

Original Research Article

Effect of Sickle cell trait on the treatment response of Individuals infected with Human Immune-deficiency virus (HIV).

Abstract

Background: Sickle cell disease is of public health importance and comes with system organ complications that can cause long-term disabilities and early death. This study was to determine the effect of co-existence between sickle cell gene (HbS) and HIV infection in a Lagos population.

Methods: The study was conducted at the anti-retroviral (ARV) outpatient clinic of Nigerian Institute of Medical Research (NIMR), Yaba, Lagos. It is a cross sectional cohort study of 292 adult HIV- positive patients who were enrolled for treatment and support in the centre between the year 2004- 2015. Review of case notes and database was done to extract haematologic, immunologic, virologic and clinical information of the patients. Additional clinical and socio-demographic information were obtained using a questionnaire. Blood samples were collected and analysed for the haemoglobin genotype of participants. Data analysis was performed using SPSS software.

Results: Out of 292 recruited patients, 274 participants had complete data of which 74.1%, 22.9% and 2.9% were HbAA, HbAS and HbAC respectively. Sickle cell trait (SCT) was found in 25.9% of the participants and there is no record of sickle cell disease (SCD). HbAA participants were found to have higher viral load (87.5%) at baseline ($P < 0.001$). For HbAA and SCT groups, treatment response over the years was similar with lower figures seen in SCT.

Conclusion: It was concluded that the lower HIV viraemia in SCT might mean delay in HIV-1 disease progression and subsequently better quality of life for such patients.

Comment [1]: The above results does not justify these conclusions

Introduction/ Background information

Sickle cell disease (SCD) is an inherited red blood cell (RBC) disorder resulting from structurally abnormal haemoglobin S (HbS) gene [1]. The disease is heralded by painful episodes which include pain in the back, chest, abdomen and extremities. SCD is a vaso-occlusive disease and haemolytic crisis are the clinical hallmarks of this disease, which results in painful episodes, known as sickle cell crisis. The swelling of hands, feet, enlargement of the spleen, heart as well as jaundice and several system organ complications can cause long-term disabilities and early death. The oxygen carrying abnormal genotype S red blood cells assume a sickle shape instead of the normal disk shape [2] showed the abnormal electrophoretic mobility of haemoglobin in an affected individual. Ingram [3] discovered that the defect of the disease was a single amino acid substitution in the haemoglobin molecule of sickle cells (HbS). There is increased rigidity of the sickle red cell with deoxygenation, which impairs passage of individual sickled red cell through the microcirculation. The ensuing reduction in blood flow may lead to regional infarction (vaso-occlusive crisis). Early symptoms appear at about five to six months of age when concentrations of foetal haemoglobin (HbF) have expectedly reduced [4].

Comment [2]: This is not correct, check your facts again

Comment [3]: The sentence is not coherent

Comment [4]: What amino-acid?

Comment [5]: Rephase

Haemoglobin S gene is particularly common in people of West African descent. High haemoglobin S gene frequencies are found in African and Mediterranean populations, and populations arising from the slave trade or voluntary emigration from Africa and the Mediterranean [1]. Sickle cell trait (SCT) (the carrier state of SCD) has a prevalence of 25%–30% in many countries in tropical Africa [5]. Worldwide, there were about 78 million carriers of sickle cell trait in 1992, with most of them (65 million) living in sub-Saharan Africa [5]. The WHO states that three quarters of 200,000 infants born with Sickle Cells in Africa annually are Nigerians [6].

Geographic overlap exists between Human Immunodeficiency Virus (HIV) and sickle cell disease (SCD). The spread of HIV virus has been particularly very high in developing countries, especially sub-Saharan Africa where most patients with SCD live. An estimated 3.1 million Nigerians are living with HIV and over 56,000 positive births annually [7]. It is expected that some degree of interaction may exist between the two diseases with HbS gene influencing the course of HIV infection/ progression.

