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THE SERO-PREVALENCE OF PARVOVIRUS ANTIBODIES AMONG CHILDREN WITH SICKLE CELL ANAEMIA IN ZARIA

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ABSTRACT
Parvovirus is an erythrovirus that infects red cell precursors in individuals with conditions characterised by a high red cell turnover like sickle cell anaemia and thalassaemia. Arthritis, vasculitis, carditis, bone marrow failure, and the slapped cheek appearance have been associated with Parvovirus B19 infection. Recurrent blood transfusion is a risk factor for the B19 serotype of Parvovirus infection, with the P antigen as the mediator for erythroid invasion presenting as transient erythroblastopaenia (TEB). Although TEB is self-limiting a few cases may progress to aplastic anaemia. Previous studies report seroprevalence rates of between 44 and 71%, but the dearth of data on the seroprevalence of B19 parvovirus strain in our region prompted this study.
Venous blood samples from 239 children aged 1to 15 years of consenting parents and guardians were screened for Parvovirus B19 IgG antibodies using the ELISA technique and antibody titer assessed spectrophotometrically. All the participants have sickle cell anaemia, but were in the steady state.
Of this serum samples from 204 (85.4%) participants were positive for IgG antibodies against *Parvovirus B19* while 35 (14.6%) were negative for the IgG antibodies.). The age-group with the highest prevalence is 10-12year group with seroprevalence rate of 88.9%. The overall seroprevalence of *Parvovirus B19* antibodies is 85.4 %.
The seroprevalence of Parvovirus B19 antibodies is high in all socio-economic groups. Antibody prevalence is higher in the non-transfused group suggesting that other factors than transfusion play a role in the spread of the B19 strain of Parvovirus B19.

INTRODUCTION

Parvovirus is of the family parvoviridae, sub family parvovirinae; which infect vertebrates as against the subfamily densovirinae which infect invertebrates. (1,2). Members of *Parvovirinae* are Amdovirus, Bocavirus, Dependovirus, Erythrovirus, Partetravirus and Parvovirus(1). Parvoviruses are erythroviruses in view of their ability to replicate independently in erythroid and megakaryocyte precursors within the bone marrow (2). Parvoviruses also infect endothelial and myocardial cells(2). While Parvovirus is selectively pathogenic to humans, other parvovirus strains are Simian, Pig-tailed macaque, and Rhesus parvoviruses depending on the nature of their hosts (2).

Parvovirus is a non-enveloped isometric virus with a diameter of 18-26nm. Crytallography at 3.5 Å determined that the parvovirus is an icosahedral virus with a polypeptide fold of 2 to 3 major capsid proteins, namely VP1, VP2 and VP3 (1,3). The genomic particle consists of 60copies of capsid protein containing single stranded Deoxyribonucleic acid (DNA). Parvovirus genome contains 5596 nucleotides, of this 4830 nucleotide sequences constitute the encoding sequences; and 383 flanking sequences on both sides form the inverted terminal repetitive sequences(2). The parvovirus B19 also possesses two non-structural proteins NS1 and NS2 which bear some homology with polyoma, papilloma and Porcine parvovirus (PPV) viruses. Parvovirus' tropism for erythroid progenitor cells is the basis aplastic crisis in persons with chronic haemolytic states like sickle cell anaemia, hereditary spherocytosis, and thalassaemia (5-8).

There are three genotypes of the Parvovirus namely B19, LaLi, and V9, but the B19 serotype is the most significant having been implicated in acute febrile illnesses of childhood like erythema -infectiosum or the slapped cheek or fifth disease indicating cytopathic effect on endothelial cells. The cellular receptor for Parvovirus is the P antigen which is present on endothelial and myocardial cells as well as precursors of erythroid and megakaryocyte lines.

Cytopathic effect following viral replication within infected erythroid precursors results in giant cell formation. The virus is spread by droplet infection through the naso-pharynx; recurrent blood transfusion and immunosuppression are also risk factors for infection(8). Foetal loss results from foetal red destruction in non-immune pregnant women (6-7). Arthritis, vasculitis, myocarditis, liver and bone marrow failure are additional clinical outcomes of parvovirus infection. Sickle cell individuals are prone to acute splenic sequestration, meningo-encephalitis, acute chest and cerebral syndromes. Diagnosis depends on demonstration of

Immunoglobulin M (IgM) antibodies within 1-2 weeks of infection and Immunoglobulin G (IgG) afterwards. Demonstration of viral nuclear components by polymerase chain reaction is the confirmatory test. But shared homology with some viruses like the porcine parvovirus poses a challenge in specificity when PCR is the basis of diagnosis, therefore real-time PCR is an alternative when cross-reactivity is suspected. (1-4N) Bone marrow involvement resulting from viraemia causes selective destruction of red cell precursors with a varying natural history, this ranges from a self-limiting transient erythroblastopaenia(TEB) and reticulocytopaenia; to a chronic transfusion dependent condition characterised by bone marrow aplasia(5, 7).

