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Seroprevalence of parvovirus B19 and its clinical effect among anaemic SCA patients in Northeastern Nigeria**AA Bukar¹, UAM Abjah¹, MB Kagu¹, AI Ladu¹, SB Zailani², AM Abba¹, MB Malah¹, JP Ambe³, BA Denu⁴**¹Department of Haematology and Blood Transfusion University of Maiduguri Teaching Hospital²Department of Medical Microbiology University of Maiduguri Teaching Hospital³Department of Paediatrics University of Maiduguri Teaching Hospital⁴Department of Internal Medicine University of Maiduguri Teaching Hospital**ABSTRACT**

Sickle cell anaemia (SCA) is a globally widespread genetic disorder affecting 5% of the world's over 6 billion people. Parvovirus infection and the resulting aplastic crisis is a recognised complication in individuals with SCA. Aplastic crisis increases the need for blood transfusion and its attendant risk of Transfusion Transmissible Infection (TTI). Hence there is a vicious cycle in which Parvovirus B19 causes aplastic crisis which in turn causes increased transfusion need; and transfusion increases risk of transfusion transmissible infection in which parvovirus B19 is included in certain parts of the world. Sickle cell anaemia is associated with foetal death and infection with parvovirus B19 increases the risk to early mortality. The objective of this study was to determine the seroprevalence of parvovirus B19 among SCA and compare with that of controls in the study area. Furthermore clinical and laboratory profile of subjects were analysed to identify possible correlation with parvovirus B19 seropositivity and explore the possibility of involvement of white cell and platelets. A total of 90 subjects comprising 45 consecutive SCA case subjects and 45 age- and sex- matched non SCA controls were studied in a cross sectional comparative study. Ten millilitres of blood was drawn from the antecubital fossa of each subject after obtaining informed consent. The 10mls of blood was divided into two aliquots, 4.5 mls was added into EDTA anticoagulated bottle and was used for basic complete blood count (CBC), while the remaining 5mls was added into a plain specimen container allowed to clot and serum obtained to test for anti- parvovirus B19 IgG and IgM using an immunochromatography based technique specifically BIOCARD™ Parvo B19 diagnostic test kit. There was male preponderance in the study. The SCA subjects comprised 26 males and 19 females (male to female ratio = 1.4:1), while the non- SCA controls comprised 25 males and 20 females (male to female ratio 1.3:1).. The analysis of anti- parvovirus B19 IgG antibody revealed a prevalence of 23.3% among SCA cases with 18.9% among controls. The haematological profile is not affected by IgG seropositivity. However pregnancy outcome revealed that the total number of stillbirths is 12 among IgG seropositive SCA cases which is higher than the 6 encountered in IgG seronegative SCA subjects; the difference is statistically significant ($p=0.04$).

INTRODUCTION

Sickle cell anaemia (SCA) runs a variable clinical course ranging from mild disease diagnosed incidentally, to crippling severe disease¹. Patients can be in relative good health termed 'steady state' which may be periodically interrupted by acute exacerbations called "Crisis" characterised by sudden onset and even fatal outcomes. The Crisis is traditionally classified as Vaso- occlusive, Aplastic,

Sequestration, and Hyperhaemolytic Crises^{2, 3, 4}. The last three are also called anaemic crises.

Parvovirus B19 attracts attention because aplastic crisis in SCA is caused mainly by Parvovirus B19 infection⁵. The anaemia induced by Parvovirus B19 infection is of minor clinical significance in healthy children and adults; however, it becomes critical in those afflicted with haemolytic diseases^{1, 5}. Parvovirus B19 was first discovered incidentally in

1975 by Yvonne Cossart and her coworkers while assessing for Hepatitis virus in a serum sample from a blood donor whence they first identified parvovirus-like particles in serum coded "number 19, panel B" hence the name Parvovirus B19⁶. It belongs to the genus *erythrovirus* of the family Parvoviridae which are single stranded small DNA viruses. The viral genome codes for three proteins; a non structural protein named NS1 and two structural proteins termed VP1 and VP2. The NS1 protein is cytotoxic to human target cells and is also responsible for packaging viral progenies while VP1 and VP2 are the minor and major components of the viral capsid with VP1 playing a major role in target cell attachment and cell entry⁷. The main cell affected by Parvovirus B19 is the developing erythroblast as the receptor which binds to the virus, identified as P blood group or Globoside, and is found in abundance on erythroblast and cells of the erythroid lineage⁸. Parvovirus B19 has a profound effect on the erythroid lineage by being cytopathic to it with resulting erythroblastopenia in the bone marrow, giving the erythroid progenitors a characteristic 'megaloblastic' appearance with peripheral reticulocytopenia⁹. There are reports of involvement of not only transient aplastic crisis (TAC) involving the red cells but also the white cell¹⁰ and thrombocytes¹¹. The present study evaluated the haematologic profile of subjects to deduce the effect of Parvovirus B19 seropositivity.

