Parvovirus B19 Antigenamia among Sickle Cell Disease Patients in Selected Hospitals in Kano Metropolis

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ABSTRACT

Human Parvovirus B19 is a small single stranded positive sense DNA virus, from the family Parvoviridae, genus Erythrovirus and Human Parvovirus B19 type species, causing transient aplastic crises in sickle cell disease patient. The aim of the study was to determine the prevalence of Parvovirus B19 in sickle cell anaemia patients in selected hospitals in Kano metropolis. A total of 90 participants with sickle cell disease were recruited for the study in four main hospitals in Kano metropolis; Murtala Muhammad Specialist Hospital, Aminu Kano Teaching Hospital, Hasiya Bayero Paediatrics Hospital and Abdullahi Wase Specialist Hospital and the socio-demographic factors were recorded using structured questionnaire. Parvovirus B19 antigen was assayed using Enzyme Linked Immunosorbent Assay kit (Melsin Medical Co., Limited). Data was analyzed using SPSS version 20.0. The prevalence of Human Parvovirus B19 antigen from the study subjects was 2 (2.2%). The positive subjects were found to be all males. It showed that the virus is in circulation among the study subjects which prompt the need for a large scale community based study to ascertain the burden of the virus in the study area.

Keywords: Parvovirus B19, Sickle Cell Disease, Patients, Hospital, Kano Metropolis

INTRODUCTION

Human Parvovirus B19V was discovered accidentally by Yvonne Cossart's group in 1974.[1] The form in which human parvovirus B19 (B19V) affects individuals is dispersing and depends on both the haematological and immunological status of the infected individual.[2] This virus targets the erythroid progenitor cells in the bone marrow by binding to the glycosphingolipid globoside (Gb4), leading to large receptor-induced structural changes triggering cell death either by lysis or by apoptosis mediated by the non-structural (Ns1) protein.[3] The virus has high tropism to red blood cells progenitor leading to infection of bone marrow and transient arrest of erythropoiesis.[4] In these diseases erythroid progenitor cell formation is increased to compensate for red blood cell lysis.[5] Parvovirus B19 infection can suppress erythropoiesis and induce acute erythroblastopenia, which is often referred to as transient aplastic crisis.[5] Aplastic crises are acute worsening of the patient's baseline anaemia, producing pale appearance, fast heart rate, and fatigue.[4] Parvovirus infection almost completely prevents red blood cell production for two to three days.[6] It was implicated as the aetiologic agent of severe aplastic crises in children with sickle-cell disease (SCD), in which this infection can evolve into various life-threatening conditions[7] including acute encephalopathy,[8] nephritic syndrome,[9] splenic sequestration[10] and fatal bone marrow embolism.[11] Sickle-cell disease (SCD) is a group of blood disorders typically inherited from one's parents.[12] The most common type is known as sickle-cell anaemia (SCA). These results in an abnormality in the oxygen-carrying capacity of haemoglobin (haemoglobin S) found in red blood cells. This leads to a rigid, sickle-like shape under certain circumstances. A number of issues may develop, such as a painful attacks "sickle-cell crisis",[13] anaemia, swelling seen in the hands and feet, bacterial infections, and stroke.[14]

The aim of this study was to determine the seroprevalence of parvovirus B19 in sickle cell anaemia patients in Kano metropolis.

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MATERIALS AND METHODS

Area of the study

This research was conducted in some selected hospitals in Kano metropolis that

included Murtala Muhammad Specialist Hospital, Aminu Kano Teaching Hospital, Hasiya Bayero Paediatrics Hospital and Abdullahi Wase Specialist Hospital. The State is located at the north-western Region of Nigeria laid between latitudeand longitude with a total land area of 20,760sq kilometer.[15] Kano State borders Katsina to the north-west, Jigawa State to the north-east, Bauchi to the South- East and Kaduna to the South-West.

Study population

Ninety (90) participants (sickle cell disease patients) were recruited in this study, and they were grouped according to age and gender.