Immunosuppression from HIV may affect the natural history of infection with other pathogens by expediting infection, modifying the disease presentation or its course [8]. On the other hand, pathogens and pathogen-derived products may upregulate HIV replication, which can alter the progression of HIV [8]. This includes malaria resulting from infection with Plasmodium species, which is a major cause of sickle cell crisis in SCD patients [8]. **Therefore, the hypothesis is that HIV may exacerbate SCD, while SCD speeds up progression of HIV to AIDS. However, available evidence on SCD in HIV-infected patients, limited as it is, contradicts this hypothesis, and suggest that despite frequent blood transfusion, SCD patients have lower risk for HIV-1 infection [9], higher frequency of HIV-1 long-term non-progressors compared with the general HIV-positive population; 44% in SCD patients vs. 13.9% in Normal controls [10]. Analysis of national hospital discharge surveys; 1997–2009 shows that SCD is associated with lower frequency of HIV-1 diagnosis; odds ratio 0.33, as compared with other diagnosis [11].**

Iron metabolism is intrinsically connected to HIV-1 infection and disease progression. The development of AIDS is accompanied by an increasing iron accumulation in macrophages, microglia, endothelial cells, myocytes, bone marrow, brain white matter, muscle and liver [12,13]. Increased iron stores correlate with rapid HIV-1 progression in AIDS patients. This is the case in iron-loaded thalassemia major patients, in HIV-positive patients administered with oral iron, and in those with the haptoglobin 2–2 polymorphism [14]. A retrospective study demonstrating the lower survival of HIV-1-infected individuals inversely correlated to higher iron stores [14]. In addition, non-anaemic HIV-1-positive women in Zimbabwe with high serum ferritin concentration are reported to exhibit increased viral load, which provides further evidence for the association of increased iron stores with more severe HIV-1 infection [15]. Elevated iron level has been suggested as a potential predictive marker for higher mortality in HIV-1-infected Gambian adults [16]. **In other words, in SCD patients with decreased iron stores, there might be a decrease in HIV-1 viral replication.**

The purpose of the present study was to establish the relationships between sickle cell gene and clinical, immunologic, biochemical and haematologic parameters of HIV patients. It is expected

Comment [6]: Not entirely correct, SCT protects against severe forms of malaria, only people with the homogenous SS have VOC, which could be triggered by malaria

Comment [7]: These are sweeping statements, you need to explain and expantiate more on these studies, were the patients having SCT or SCA?, cohort or cross-sectional studies?

Comment [8]: This is an incorrect assumption, where is your reference

that the outcome of this study will positively influence the management of those with the co-morbidity (HIV /HbS gene).

General Objective

To determine the effect of co-existence between HbS gene and HIV infection in a Lagos population.

Specific Objectives

1. To determine the haematological, immunological, biochemical and virologic status of HIV positive individuals with HbS gene at the time of commencing Highly Active Antiretroviral Therapy (HAART).
2. To compare the parameters among patients with genotypes AA and AS, AC, SS & SC.
3. To determine the trend of the parameters overtime in these patients.

Materials and Methods

Study Centre

This study was conducted at the anti-retroviral (ARV) outpatient clinic of Nigerian Institute of Medical Research (NIMR), Yaba, Lagos; a large HIV care and treatment centre in Lagos, Nigeria established in 2002. Over 25,000 HIV positive adult and children are receiving care, treatment and support at the centre. Patients are referred from clinics and hospitals within and outside Lagos State to this centre.

Study Design

The study was a [cross sectional cohort study](#) of purposively selected adult patients (HIV-positive) who were enrolled for treatment and support in the centre between the year 2004- 2015 and are HAART experienced, who have been on treatment for at least 5 years.

Comment [9]: How? A retrospective study being cohort? What was their selection criteria? A purposive sampling technique being cohort?

Review of case notes and database was done to extract haematologic, immunologic, virologic and clinical information of the patients.

Additional clinical and socio-demographic information was obtained using a questionnaire, blood samples were also collected to ascertain the genotype of these individuals through laboratory assay.

Sample Size Determination

Two hundred and ninety two (292) purposively selected adult HIV positive individuals who were enrolled for HAART treatment within the stated study period were recruited for this study. This sample size was calculated based on the WHO cross sectional sample size formula

$$n = \frac{Z^2 [P(1-P)]}{D^2} [17]$$

P = 25% [5]. Z = 1.96 D = 5% (0.05)

where P is the expected prevalence, Z the value of the reference normal distribution for the desired confidence level (1.96) for 95% confidence level. D is the highest acceptable error in the estimate [17].

Study Population

The population studied included adult HIV-1 positive individuals that are HAART experienced, who have been on treatment for at least 5 years.

Inclusion Criteria

Individuals recruited were consented confirmed HIV positive adult patients that are 18 years and above at the time of enrolment, who have been under HIV care for not less than 5 years.

Exclusion Criteria

Individuals who were pregnant or with any other co-morbidity (Tb, Hep B, Hep C, diabetes) were excluded from this study.