In addition to the haematological effect, intrauterine infections of fetuses in pregnant women do occur resulting in hydrops fetalis and foetal death¹². Parvovirus B19 has been implicated as a causative agent in fulminant hepatitis even in healthy individuals¹³. Parvovirus B19 can be transmitted through respiratory secretions, transplacentally and by transfusion of blood or blood products^{14, 15}. The study also tried to explore the relationship between stillbirth, history of transfusion and Parvovirus B19 in the study population.

Antibody response in Parvovirus B19 infection is directed against two capsid proteins namely VP1 and VP2, the majority of virus neutralizing antibodies responsible for life-long protection against reinfections are directed against the VP1-unique region^{16, 117}. IgM is mainly directed against VP2-specific epitopes¹⁷. These IgM antibodies may be present for a rather short period of two to ten weeks after acute infection^{16, 17}. IgG-antibodies against the nonstructural protein NS1 are preferentially found in patients who are unable to eliminate the virus and develop persisting viraemia or virus persistence in

distinct organs, e.g. synovial fluid, liver, bone marrow¹⁶.

Two detection methods are currently utilized for the identification of Parvovirus B19 infection, nucleic acid testing techniques and antibody detection methods¹⁷. The main nucleic acid testing (NAT) technique used for B19 detection is polymerase chain reaction (PCR) and both qualitative and quantitative PCR strategies exist for the detection of all three B19 genotypes¹⁸. Apart from total genotype detection, the balance between PCR assay sensitivity and specificity to facilitate detection of acute infection only is important¹⁸.

The second method for detection of Parvovirus B19 infection is the antibody detection method. And two types are utilized in the clinical setting namely Enzyme-linked immunosorbent assay (ELISA) and immunochromatographic techniques^{17, 18}. The sensitivity of ELISA was compared to nucleic acid technique by Sato et al¹⁹ and was found to be less sensitive and specific. However ELISA methods remain a very popular and valid method for the detection of Parvovirus B19²⁰

The search for prompt and type specific method for the detection of Parvovirus B19 infection led to the development and utilization of an immunochromatographic technique^{21, 22}. The immunochromatographic method in principle resembles ELISA but the enzyme is substituted by chromatographic materials that appear as visible colours on antigen- antibody reaction in the presence of positive Parvovirus B19 infection. The reaction targets a Parvovirus B19 Epitope- type specific VP2 Immunoglobulin G. Kaikkonen et al²² compared the sensitivity and specificity of this method to other EIA methods and found the diagnostic sensitivity to be 98% and a diagnostic specificity of 94%. In combination with conventional approaches, the epitope-type assays increase greatly the accuracy of Parvovirus B19 diagnosis besides the added advantages of enhancing prompt diagnosis and being less expensive²². The study went to utilise the immunochromatographic method to determine the seroprevalence of Parvovirus B19 infection.

METHOD

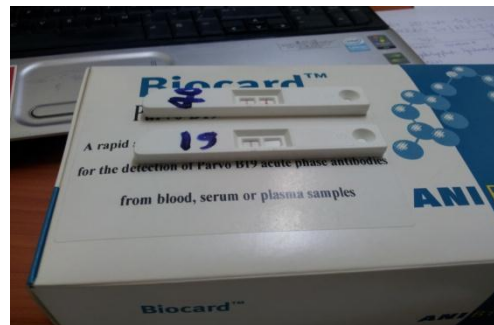
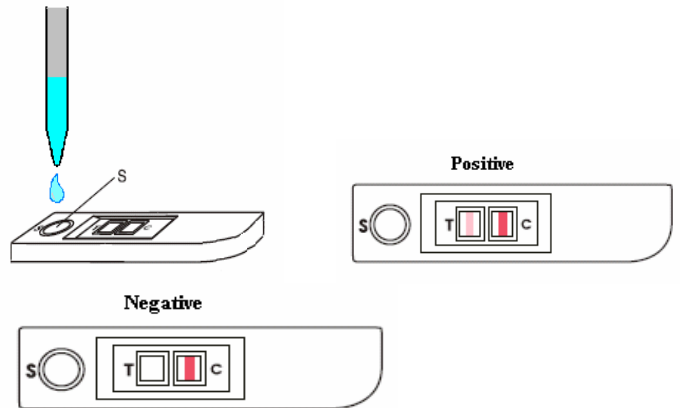
This study was carried out at Department of Haematology and Blood Transfusion of University of Maiduguri Teaching Hospital (UMTH) Maiduguri North Eastern Nigeria. Ninety consecutive subjects were recruited and these comprised forty five subjects confirmed to be HbSS and forty five HbAA

