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SEROPREVALENCE OF PARVOVIRUS BI9 ANTIBODY IN BLOOD DONORS AND SICKLE CELL DISEASE PATIENTS AT LAGOS UNIVERSITY TEACHING HOSPITAL (LUTH): A COMPARATIVE STUDY.

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ABSTRACT

INTRODUCTION: Parvovirus B19 (PVB19) is a DNA virus transmissible by blood transfusion. It is a major cause of aplastic crisis especially in chronic haemolytic anaemic patients such as sickle cell disease patients.

OBJECTIVE: The study was aimed to determine the seroprevalence of PVB19 in blood donors and sickle cell anaemia (SCA) patients and to evaluate its association with blood transfusion in SCA patients.

METHODS: This is a cross sectional study conducted at the Lagos University Teaching Hospital, Lagos Nigeria. Three hundred participants, consisting of 150 voluntary blood donors and 150 sickle cell anaemia subjects were enrolled into the study. Seroprevalence of parvovirus was determined using ELISA kits for IgG and IgM anti-PVB19 antibodies by Immuno-Biological Laboratories, (IBL) inc. Minneapolis, USA. Results was analyzed with SPSS 11 software and presented in tables. Fishers Exact test, Chi-square and student T-test were used as appropriate to compare variables between both groups. P-values <0.05 were considered significant.

RESULTS: Ninety nine (66%) blood donors were positive for anti-PVB19 IgG antibody while ninety two (61.3%) sickle cell patients were positive. Two (1.3%) blood donors were positive anti-PVB19 IgM antibodies while 8 (5.3%) SCD patients were positive for anti-PVB19 IgM antibodies. There was no significant difference in the seroprevalence of IgG and IgM antiPVB19 virus in both groups. There was no association of parvovirus seroprevalence with blood transfusion.

CONCLUSION: The study has shown a high seroprevalence of IgG anti-PVB19 antibodies in both blood donors and SCA patients. Therefore routine screening for parvovirus infection for donor blood is not justified. However seronegative SCA patients who require blood transfusion should have the blood screened for parvovirus to reduce the risk of associated aplastic crisis.

Key words: Seroprevalence, parvovirus B19, blood donors, sickle cell anaemia

INTRODUCTION

Parvovirus B19 (PVB19) a DNA virus belonging to the parvoviridae family and erythrovirus genus (1). It is one of the transfusion transmissible viruses. Others include hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human T cell leukaemia virus I (HTLV1), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) (1).

It is non-enveloped, with icosahedral nucleocapsid symmetry, and measures 22-24 nm in diameter. The virus has a linear nucleic acid (DNA) of ~5.6 kb in length. There are 3 genotypes 1, 2 and 3. Genotype 1 is responsible for the majority of human infections worldwide; genotypes 2 and 3 appear to have some geographic and temporal variation in distribution. The virus is resistant to dry heat, freezing and lipid solvents. It is inactivated by formalin, β-propiolactone and gamma irradiation (1).

The virus is transmitted mainly as droplet infections, vertically through placental to fetus and through blood transfusion. At risk population include children, pregnant women, immunocompromised and those with chronic haemolytic anemia such sickle cell and Thalassemia patients (1, 2).

Clinical manifestations range from asymptomatic disease in immunocompetent to symptomatic disease in the immunocompromised. Clinical features include erythema infectiosum (fifth disease) in children, arthropathy, aplastic anemia/crisis and fetal hydrops (1).

Acute infection is associated with a viremic phase shortly followed by IgM antibody production (10 – 14 days post-infection). This is followed by IgG antibody production against the viral capsid. Viraemia declines with IgM production; IgM declines after a few months but IgG persists longer to convey immunity against reinfection. Infrequently, low level PVB19 nucleic acid may persist with IgG for months or years.

Parvovirus B19 infection has been reported globally. Recent infection is associated with the secretion of immunoglobulin M (IgM) antibodies in plasma while IgG signify previous exposure.

Anti-parvovirus IgG antibody seroprevalence rates are similar in the United States, Europe, and Asia (3 – 5).