Research design

This was a hospital based Cross sectional study which was conducted from June to November, 2018.

Ethical consideration and clearance

Ethical approval to conduct this research was granted by the Research Ethics Committees of Aminu Kano Teaching Hospital and from Hospital Management Board in Kano metropolis. The participants' consent was also obtained.

Sample collection and processing

Three milliliter (3ml) of venous blood sample was collected aseptically from all the participants and dispensed into a plain container as described by Cheesbrough.[16] The sample was allowed to clot, and then centrifuged at 3000rmp for 5minutes.The serum was transferred into pre-labeled 5ml plain containers and it was stored at -20^{0}C until required for analysis.

Sample analysis

Serum sample was assayed for parvovirus B19 Antigen, using Human Parvovirus B19 Antigen ELISA assay kit technique (Melsin Medical Co., Limited). All reagents were prepared before starting the assay procedure.

Fifty microliter (50 l) of positive control and negative control was added separately to the positive and negative well. Ten microliter (10 l) of test sample was added, and 40 l of sample diluent was added to testing sample well, while the well for blank nothing was added.

Hundred microliter (100 l) of horseradish conjugate reagent was added to each wells, it was covered with an adhesive strip and incubated for 60 minute at 37^{0}C. Each well was aspirated and washed, the process was repeated four times for a total of five washes, it was washed by filling each well with wash solution. Remaining wash solution was removed by decanting; the plate was inverted and blotted against clean paper towels. Fifty microliter (50 l) of chromogen solution A and chromogen solution B was added to each well; it was gently mixed and incubated for 15 minutes at 37^{0}C. Fifty microliter (50 l) stop solution was added to each well, the plate was gently tapped to ensure thorough mixing. The Optical Density was read at 450nm using a micro titer plate, it was read within 15 minutes.

Statistical analysis

Data obtained was analyzed using SPSS version 20.0 packages, using a ninety-five (95%) confidence interval, a p-value of less than 0.05 was considered as significant difference. The mean, median, standard deviation and other parameters of statistical location were generated.

RESULTS

Out of 90 participants, 2 were positive while 88 were negative for Parvovirus B19 infection. Seroprevalence of 2(2.2%) Parvovirus B19 infection was found among sickle cell anaemia patient in Kano metropolis.

Table 1 shows that females 56(62.2%) were higher than male participants 34(37.8%). Among age groups, those found to fall among (11-20) years are said to be higher with a frequency of 67(74.4%), followed by a frequency of 11(12.2%) among (21-30) years age group, participants between (0-10) years are 8(8.9%), and those found to fall among (31-40) years were 4(4.4%). Among the marital status, participant that are single are said to have a maximum frequency of 83 and a total of (93.3%), followed by those that are married with a frequency of 5 and a total of 5.6%, and 1(1.1%) participant that was separated. Among the educational level, participants that have an SSCE qualification and those that are still in secondary school are said to have a high frequency of 40(44.4%), these was followed by those in junior secondary school 24(24.6%),

participants in primary school are 19(21.1%), participant in tertiary institution are 5(5.6%), those that are not in school have a frequency 2(2.2%). Occupational level of the participants showed that students are dominant 74(82.2%), those without anything doing 10(11.1%), participant that are traders 5(5.6%), and only 1(1.1%) participant that was civil servant.

Table 2 shows distribution of the virus in the study population. Two (2.2%) of the study subjects were positive for the Parvovirus antigens. The antigen positive subjects were found to be males, while all the females were found to be negative, though there was no statistical significant relationship between the presence of the virus and gender (p>0.05).

Distribution of Parvovirus B19 among age groups is shown in table 3. Age group 11-20 years has the highest participants 67 among the study subjects in which 2(2.2%) were positive for Parvovirus B19. Eight (8) recruited participants were in the age group 0-10years. Eleven (11) participants were in age group of 21-30 years, and 4 participants within the age group of 31-40years. There was no statistical significant relationship between the presence of the virus and age p-value (>0.05).

