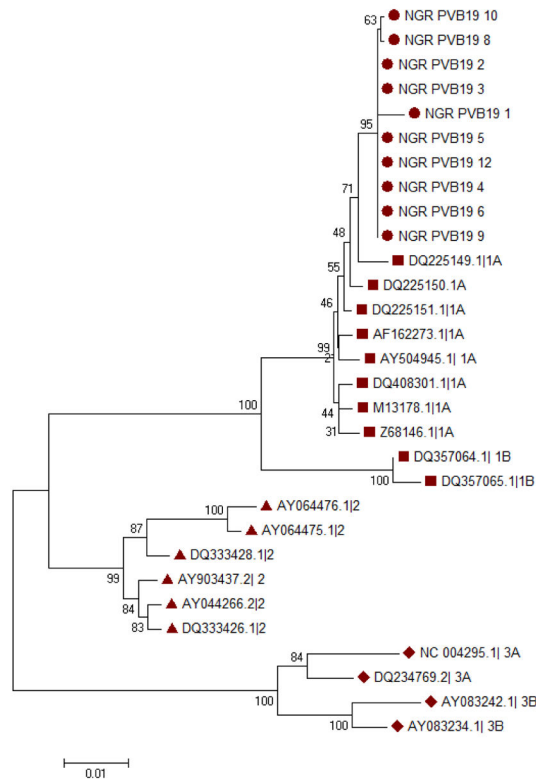


Figure 2. Phylogenetic tree of the NS1/VP1u region of the Human Parvovirus B19 genome. The genotypes circulating in the study population are indicated by circles while the reference strains genotypes 1, 2 and 3 are indicated by squares, triangles and diamonds respectively. Multiple sequences alignment and phylogenetic tree were constructed using Clustal W and neighbour joining algorithm in MEGA 5.05 software. Statistical significance of the tree topology was tested by 1000 bootstrap replication. Only bootstrap values above 50% are displayed at the nodes.

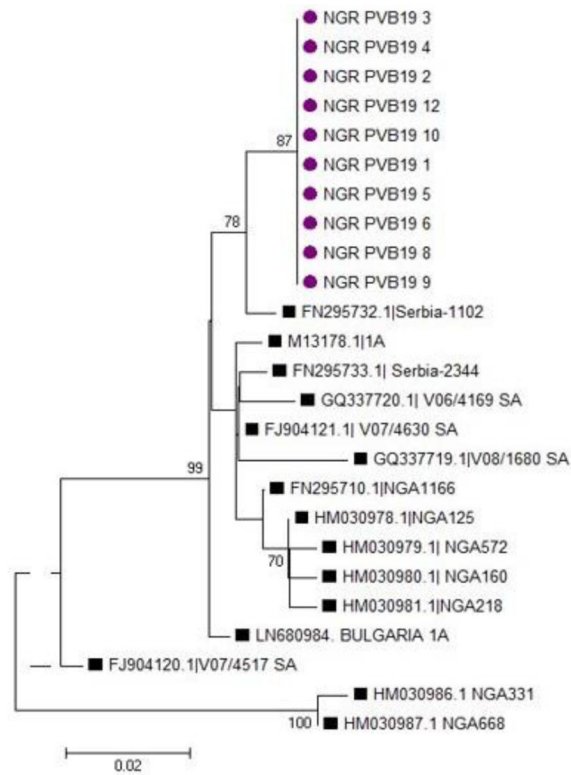


Figure 3.

Phylogenetic tree of the NS1/VP1u region of the Parvovirus B19 genome showing comparison of samples with genotype 1 from different countries. The genotype 1A circulating in the study population is indicated by circles while the reference strains are indicated by squares. Multiple sequences alignment and phylogenetic tree were constructed using Clustal W and neighbour joining algorithm in MEGA 5.05 software. Statistical significance of the tree topology was tested by 1000 bootstrap replication. Only bootstrap values above 50% are displayed at the nodes.

Table 1

Demographic Characteristics of Participants

Characteristics	Parvovirus B19		χ^2	<i>p</i>
	Positive (%)	Negative (%)		
Sex				
Male	1 (1.1%)	92 (98.9%)	0.066	0.798
Female	1 (1.5%)	64 (98.5%)		
Age				
2 years	1 (14.3%)	6 (85.7%)		
3–5 years	0 (0%)	16 (100%)	22.423	0.169
6–10 years	1 (1.4%)	68 (98.6%)		
>10 years	0 (0%)	66 (100%)		

Table 2

Clinical Characteristics of Participants

Characteristics	Parvovirus B19		χ^2	<i>p</i>
	Positive (%)	Negative (%)		
Anaemic status				
<i>None</i>	0 (0%)	22 (100%)	10.444	0.015
<i>Mild</i>	0 (0%)	52 (100%)		
<i>Moderate</i>	1 (1/3%)	76 (98.7%)		
<i>Severe</i>	1 (14.3%)	6 (85.7%)		
CD4% Value				
<i>Below Normal</i>				
<i>Range (<40%)</i>	2 (1.4%)	136 (98.6%)	0.260	0.610
<i>Normal</i>				
<i>Range (40%)</i>	0 (0%)	20 (100%)		

Table 3

Nucleotide sequence changes in the NS1/VP1u region sequenced

Position	2161	2162	2163	2193	2195	2404	2424	2448	2451	2532	2663	2711	2758	2196	3104	3105	3106	3136	HIV Status	
Nucleotide sequence Changes	A-G	G-C	C-T	T-A	A-?	T-C	A-G	G-C	G-A	A-G	A-G	G-C	T-C	G-A	G-A	T-?	A-?	G-C		
Virus ID No	NGR PVB19 1	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	Positive
	NGR PVB19 2	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	Y	Y	Positive
	NGR PVB19 3	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	Negative
	NGR PVB19 4	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	Negative
	NGR PVB19 5	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	Negative
	NGR PVB19 6	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	Negative
	NGR PVB19 8	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Negative
	NGR PVB19 9	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	Negative
	NGR PVB19 10	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Negative
	NGR PVB19 12	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	Negative

Key: Y=Yes; N=No

Table 4

Amino Acid Mutations in the NS1/VP1u Region Sequenced

Position	721	731	878	904	920	1035	1046
Amino acid changes	S-A	F-L	K-R	G-A	Stop-G	G-D	D-H
	(Ser to Ala)	(Phe to Leu)	(Lys to Arg)	(Gly to Asp)	(Stop to Glu)	(Gly to Asp)	(Asp to His)
Virus ID No	NGR PVB19 1	NGR PVB19 1	NGR PVB19 1	NGR PVB19 1	NGR PVB19 1	NGR PVB19 10	NGR PVB19 2
			NGR PVB19 2	NGR PVB19 2	NGR PVB19 2		
			NGR PVB19 3	NGR PVB19 3	NGR PVB19 3		
			NGR PVB19 4	NGR PVB19 4	NGR PVB19 4		
			NGR PVB19 5	NGR PVB19 5	NGR PVB19 5		
			NGR PVB19 6	NGR PVB19 6	NGR PVB19 6		
			NGR PVB19 8	NGR PVB19 8	NGR PVB19 8		
			NGR PVB19 9	NGR PVB19 9	NGR PVB19 9		
			NGR PVB19 10	NGR PVB19 10	NGR PVB19 10		
			NGR PVB19 12	NGR PVB19 12	NGR PVB19 12		