Data Collection: Data was extracted from the database. Clinical history, laboratory and socio-demographic information of the patients were obtained from the database. The clinical history includes history at presentation, presence of opportunistic infections, and other non- HIV related history. The laboratory information obtained were CD4 cell counts, viral load, Full blood counts, liver function tests, renal function tests.

A semi- structured questionnaire was used to obtain additional clinical and socio- demographic information- weight/ height (Body Mass Index), level of income e.t.c.

Sample Collection: 2mls blood samples were collected by venepuncture using vacutainers from each participant. Each blood sample was labelled accordingly and whole blood samples were used for genotype analysis.

Laboratory Procedures

The sickling status of each sample was determined by using cellulose acetate electrophoresis method. The machine was set at 250V and the separation was read after 20 minutes.

Following electrophoretic migration, visualization of haemoglobin bands were accomplished and compared with known standards. This method yields rapid and reproducible separation of HbA, HbF, HbS and HbC as well as other variant haemoglobins with minimal preparation time.

Data Analysis

Data analysis was done using SPSS software. Data was collected, collated, entered and analysed using statistical package for social sciences (SPSS) version 20. Descriptive statistics was performed on the socio-demographic data (age, sex, occupation, ethnic group, religion, income). Data was summarized using frequency tables and bar charts. Chi-square was used to test association between categorical variables. P value < 0.05 was considered to be statistically significant. For multivariate analysis, factors that were found by summary statistics to have a significant ($P \leq 0.05$) association with SCD were entered into logistic regression.

Ethical Considerations

This study was approved by NIMR institutional review board (IRB). Willingness to participate was solely the patients' decision and the participants could decide to withdraw at any time. Hence, withdrawal and refusal to participate did not attract any penalty whatsoever. In other words, only individuals who consented to be part of this study were recruited.

Results

Socio-Demographic Characteristics of Respondent

Two hundred and ninety two patients were recruited into this study. The females were higher (73.8%) than males. The preponderance age group was 41-50, (46%) with majority (47.1%) having secondary education and about half of the participants are traders (49.3%). About two third are married (61.9%) with 14.7% single. Majority of the participants belong to low income class.

Table 1 shows the baseline clinical and laboratory characteristics of the participants. The distribution of Hb genotypes among study participants is as described. Out of the two hundred and ninety two (292) participants that were sampled, two hundred and seventy four (274) had result for Hb genotype, 74.1%, 22.9 and 2.9% were HbAA, HbAS and HbAC respectively. Sickle cell trait (SCT-HbAS + HbAC) was found in 25.9% of the participants and no HbSS was recorded.

Table 2 compares the baseline laboratory parameters of HbAA and sickle cell trait (SCT). There was no significant difference in the baseline CD4, WBC, CRT and Hb of HbAA and SCT participants. However, high baseline VL was significantly associated with HbAA genotype.

There was no significant difference in age between SCT and HbAA, with age 41-50 being predominant in both groups. There was no significant difference in sex (females were higher than males at 1:3 ratio), level of education, occupation, marital status and level of income (Table 3).

No significant difference was observed in the mean BMI between HbAA (-1.31±2.6) and SCT (2.07±0.9), $P>0.05$; ART regimen ($P= 0.431$), Duration of ART treatment, and presence of opportunistic infections at diagnosis ($P= 0.221$). The mean WHO clinical stage (HbAA: 1.04±0.07; SCT: 1.06±0.03; $P= 0.033$) was significantly different in the two groups. History of blood transfusion (10.8%, $P= 0.046$) and presence of anaemia at diagnosis (14.8%; $P= 0.006$) was significantly more common in HbAA genotype (Table 4).

The trend over 5 years in CD4 cells rise was low in SCT compared to HbAA. There was higher number of HbAA with normal WBC counts over 5 years period than SCT and the rise peaks at 2 years and 5 years in HbAA and SCT respectively with a drop at 2 years in SCT. Most of the participants are anaemic in both HbAA and SCT with majority having normal CRT level at baseline which drops gradually from 6 months to 12 months; plateaus from 12 months to 24 months and rises again from 24 months. Lower CRT level was high between 12 months to 24 months of treatment (Figure 1). In general, both groups' response over the years was similar with lower figures in SCT. Very high viral load was seen at baseline in both groups which gradually drops in 5 years. However, majority of HbAA had higher viral load than SCT.