subjects as controls. There was male preponderance in the study with the SCA patients comprising 26 males and 19 females (male to female ratio= 1.4:1) while the non SCA controls comprised of 25 males and 20 females (Male to female ratio= 1.3:1). Nineteen (42.2%) of the SCA subjects were less than 15 years of age and twenty six (57.8%) were greater than or equals to 15 years of age. Among the Controls twenty (44.4%) were below 15 years of age while 25 (55.6%) were greater than or equals to 15years of age. After recruitment, Clinical assessment of study subjects were done and included administering a questionnaire containing information on demographic data, number of pregnancy and stillbirths. Subsequently 10mls of venous blood was obtained from the subjects and control via the antecubital vein. This was separated into two aliquots as follows: 4.5mls was put into specimen bottle containing Ethylenediamine Tetra-acetic acid (EDTA) and the remaining 5mls into a plain specimen bottle.

The blood sample in the EDTA bottle was used for analysis of haematologic parameters according to method described by Dacie and Lewis²³. The parameters assessed were packed cell volume/ haematocrit, total white blood cell count, absolute platelet count, Peripheral Blood films were also reviewed to assess the differential leucocytes count and reticulocytes count.

The second aliquot of 5mls blood in the plain container was allowed to clot and serum was obtained for analysis of Parvovirus B19 antibodies using an immunochromatography based technique specifically BIOCARD™ Parvo B19 diagnostic test kit. The test was carried out according to the manufacturer’s specification as follows: At room temperature, 6.7µL of serum sample was added to 1.0mL tube of sample diluent buffer solution to make 1:150 dilution and then mixed properly. Few drops of diluted sample were removed with the aid of pipette. Holding the pipette containing the diluted blood sample vertically, 3 drops of the sample was added

onto the round application field (S). After applying the drops, care was taken not to touch and move the test card for 2 minutes. The test is read after 10 minutes. A positive result was indicated as a distinctly visible red line in the test indicator window and a red line in the control indicator window. A negative test shows only the control line.



RESULTS

Table 1 shows the seroprevalence of parvovirus B19 IgG antibody. This revealed a prevalence of 23.3% among SCA cases with 18.9% among controls. The difference is however not statistically significance P<0.05.

Table 1: Seroprevalence parvovirus B19 infection among Study subjects based on IgG seropositivity

Characteristics	SCA Cases, n (%)	non-SCA Controls	P value
IgG positive	21 (23.3)	21 (23.3)	0.393
IgG positive	24 (26.7)	28 (31.1)	0.393

Categories of subjects with IgG seropositivity:

Table 2 shows the categories of SCA subjects found to be IgG positive. This revealed that out of the 21 IgG seropositive SCA cases, 8 were of paediatric age while 13 were adults; 11 were males and 10 were females. Thus 61.9% of seropositive SCA subjects are adults compared to only 38.1% paediatrics subjects.

Table 2: Categories of subjects with IgG seropositivity

Category	IgG positive (n=21)	IgG negative (n=24)
Age Group		
Paediatrics (<15years of age)	8 (38.1%)	10
Adults (≥15 years of age)	13 (61.9%)	14
Gender		
Male	11 (52.4%)	15
Female	10 (46.6%)	19

Table 3 compares the mean haematological variables between parvovirus B19 IgG seropositive and IgG seronegative SCA subjects. This shows the mean PCV among IgG seropositive SCA subjects is 12.1(±2.0) % compared to 12.0(±2.3) % among IgG seronegative SCA subjects, the difference is not statistically significant (p=0.260). The mean total leukocyte count among parvovirus B19 IgG seropositive and seronegative SCA subjects are 11.8(±3.2) x 10⁹/L and 11.0 (±3.6) x 10⁹/L respectively, the difference is however not statistically significant (p=0.213). The mean reticulocyte index among parvovirus B19 IgG seropositive is 3.0(±2.2) % which is almost equal to the mean reticulocyte index of 3.1(±2.3) % among IgG seronegative SCA subjects, the difference is not statistically significant (p=0.677). Similarly, the mean absolute platelet count among seropositive IgG SCA subjects is 240(±82) x 10⁹/L compared to 275(±65) x 10⁹/L among IgG seronegative SCA subjects, the difference is also not statistically significant (p=0.120). Hence overall there is no effect of IgG seropositivity on haematological profiles of subjects which is not surprising as aplastic crisis is an acute event which should be associated with acute Parvovirus B19 infection as represented by IgM.