The virus has a direct cytopathic effect on erythroid progenitors in bone marrow leading to an arrest in the maturation and subsequent anaemia. The clinical manifestation varies widely depending on the immunological and haematological status of the host. In individuals with underlying haemolytic disorders, as in patients with sickle cell anaemia (SCA), PVB19 infection may cause transient erythroblastopenia (TEB), characterized by a fall in haemoglobin level with reticulocytopaenia (6). Other manifestations of parvovirus infection include arthritis, vasculitis, myocarditis, liver failure and fetal loss.

Sickle cell anaemia is one of the most prevalent genetic diseases in Nigeria (7). SCA patients have accelerated premature haemolysis with significant reduction in red cell half life. Generally, the chronic haemolysis and resulting anaemia is well tolerated however, a reduction in the level of haemoglobin below the steady state may be detrimental to the patient; necessitating blood transfusion (8, 9). Transfusion may predispose them to increased risk of immunological and infectious complications.

Accurate epidemiologic data on the frequency of Parvovirus B19 infection in patient with sickle cell anaemia are essential for assessing the potential effect of viral prevention programs in this patient population (10). However there are limited data on the seroprevalence of PVB19 infection in our environment hence the justification for this study.

The findings from this work may justify need for routine parvovirus B19 screening of blood products before transfusing sickle cell patients or justify the need for institution of vaccination program SCA patients.

OBJECTIVES

The study was aimed at determining the seroprevalence of parvovirus B19 antibodies (IgG and IgM) among SCA patients and blood donors; to compare the seroprevalence rates between both groups and to determine its association with blood transfusion.

METHODOLOGY

Study design: This was a cross sectional study conducted at the Lagos University Teaching Hospital, Idi-Araba.

Sample size: This was calculated using the Kirkwood formula for cross sectional study. An estimated prevalence rate of 50% was used. A total of 300 subjects were recruited for the study.

Study participants: There are 2 study groups. Group 1 comprises 150 voluntary blood donors recruited from the donor clinic. Group 2 comprises 150 SCA patients attending clinic at the Sickle Cell Centre in LUTH. A structured questionnaire was used to obtain personal and medical data from the subjects and venous blood was collected for serological and haematological analysis.

Ethical Considerations: The study was approved by the ethical committee of the hospital. An informed consent was given by all participants above 18 years while consent was obtained from guardians for patients below 18 years in the language best understood by them.

Inclusion and Exclusion Criteria

Inclusion criteria for blood donors: Age between 18-60 years; Weight >50 kg; Hb >12.5g/dl; Normal blood pressure, pulse, and temperature.

Exclusion criteria for blood donors: History of chronic illness e.g. Hypertension, Diabetes, Asthma; commercial sex workers and Intravenous drug users.

Inclusion criteria for sickle cell patients include SCA aged 5 years and above.

Sample Collection: Ten milliliters (mls) of venous blood was collected from the antecubital fossa using aseptic technique. Five mls was dispensed into a sterile plain bottle and allowed to stand at room

temperature until clotted. The samples was centrifuged, serum separated into another sterile bottle stored at -20^{o}C until the required sample size were obtained.

The other 5mls was dispensed into EDTA bottle for full blood count, reticulocyte count and red cell indices. Samples were analyzed within 2 hours of collection using automated haematology analyzer. The reticulocyte count was performed manually as described in Decie and Lewis (11).

Parvovirus B19 IgG and IgM Assays

IgG and IgM assay were determined using a solid phase enzyme – linked immunosorbent assay (ELISA) kits for IgG and IgM anti-PVB19 antibodies by Immuno-Biological Laboratories, (IBL) inc. Minneapolis, USA.

Assay Procedure

The required number of microtitre strips or wells were selected and inserted into the holder. Wells were filled with 300µL of diluted wash solution and allowed to soak for 5 minutes and aspirated off. Sample was dispensed into each properly identified well using the work sheet as a guide. 100 µL each of negative control, cut-off control, positive control, diluted samples were dispensed into appropriate wells and a microwell was left for substrate blank. Wells were covered with foil and incubated for 60 minutes at room temperature.

The contents of the wells were briskly shaked out and rinsed five times with diluted wash solution and then strike on absorbent paper to remove residual droplets.

100µL of enzyme conjugate was dispensed into each well except the blank. All the wells were covered with foil and incubated for 30 minutes at room temperature. The contents of the wells were briskly shake out and rinsed five times with diluted wash solution and then strike on absorbent paper to remove residual droplets.