The prevalence of parvovirus antibodies ranges from 44 to 71% with an overall prevalence of 53% in one study. The team from the children's hospital of Philadelphia reported an IgG prevalence rate of 70%. The paucity of data on the prevalence of Parvovirus B19 infection despite reported cases of transfusion dependence in persons in this environment prompted this study.

MATERIALS AND METHODS

Two hundred and thirty nine children aged 1 to 15 years with sickle cell anaemia who attend the sickle cell clinics of Ahmadu Bello University Teaching Hospital, Shika Zaria and Barau Dikko Specialist Hospital, Kaduna but were in the steady state were the participants in this cross-sectional study. Consent was obtained from their parents and guardians. Their serum samples were screened for *Parvovirus B19* IgG antibodies using the Enzyme Linked Immunosorbent Assay (ELISA) technique applying kits manufactured by DRG instruments GmbH Frauenbergstrate 18, 35039 Marburg Germany.

ELISA Procedure for Anti-Parvovirus IgG Assay
Quality Control Measures

The kits are purchased in packs of 96 tests per kit.

Each test kit contains an internal quality control pack to validate the efficacy of the assay and results according to the manufacturer's instructions.

Each test kit has 5 wells reserved for blanks and controls.
Low positive was tested in duplicate.

A well was left empty for the substrate blank which was mixed by gently tapping all sides on a flat surface.

ELISA method

About 100µl of conjugate was transferred to each well of the micro plate except the blank. Bubbles were avoided upon addition. It was then mixed well by gently tapping all microplates sides on a flat surface.
The plates were covered with an adhesive seal and incubated for 30 minutes at room temperature (30-32°C). The adhesive seal was removed and discarded. Wells were aspirated and washed.
About 100µl of substrate was added to each well including the blank
After this, it was incubated for 15 minutes at room temperature and (30-32°C) and protected from light
Finally, the reaction was stopped by adding 100µl of stop solution in each well including the blank, in the same sequence and time intervals as for the substrate addition. The plate was gently tapped to mix the content of the wells.
Plates were read according to manufacturer's instructions as follows.
Dark precipitates of the chromogen to yellow colour indicated positive result
Pale yellow colour indicated negative result.
The intensity of the reaction was photometrically quantitated with a dual filter Enzyme Immuno Assay Reader (Sigma diagnostics EIA Multi-well Reader 11) after one hour using O.D. at 450nm, 630nm.

RESULTS

Of 239 participants, 120 were males and 119 were females. Serum samples from 204 (85.4%) out of 239 participants were positive for IgG antibodies against *Parvovirus* while 35 (14.6%) were negative for the IgG antibodies. Therefore, the seroprevalence of *Parvovirus* antibodies is 85.4 %(Table1). The seroprevalence rate by gender is 99(82.5%) and 105(88.2%) for males and females respectively but there is no statistically significant difference between the genders (Table2). The age-group with the highest prevalence is 10-12year group with seroprevalence rate of 88.9% (Figure1).

The prevalence rate for *Parvovirus* antibodies was more than 80% in all socio-economic groups and the difference in prevalence rates between the groups was not statistically significant with the children of persons in the higher socio-economic groups having highest prevalence rates. Fifty three (22.2%) were previously transfused and 186(77.8%) were not transfused. The sero-prevalence by transfusion is 43/53(81.1%) and 161/186(86.6%) for the transfused and non transfused group respectively. There is no statistically significant difference between the prevalence rates.

TABLE 1: PREVALENCE OF *PARVOVIRUS B19* IG G AMONG CHILDREN WITH SICKLE CELL DISEASE

Status	No. of children screened	Percentage positive
Negative	35	14.6
Positive	204	85.4
Total	239	100%

$X^2 = 0.001$, $P < 0.05$ at 95% CI

There is a significant association between Immunoglobulin G antibodies to *Parvovirus B19* and sickle cell disease.

TABLE 2: DISTRIBUTION OF *PARVOVIRUS B19* IG G AMONG THE DIFFERENT SEXES IN CHILDREN WITH SICKLE CELL DISEASE

Sex	No. of samples screen	No. of positive samples	percentage
Male	120	99	82.5%
Female	119	105	88.2%
Total	239	204	85.4%