Discussion

The prevalence of HbAA and Sickle cell trait (SCT) among HIV infected adults from this study is 74.1% and 25.9% respectively. There is no record of Sickle cell disease (SCD) among the study population. The zero prevalence of SCD could either be due to early death, as about 3% of births in Africa accounts for SCD, which is believed to be associated with high child mortality [18] or due to low susceptibility of SCD to HIV virus infection. However, available evidence suggests that SCD has lower risk for HIV-1 infection [9] and this might explain the zero prevalence in this study. The SCT prevalence is in agreement with the recorded prevalence (25-30%) in Nigeria and sub-Saharan African countries. [8].

There was no significant difference in the baseline CD4, WBC, CRT and Hb of HbAA and SCT participants except on viral load. High baseline VL was significantly associated with HbAA genotype. The lower HIV viraemia in SCT could be due to lower iron metabolism as demonstrated by [19]; there was inhibition of HIV-1 replication in vitro under the conditions of low intracellular iron or heme treatment and this suggests a potential restriction of HIV-1 infection in SCD. Iron metabolism is intrinsically connected to HIV-1 infection and disease progression. The development of AIDS is accompanied by an increasing iron accumulation in macrophages, microglia, endothelial cells, myocytes, bone marrow, brain white matter, muscle and liver [12,13]. Increased iron stores correlate with rapid HIV-1 progression in AIDS patients. This is evidenced by a retrospective study demonstrating the lower survival of HIV-1-infected individuals inversely correlated to higher iron stores [14]. Elevated iron level has been suggested as a potential predictive marker for higher mortality in HIV-1-infected Gambian adults [16]. In other words, SCT patients with lower iron stores is evidenced by decrease in HIV-1 replication.

Most of the participants were diagnosed at stage 1 of HIV disease and this could be due to provider-initiated counselling and testing and referral to care, put in place to diagnose apparently healthy individuals with little or no symptom to HIV. Anaemia and history of blood transfusion were recorded in most of the participants with higher significant difference in the HbAA group. Anaemia has been implicated in HIV infected population and it is predictive of the disease progression [20].

All the study participants after recruitment were exposed to HIV treatment, and most of them had very high viral load at baseline before commencement of treatment. Over the years, the Highly Active Antiretroviral Therapy (HAART) had reduced the disease to a chronic condition with clinical improvement evidenced in both groups. However, treatment response was better in SCT group than in HbAA group in respect to their viral load (Figure 1), although CD4 response in SCT was slow.

Limitations

None.

Conclusion

Comment [10]: cant make this assumption because their iron status was not done

Comment [11]: Cant apply these here

Comment [12]: Apart from the genotype of the patients which was done after their enrollment into the study, what other criteria were used to separate them into groups?

Comment [13]: There are limitations, e.g the study was retrospective, the sampling and results were not gotten at the same time

High baseline VL was significantly associated with HbAA genotype. Treatment response over the years was similar in both groups, with lower figures seen in SCT. It appears that sickle cell gene contributes to delays in HIV disease progression. It was concluded that the lower HIV viraemia in SCT might mean delay in HIV-1 disease progression and subsequently better quality of life for such patients.

Comment [14]: The study cant give this conclusion

What is already known on this topic: there is paucity of data on this topic but available evidence suggests that individuals with high iron load exhibit an increase in HIV viral load.

What this study adds: sickle cell trait contributes to delay in HIV disease progression.

Comment [15]: Really?

Tables and Figures

Table 1: Baseline Clinical and Laboratory Characteristics of Participants

Table 2: Baseline laboratory Parameters among Participants (SCT and HbAA)

Table 3: Relationship between Socio-demographics and HbAA and SCT

Table 4: Association between HIV Characteristics and HbAA and SCT

Figure 1: Comparative trend in treatment response of study participants over 5 year period.

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Comment [16]: Not properly referenced

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Comment [17]: Six name sbefore et al

Comment [18]: Same as above

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Table 1: Baseline Clinical and Laboratory Characteristics of Participants

Characteristic	Mean ± SD
Age at HIV Diagnosis (years):	44.20 ± 9.02
Baseline HIV Viral Load (copies/ml)	4.518 ± 0.075
Baseline CD4 Count (cells/μL):	241.54 ± 198.50
Baseline Haemoglobin (Hb) level (g/L):	122.3 ± 8. 5
WHO Clinical Stage at Diagnosis	1.04 ± 0.29
Baseline creatinine (CRT) level	98.68 ± 48.10
Baseline white blood cell count (WBC)	4.59 ± 1.60

Table 2: Baseline laboratory Parameters among Participants (SCT and HbAA)