100 µL of substrate solution was added into all wells. Wells were covered with foil and incubated for exactly 15 minutes at room temperature. Enzymatic reaction was stopped by adding 100µL of stopped solution to each well. There is a colour change from blue to yellow. The intensity of the colour is proportional to the antibody titre.

Optical density was read at 450nm blanking the instrument with the blank microwell.

Results were interpreted according to manufacturer's instructions.

Internal Quality Control Measures

Negative, cut-off, positive controls and blanks were used in each run.

For IgM assay, patient serum samples are diluted and simultaneously absorbed with sample diluents containing hyper immune anti-IgG-class antibody to eliminate competitive inhibition from specific IgG and to remove rheumatoid factor.

Statistical analysis

The results were analyzed using statistical package for social science (SPSS 11.0), and Epi-info 6.0. The results were presented with in frequency tables. Comparism of the seroprevalence of IgG and IgM anti-PBV19 antibodies in both groups was made using chi-square and Fishers Exact test as appropriate. Other numerical parameters were compared using the student T-test. The association of anti- PVB19 antibody with blood transfusion was determined using odd ratio. Significance level was set at $p< 0.05$**.**

RESULTS

Demographic parameters of the subjects

A total of 300 subjects were studied consisting of 150 sickle cell patients and 150 blood donors. Their mean ages (in years) were 20.29 ± 11.27 and 30.43 ± 9.58 for the sickle cell subjects and blood donors respectively. The SCA subjects included 70 (46.7%) females and 80 (53.3%) males while blood donors included 6 (4%) females and 144 (94%) males. Tables 1and 2 show the age and sex distribution of the participants.

TABLE 1: SHOWS THE MEAN AGE, SEX, SEROPREVALENCE AND HAEMATOLOGICAL PARAMETERS OF THE STUDY SUBJECTS

Variables	Blood Donors (N = 150)	SCA (N = 150)	P values
Age (Mean ± SD)	30.43 ± 9.58	20.29 ± 11.27	<0.01
Sex			
Males	80	144	
Females	70	6	
Positive history of Blood Transfusion	13 (8.67%)	76 (50.67%)	
Seroprevalence of PVB19			
IgG	99 (66.0%)	92 (61.3%)	0.47
IgM	2 (1.3%)	8 (5.3%)	0.11
IgM and IgG	1 (0.67%)	4 (2.67%)	
Haematological Parameters			
WBC/mm³	9.85 ± 3.87	4.28 ± 1.01	<0.01
Neut/mm³	5.16 ± 2.48	2.13 ± 0.80	<0.01
Lymph/mm³	3.84 ±1.68	1.81 ± 0.51	<0.01
Platelet x 10⁹/l	355 ± 150	227 ± 168	<0.01
Hb (g/dl)	7.51 ± 1.62	13.72 ± 1.54	<0.01
Reticulocyte (%)	5.30 ± 2.43	1.22 ± 0.47	<0.01
MCV (fl)	81.53 ± 5.54	84.24 ± 10.35	<0.01
MCHC (g/dl)	32.51 ± 13.66	32.15 ± 12.40	0.02
MCH (pg)	26.88 ± 3.22	27.22 ± 2.77	0.33

TABLE2: AGE AND SEX DISTRIBUTION OF THE STUDY SUBJECTS

Age (Yrs)	SCA (N = 150)		Blood donors (N = 150)	
	Female	Male	Female	Male
< 11	9	24	0	0
11 – 17	19	15	0	0
18 – 24	19	22	2	47
25 – 31	8	6	3	46
32 – 38	8	6	0	20
39 – 45	4	6	0	21
46 – 52	3	1	0	6
>52	0	0	1	4
Total	70	80	6	144

History of blood transfusion
Seventy six (50.67%) of the Sickle cell patients had a history of previous blood transfusion while 13 (8.67%) of the blood donors had been transfused in the past.

Seroprevalence of parvovirus antibodies in the participants
A total of 92 (61.3%) sickle cell patients were anti-PVB19 positive and 99 (66.0%) of the blood donors were also positive for IgG. For IgM anti-PVB19, 8 (5.3%) sickle cell anaemia patients were positive while 2 (1.3%) of the blood donors were positive. There was no significant difference in the prevalence of IgG and IgM anti-PVB19 antibodies between both group (P = 0.471 and 0.1078 respectively). Four of the sickle cell anaemic patients were positive for both IgG and IGM anti-PVB19 antibodies while only one of the healthy donors was positive for both antibodies as shown in Table 1.