Distribution of parvovirus B19 infection in relation to clinical manifestation, table 4 showed that among those positive, one has myocardial infarction the other does not. Forty eight (48) of the negative participants for parvovirus B19 had myocardial infarction while 40 do not. Among the positive subjects, 1 had lethargy the other did not, while 71 participants negative for the infection were found to have lethargy and 17 of them do not. Fifty (50) of the negative participant were found to have nephritic syndrome while 38 of the negative participant do not, one positive participant is said to have nephritic syndrome the other do not (p >0.05). The positive participants were found to have chest pain. Among the 88 participant found to be negative, 69 have chest pain while 19 do not (p >0.05). Two participants that were positive both had splenic sequestration, while 59 participants found to be negative with the virus have splenic sequestration while 29 do not (p>0.05).

Distribution of Parvovirus B19 infection in relation to transfusion history was shown in table 5 which showed that the 2 positive participants for the infection fall under the transfusion history category of rarely and none. Among those negative subjects, 12 were frequently transfused, 32 were occasionally transfused, 16 were rarely transfused and 28 were never transfused.

Distribution of Parvovirus B19 infection among hospitals used shows that among the four hospitals used, a total of 56 participants were recruited from Murtala Muhammad Specialist Hospital out of which 1 was positive and 55 negative, 6 participants were recruited from Aminu Kano Teaching hospital, 1 among this 6 participants was positive, 8 and 20 participants were recruited from Hasiyah Bayero Paediatric Hospital and Abdullahi Wase Specialist Hospital in which all were negative as presented in table 5.

Table 1: Distribution of Socio-demographic data base on frequency and percentage

Variables	Frequency	Percentage (%)
Gender		
Male	34	37.8
Female	56	62.2
Age (years)		
0-10	8	8.9
11-20	67	74.4
21-30	11	12.2
31-40	4	4.4
Marital status		
Single	84	93.3
Married	5	5.6
Separated	1	1.1
Educational level		
Primary	19	21.1
Junior secondary	24	26.7
Senior Secondary	40	44.4
Tertiary	5	5.6
None	2	2.2
Occupation		
Civil servant	1	1.1
Trader	5	5.6
Student	74	82.2
None	10	11.1
Total	90	100.0

Table 2: Distribution of Parvovirus B19 infection among genders

Gender	Parvovirus B19		
	Positive	Negative	P-value
Male	2	32	0.140
Female	0	56	
Total	2	88	

Table 3: Distribution of parvovirus B19 infection among age groups

	Parvovirus B19		
Age (years)	Positive	Negative	P-value
0-10	0	8	0.873
11-20	2	65	
21-30	0	11	
31-40	0	4	
Total	2	88	

Table 4: Distribution of parvovirus B19 infection in relation to clinical manifestation

Clinical manifestation	Parvovirus B19		
	Positive	Negative	P-value
Myocardial infection			
Yes	1	48	
No	1	40	0.9999
Lethargy			
Yes	1	71	
No	1	17	0.7236
Nephritic syndrome			
Yes	1	50	
No	1	38	0.9999
Chest pain			
Yes	2	69	
No	0	19	0.9999
Splenic sequestration			
Yes	2	59	
No	0	29	0.9999

Table 5: Distribution of Parvovirus B19 infection in relation to transfusion history

	Parvovirus B19		
Transfusion History	Positive	Negative	P-value
Frequently	0	12	0.522
Occasionally	0	32	
Rarely	1	16	
None	1	28	
Total	2	88	

Table 6: Distribution of Parvovirus B19 infection among hospitals used

	Parvovirus B19		
Hospitals	Positive	Negative	P-value
MMSH	1	55	0.092
AKTH	1	5	
HBPH	0	8	
AWSH	0	20	
Total	2	88	

Keys: MMSH-Murtala Muhammad Specialist Hospital; AKTH-Aminu Kano Teaching Hospital; HBPH-Hasiya Bayero Pediatric Hospital; AWSH-Abdullahi Wase Specialist Hospital.