Characteristics	SCT	HbAA	P-value
CD4_baseline	SCT (n = 59)	HbAA (n =185)	
< 100	13(22.0)	47(25.4)	0.576
101-200	13(22.0)	49(26.5)	
> 200	33(55.9)	89(48.1)*	
WBC Baseline	SCT (n = 60)	HbAA (n =179)	
< 4.5	31(51.7)	102(57.0)	0.632
4.5-11	29(48.3)	76(42.5)	
>11	0(0)	1(0.6)	
CRT_Baseline	SCT (n = 61)	HbAA (n =173)	
< 44	1(1.7)	2(1.2)	0.912
44-115	50(83.3)	142(82.1)	
>115	9(15.0)	29(16.8)	
VL_Baseline	SCT (n = 61)	HbAA (n =160)	
< 2.0	11(19.0)	2(1.3)	<0.001
2.0-3.1	47(81.0)	18(11.3)	
>3.1	0(0.0)	140(87.5)	
HB Baseline	SCT (n = 61)	HbAA (n =179)	
<135	61(100)	171(99.4)	0.551
135-175	0(0)	1(0.6)	

Table 3: Relationship between Socio-demographics and HbAA and SCT

Characteristic	No of participants (%) n = 292	SCT (n = 71)	HbAA (n =203)	OR (95% CI)	P value
Age (years)					
< 31	13(4.5)	4(5.6)	8(3.9)		0.555
31-40	86(29.8)	18(25.4)	64(31.5)		
41-50	133(46.0)	37(52.1)	89(43.8)		
51 & above	57(19.7)	12(16.9)	41(20.2)		
Sex					
Male	76(26.2)	15(21.1)	55(27.1)	0.72 (0.37-1.37)	0.311
Female	214(73.8)	56(78.9)	147(72.4)	1.0	
Level of Education					
None	10(3.6)	3(4.2)	6(2.9)		0.419
Primary	40(14.6)	9(12.7)	29(14.3)		
secondary	129(47.1)	35(49.3)	93(45.8)		
Tertiary	88(32.1)	21(29.6)	59(29.1)		
Others	7(2.6)	4(5.6)	3(1.5)		
Occupation					
Unemployed	37(13.4)	9(12.7)	26(12.8)		0.697
Artisan	14(5.1)	6(8.5)	7(3.4)		
Trader	136(49.3)	35(49.3)	97(47.8)		
Civil Servant	35(12.7)	6(8.5)	27(13.3)		
Professional	33(12.0)	12(16.9)	20(9.9)		
Others	21(7.6)	3(4.2)	15(7.4)		
Marital Status					
Married	169(61.9)	41(57.7)	117(57.6)		0.238
Single	40(14.7)	8(11.3)	31(15.3)		
Divorced	16(5.9)	5(7.0)	11(5.4)		
Separated	15(5.5)	3(4.2)	11(5.4)		
Widowed	33(12.1)	14(19.7)	19(9.4)		
Level of Income					
<20,000	99(43.8)	32(45.1)	89(43.8)		0.098
20,000-50,000	64(28.3)	19(26.8)	55(27.1)		
51,000-100,000	29(12.8)	11(15.5)	32(15.8)		
>100,000	34(15.0)	9(12.7)	27(13.3)		

Table 4: Association between HIV Characteristics and HbAA and SCT

Characteristic	SCT (n = 71)	HbAA (n =203)	OR (95% CI)	P value
BMI/Weight for Age Z Score Mean (± SD)	-2.07(1.9)	-1.31(2.6)		0.245
WHO Clinical Stage: Mean (± SD)	1.06(0.03)	1.04(0.07)		0.033
ART				
1 st Line	54(76.1)	123(60.6)	1.29 (0.68-2.44)	0.431
2 nd Line	17(23.9)	50(24.6)	1.0	
ART Duration (years): (Mean ± SD)	50(70.4)	147(72.4)		
History of Blood Transfusion	71	183		
Yes	6(8.5)	22(10.8)	0.13 (0.02-0.96)	0.046
No	65(91.5)	161(79.3)	1.0	
Presence of Opportunistic Infections at Diagnosis	71	153		
Yes	8(11.3)	27(13.3)	0.59 (0.25-1.37)	0.221
No	63(88.7)	126(62.1)	1.0	
Diagnosed with Anaemia/Shortage of Blood				
Yes	5(7.0)	30(14.8)	0.39 (0.15-1.05)	0.006
No	66(93.0)	155(76.4)	1.0	

Figure 1: Comparative trend in treatment response of study participants over 5 year period.