The seroprevalence by age and sex are presented in Tables 3 and 4. Tables 5 and 6 represent its association with blood transfusion in the study subjects.

Haematological parameters
The haematological parameters of the participants are as presented in Table 1. Table 7 compares haematological parameters in SCA subjects with IgG and IgM antibodies. Reticulocyte counts were significantly reduced in SCA subjects with IgM antibodies.

DISCUSSION
Parvovirus B19 is one of the emerging transfusion transmissible infections. It has been widely studied in various countries among healthy blood donors and

sickle cell patients with results indicating a high seroprevalence of the virus in the study areas however there are limited publications on parvovirus in SCA patients and blood donors in our environment. In this study, we found a seroprevalence rate of 66% for IgG antibody in healthy blood donors.

Abraham et al and Munoz et al reported seroprevalence of 65% each for IgG antibody in blood donors in India (12) and Salamanca, Spain (13) respectively. In various studies in developed nations, rates between 55 – 77% were reported (3 – 5). The seroprevalence rates from these studies were comparably the same with our findings. However, Mata et al reported a low seroprevalence of 9.8% (anti-IgG) in a cross sectional study conducted among 92 blood donors in Galicia in Spain (14). This is significantly lower than what we found in our study. This shows that there are geographical variations in the seroprevalence of parvovirus infection.

TABLE3: SEROPREVALENCE OF IGG AND IGM ANTIBODIES BY AGE IN SCA PATIENTS AND BLOOD DONORS

Age (Yrs)	SCA (IgG Positive)	Blood donors (IgG Positive)	SCA (IgM Positive)	Blood donors (IgM Positive
< 11	18	0	0	0
11 – 17	20	0	0	0
18 – 24	28	33	2	47
25 – 31	10	31	3	46
32 – 38	6	14	0	20
39 – 45	7	16	0	21
46 – 52	3	1	0	6
>52	0	4	1	4
Total	92	99	6	144

TABLE 4: ASSOCIATION OF IGG PARVOVIRUS ANTIBODIES WITH SEX IN SCA PATIENTS AND BLOOD DONORS

Sex	SCA IgG status			Blood donors IgG status		
	Negative	Positive	Total	Negative	Positive	Total
Female	22	48	70	1	5	6
Male	36	44	80	50	94	144
Total	58	92	150	51	99	150
P-value	0.089			0.360		

TABLE 5: ASSOCIATION OF IGG PARVOVIRUS ANTIBODIES WITH BLOOD TRANSFUSION IN SCA PATIENTS AND IN BLOOD DONORS

Transfusion Status	SCA IgG status			Blood donors IgG status		
	Positive	Negative	Total	Positive	Negative	Total
Transfused	44	32	76	6	7	13
Not transfused	48	26	74	93	44	137
Total	92	58	150	99	51	150
	P-value = 0.4785			P-value 0.2026		

TABLE 6: ASSOCIATION OF IGM PARVOVIRUS ANTIBODIES WITH BLOOD TRANSFUSION IN SCA PATIENTS AND IN BLOOD DONORS

Transfusion Status	SCA IgM status			Blood donors IgM status		
	Positive	Negative	Total	Positive	Negative	Total
Transfused	1	75	76	0	13	13
Not transfused	7	67	74	2	135	137
Total	8	142	150	2	148	150
	P-value = 0.0635			P-value 0.4085		

The seroprevalence of Parvovirus B19 IgG antibody in SCA patients was found to be 61.3% in this study. Ujo et al (15) in a cross sectional study in paediatric SCA patients in Zaria found a seroprevalence rate of 85.4%.

The study also found that there is no sex association in the seroprevalence of the virus in both SCA patients and healthy blood donors. This is in agreement with the findings of Ujo et al (15) and Teuscher et al (16) in their separate studies.

This study also found that though blood donors have a slightly higher seroprevalence than SCA patients, this was not statistically significant. Teuscher et al (16) and Serjeant et al (6) in their separate studies also found no significant difference in the seroprevalence of the virus in both study groups. This suggest that SCA patients are not at increased risk when compared to the general populace however due to the fact that they have a lower stable haemoglobin value, parvovirus infection in them may result in symptomatic anaemia necessitating transfusion.