DISCUSSION

Infection with Parvovirus B19 among sickle cell anaemia can result into various life-threatening conditions including acute encephalopathy, nephritic syndrome, splenic sequestration and fatal bone marrow embolism.[11,17] Prevalence of parvovirus B19 among sickle cell anaemia patients was 2.2% in the current study. Our finding was lower than that of the previous studies conducted in some parts of the country. Ujo *et al.*[18] reported a prevalence of (85.4%) SCA patients were positive for IgG antibodies against parvovirus B19; Iheanacho *et al.*[19] reported that 61.3% and 5.3% SCA patients were human Parvovirus B19 IgG and IgM positive respectively. Eleven percent (11%) Parvovirus B19 IgM prevalence was reported by Ayolabi *et al.*[20] in his study in Lagos among sickle cell disease patients. This difference could be due

to the approach followed for the detection of the Parvovirus B19 because in the current study viral antigen was targeted, depicting actual presence of the virus while previous studies targeted Parvovirus B19 antibodies. Iwalokun *et al.*[21] reported that Parvovirus B19 DNA was found in 2 (11.1%) of the 18 IgM seropositive SCA serum samples screened in his study. In Tanzania, B19-specific IgM and IgG antibodies were detected in 24 (9%) and 46 (17.4%) children, respectively.[22]

A total of 34 (37.8%) males were recruited for the study, 2 were positive, while 56 (62.2%) were female participants in the study. It has been found that there is no statistical relationship between parvovirus B19 infection and gender. This finding was in agreement with the report of Iwakolun *et al.*[21] in which the detection of Parvovirus B19 IgM was found be higher in males (7.2%) and slightly lower in females (7.1%). Our finding was similar to that of Sant`anna *et al.*[23] who also did not find statistical association between sex and parvovirus B19 infection from their study. Jegede *et al*. [24] reported an IgG prevalence of (42.6%) in females and (40.4%) in males which contrast our finding, though, the approach for the detection of the virus differs. Current study found that the positive cases where among age group 11-20 years (2.2%). Although, there was no statistical significant relationship found between the presence of the infection and age group in our current study.

Higher percentages (93.3%) of our participants were single and this study has not found statistical relationship between marital status and presence of human Parvovirus B19 infection.

Current study showed that those with the infection were at senior secondary school educational level. In respect to the socio-economic status of the participants, high prevalence was recorded among students this may be due to frequent outdoor activity that may led to exposure to the virus.

Clinical manifestations such as myocardial infarction, lethargy, nephritic syndrome, chest pain, splenic sequestration showed no statistical significance, those positive might be in their acute state, contrary to the study done by Iwalokun[21] and Obeid[25] in which similar results were reported showing that there is association between this clinical manifestations with parvovirus B19 among SCA patients.

History of transfusion showed no statistical significant relationship to viral infection, positivity was found among ones that were rarely and other that have never undertaken blood transfusion this agrees with the study conducted by Ujo *et al.*[18] who recorded high prevalence of HPV B19 among non blood transfused participants. The virus might have been transmitted through its major route such as through respiratory droplet.

In this study, there was no correlation of the various hospitals used with the virus, as it was found to be statistically non-significant, positivity was found among sample collected from MMSH and AKTH.

CONCLUSION

In this study the seroprevalence of Parvovirus B19 among SCA was 2.2%, and there was no statistical association between age and gender with respect to human Parvovirus B19 infection among SCA patients. Our result could improve the awareness of hospitals and laboratories by raising alertness of pathogens such as parvovirus B19 that may be hidden in plain sight among sickle cell disease patients.

It is recommended that every patient with Sickle Cell Disease who presents with abrupt fall in haemoglobin, reticulocytopenia, and an acute swelling of spleen should be tested for the presence of human Parvovirus B19.

Patient who are positive for human Parvovirus B19 infection should be focused on, as they are at risk of transient aplastic crises, and further studies should be carried out with a larger sample size.

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