Table 3: Comparison of the mean haematological variables between IgG seropositive and seronegative SCA case subjects

Variable	IgG seropositive, n=21 Mean (±SD)	IgG seronegative, n=24 Mean (±SD)	p value
Packed cell volume (%)	12.1 (2.0)	12.0 (2.3)	0.266
Total Leucocyte count (X10 ⁹ /L)	11.8 (3.2)	11.0 (3.6)	0.213
Absolute Neutrophil count (x10 ⁹)	7.8 (1.0)	7.7 (0.6)	0.09
Reticulocyte index (%)	3.0 (2.2)	3.1 (2.3)	0.677
Absolute platelet count (X10 ⁹ /L)	240 (82)	275 (65)	0.120

Table 4 compares pregnancy and outcome (stillbirths) in Parvovirus B19 IgG seropositive and IgG seronegative SCA subjects. This revealed that 7 (33%) of the 21 IgG seropositive SCA subjects are females within the reproductive age group with all being married whereas 7 (29%) of the 24 IgG seronegative SCA subjects are females of the reproductive age of which 6 are married (P=0.335).

The total number of pregnancies encountered in IgG seropositive SCA subjects is 18 compared to 21 in IgG seronegative SCA subjects (P= 0.01). The pregnancy outcome revealed that the total number of stillbirths is 12 among IgG seropositive cases which is higher than the 6 encountered in IgG seronegative SCA subjects; the difference is statistically significant (p=0.04).

Table 4: Comparison of pregnancy and outcome (Stillbirths) in IgG seropositive and seronegative SCA subjects

Variable	IgG seropositive, n=21 Mean (\pm SD)	IgG seronegative, n=24 Mean (\pm SD)	p value
No of females in Reproductive age	7 (33%)	7 (29%)	0.335
Number Married	7 (33%)	6 (25%)	0.332
Total No. of pregnancies	18	21	0.01
Total No. of stillbirths	12	6	0.04

DISCUSSION

Parvovirus B19 infection and its attending consequences is a recognized complication of SCA and other haemolytic anaemias^{5, 6}. Seroprevalence of the disease can be obtained by antibody detection techniques in which the various methods notably Nucleic acid test¹⁸ (NAT) using PCR, antibody detection using ELISA techniques²¹. The search for prompt and type specific method for the detection of Parvovirus B19 infection led to the development and adoption of an immunochromatographic technique^{21, 22}. Utilizing the immunochromatographic method in this study, the seroprevalence of anti-parvovirus B19 IgG was found to be 23.3% among SCA subjects as compared to 18.9% among control subjects. A study by Abraham et al²³ in India found a prevalence of 50%, however in Europe, a study in Serbia²⁴ revealed a prevalence of acute parvovirus B19 to be 32% among subjects and even higher (43%) among children. Their results were found to be closer to the value obtained in the index study.

The notable effect of Parvovirus B19 infection from previous studies was marked on the developing red cells, but few reports indicated the involvement of white cells¹⁰ and platelets¹¹. This study evaluated the haematological profile of SCA patients with and without IgG seropositivity. It revealed a higher mean total leukocyte count among IgG seropositive than in IgG seronegative SCA subjects, but the difference is not statistically significant ($P=0.213$). This tallies with earlier reports by Girei et al²⁵ and Alao et al²⁶ who found no significant difference in the total leukocyte count between IgG seropositive and IgG seronegative SCA subjects. Furthermore, the index study found the mean absolute platelet count to be lower among IgG seropositive SCA subjects as compared to IgG seronegative SCA subjects; the

difference is however not statistically significant ($p=0.120$). This differs from the finding in the study by Girei et al²⁵ who reported statistically significant difference in mean absolute platelet count between IgG seropositive and IgG seronegative subjects. The disparity seen in the index study might be due to the recruitment of anaemic subjects in the index study. The index study also found a lower mean reticulocytes index among IgG seropositive subjects as compared to IgG seronegative subjects but this is not statistically significant ($P=0.677$). The finding of low reticulocytes index is consistent with the finding in aplastic crisis^{5, 26}. This happens to be a prominent complication of acute parvovirus B19 infection among individuals with SCA²⁶.

In addition to the haematological effect intrauterine infection of fetuses in pregnant women occur resulting in hydrops fetalis and fetal death¹². We noted a statistically significant relationship between IgG seropositivity and stillbirths ($p=0.04$). These tallies with the report by Heegard et al¹⁰ which implicated a vertical transmission of parvovirus B19 and qualifies its role in causation of abortion and stillbirths.

CONCLUSION

The prevalence of Parvovirus B19 in our community is noted to be 23.3% and 18.9% among SCA and non SCA subjects respectively. There are no strong association between the haematologic parameters and Parvovirus B19 seropositivity. However we found an association of the viraemia with foetal outcome.

In the light of the above, we recommend that the screening for Parvovirus B19 in SCA with poor foetal outcome be undertaken. And likewise the screening of blood and blood products for Parvovirus B19 be established for SCA patients who are pregnant.

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