TABLE 7: COMPARISM OF HAEMATOLOGICAL PARAMETERS IN SCA PATIENTS WITH IGG AND IGM PARVOVIRUS ANTIBODIES

Haematological Parameters	IgG Mean ± SD N = 92	IgM Mean ± SD N = 8	P value
WBC x 10^9	9.93 ± 4.29	9.81 ± 3.80	0.94
Neutrophils x 10^9	5.25 ± 2.72	4.58 ± 1.80	0.49
Lymphocytes x 10^9	3.90 ± 1.93	4.40 ± 2.11	0.49
Platelets x 10^9	348.66 ± 149.69	293.63 ± 176.96	0.33
Hb (g/dl)	9.46 ± 1.57	7.15 ± 1.97	0.60
Reticulocyte (%)	5.48 ± 2.42	1.19 ± 0.59	<0.01
MCV (fl)	82.30 ± 8.33	80.18 ± 9.35	0.50
MCH (pg)	27.11 ± 3.37	26.29 ± 3.36	0.51
MCHC (g/dl)	32.64 ± 1.31	32.74 ± 0.89	0.83

Studies have shown that by 15 years of age, about 50% of some populace are positive for PVB19 IgG antibodies (1, 17, 18). Some studies have noted an increase in seroprevalence of the virus with age.[18] Kim et al in their study on the epidemiology of parvovirus in sickle cell disease patients reported an increase in seroprevalence with age (19).In this study, we found a peak age prevalence to be 18 – 24 years in SCA patients and blood donors. This is higher than that reported by Ujo et al (15).

The seroprevalence of parvovirus B19 IgM antibody in sickle cell anaemia subjects and blood donors were found to be 5.3% and 1.3% respectively. Doyle and his coworker found seroprevalence of 1% prevalence among American blood donors (4) while Munoza reported 0% in Spanish blood donors (20). The seroprevalence of PVB 19 may vary with the sensitivity of the test used in addition to geographical and seasonal variations. The possibility of transmission of PVB19 by blood and blood products raises several blood safety questions still unanswered (21 – 23). Human blood and its components are widely used as life saving therapy in hospital practices. However, there is an associated risk of transmission of infections such as HIV, hepatitis and parvovirus inclusive due to infected donor blood (23). Parvovirus lacking a lipid envelope is not susceptible to the solvent-detergent treatment which can inactivate pathogens with envelopes such as HIV, hepatitis B and C among others transmissible by blood. The virus is stable in heat and remains infective even after treatment with dry heat at 80°C for 72 hours, which was used for treating some blood products. Hence the reports on a high prevalence of PVB19 in haemophilic patients receiving pooled blood products (24, 25). In non-immune sickle cell anaemia patients, the clinical manifestation of the virus upon infection may include transient aplastic crisis, which is indicated by a fall in haemoglobin with reticulocytopaenia. Serjeant et al in their study of epidemiology of human parvovirus B19 infection in Jamaica in homozygous sickle cell disease found that PVB19 infection account for most if not all aplastic crisis in SS disease (6).

The lack of a statistically significant difference between the seroprevalence of PVB19 antibodies between blood donors and SCA patients will suggest that blood transfusion may not the major means of transmission of parvovirus SCA patients. This finding highlights the importance of investigating other means of transmission other than blood transfusion as recommended in the report of the Committee on Infectious Diseases (17). The reticulocyte percentages of IgG antibody positive SCA patients are generally higher than that of IgM positive SCA subjects. This affirms the risk of transient aplasia associated with acute or persistent parvovirus infection documented in some previous studies (19). This is understandable

in the light of the cytopathic effect of PVB19 on haemopoietic precursor cells in the bone marrow. In conclusion, this study has shown a high seroprevalence of IgG anti-PVB19 antibodies in sickle cell patients and voluntary blood donors in our environment. This suggests that it may not be cost effective to recommend routine donor screening for PVB19 antibodies. However, because of the increased risk of aplastic crisis in SCA patients, SCA patients who require transfusion and are seronegative for PVB19 should have the blood screened for PBV virus.

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