$X^2 = 0.210$ 95% CI

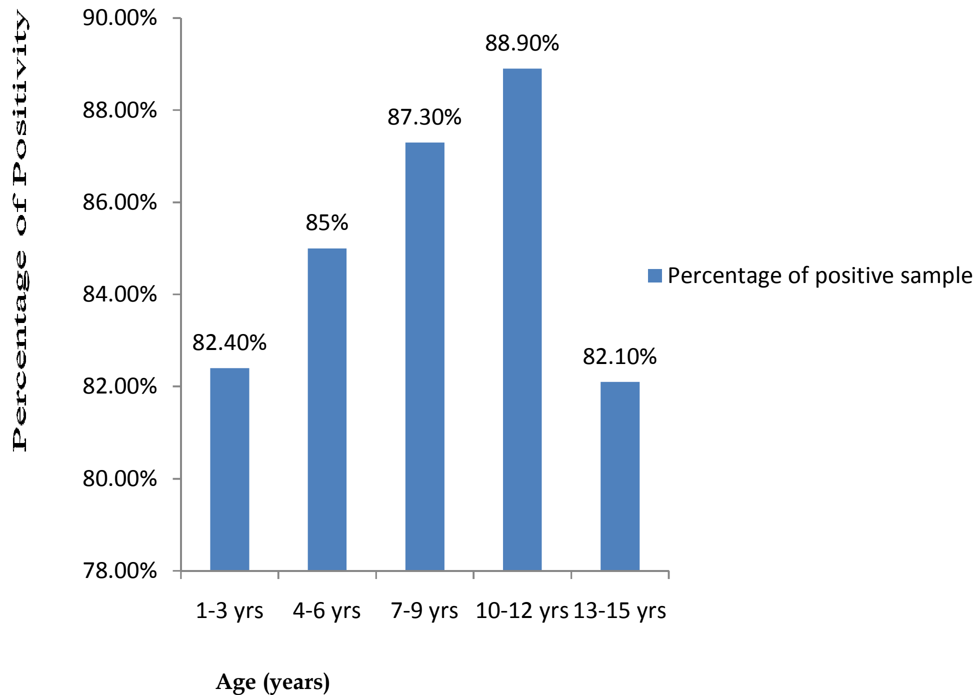
There is no significant association between the presence of IgG to *Parvovirus B19* and gender. $P > 0.05$

DISCUSSION

The majority formed by males in this study is contrary to the observations by Konotey Ahulu, Aliyu et al, and Mamman et al in which females were the majority (10-12). This may be explained by the fact Ahulu, Aliyu and Mamman conducted their studies on adult patients and females are more likely to comply with clinic attendance than their male counterparts. The prevalence rate of 85.4% obtained in this study is higher than 70%, 60% and 56.5% reported by Eis-Hübinger, Dayana and Regaya *et al.* (13-15). The highest prevalence was in the 10 to 12 year age group. This is at variance with Ohene Frempong study in which the age 1 to 5 year group had the highest prevalence (9).

The proportion of participants who had a previous history of blood transfusion is 22.2% in this study. This is less than 60.8% reported by Mamman and Durosinmi (12). This suggests that transfusion demand increases with age as complications manifest. Paradoxically, the non-transfused group had a higher IgG antibody prevalence rate of 86.8% than 81.1% observed in the transfused group. Therefore, other factors than blood transfusion contribute to the transmission of *Parvovirus*.

FIGURE 1: THE BAR CHART SHOWS THE OVERALL AGE GROUP DISTRIBUTION OF *PARVOVIRUS B19* IMMUNOGLOBULIN G AMONG CHILDREN WITH SICKLE CELL DISEASE IN SOME PARTS OF KADUNA STATE.



Age (years)

$X^2 = 0.875$, $P > 0.05$, at 95% Confidence Interval

There is no significant association between the presence of Ig G to B19 virus and age group.

TABLE 3: THE DISTRIBUTION OF *PARVOVIRUS B19* IGG AMONG CHILDREN WITH SICKLE CELL DISEASE OF DIFFERENT SOCIO-ECONOMIC STATUS BASED ON THEIR FATHERS' OCCUPATION.

Socioeconomic status / Fathers' occupation	No. children screened	No. positive	Percentage positive
Farmers/Cottage craft/ Unemployed	125	107	85.6%
Business men/Traders/ Civil servants	95	80	84.2%
Professionals/Politicians	19	17	89.5%
Total	239	204	85.4%

$X^2 = 0.834$, $P > 0.05$, 95% Confidence Interval

There is no statistically significant association between the presence of Parvovirus Ig G and socio-economic status.

prevalence rate of 82.4% in the first three years of life suggests early infection probably due to droplet or aerosol spread (5, 6). The ubiquitous nature of the virus is indicated by near identical prevalence rates across all socio-economic groups as children of farmers, cottage craftsmen had a prevalence rate of 85.6% on the one hand while children of highly skilled professionals and politicians had a prevalence rate of 89.5% on the other.

TABLE 4: DISTRIBUTION OF *PARVOVIRUS B19* IG G IN BLOOD TRANSFUSED AND NON BLOOD TRANSFUSED CHILDREN WITH SICKLE CELL DISEASE.

Blood transfusion Status	No. children screened	No. positive	Percentage positive
Non-transfused	186	161	86.6%
Transfused	53	43	81.1%
Total	239	204	85.4%

$X^2 = 0.324$, $P > 0.05$, 95% Confidence Interval
There is no significant association between blood transfusion and *Parvovirus B19*.

Although IgG antibodies were detected by ELISA, this is limited by cross-reactivity with other parvoviruses, therefore Nucleic Acid Testing and PCR are confirmatory tests considering their sensitivity and specificity. Neither PCR nor NAT were carried out in this study due to logistic and infrastructural challenges in our settings in which Nigeria is 156th on the Human Development Index (16). In conclusion, this calls for studies that will assess the prevalence of IgM activity with the aim of detecting on-going infection, PCR based sero-epidemiology that will provide a basis for vaccine development.

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