Introduction

Haemoglobinopathies are fast becoming a global health problem. It is estimated that about 300,000 children are born each year with a severe inherited disorder of haemoglobin and that approximately 80% of these births occur in low- or middle-income countries particular in Africa [1]. Haemoglobinopathies are inherited disorders of haemoglobin. They are the most common gene disorders with 7% of the world's population being carriers. An estimated 300,000 children are born with sickle cell disease (SCD) worldwide every year [2]. Sickle cell disorders are found very frequently in the Afro-Caribbean populations and sporadically throughout the Mediterranean region, India and the Middle East [1]. These sickling disorders include the homozygous state for haemoglobin S or the sickle cell trait (AS), the homozygous state for HbS or sickle cell anaemia (SS) and the compound heterozygous state for HbS together with haemoglobin C, D, E or other structural variants. Haemoglobin S differs from haemoglobin A by the substitution of valine for glutamic acid at position 6 in the β–chain [3].

Haemoglobinopathies are the commonest genetic defect worldwide with an estimated 269 million carriers [4]. Certain populations are particularly at risk of having a haemoglobinopathy, for example, in South East Asia, there are 90 million carriers, about 85 million in sub-Saharan Africa and 48 million in the West Pacific region. Sickle haemoglobin (HbS) is the most common and clinically significant haemoglobin structural variant [5]. It is anticipated that the global economic burden of the haemoglobinopathies on public health will increase over the coming decades [6].

Early detection and characterization of the haemoglobinopo-
Table 1. Distribution of haemoglobin types among subjects based on age groups.

| Age group (years) | Hb AA | Hb AS | Hb AC | Hb SC | Hb SS |
|-------------------|-------|-------|-------|-------|-------|
| 0–10              | 74 (18.50%) | 34 (8.50%) | -     | 3 (0.75%) | 17 (4.25%) |
| 11–20             | 140 (35.00%) | 20 (5.00%) | 3 (0.75%) | -     | 2 (0.5%) |
| 21–30             | 11 (2.75%) | 7 (1.75%) | 2 (0.5%) | -     | -     |
| 31–40             | 13 (3.25%) | 30 (7.50%) | -     | -     | -     |
| 41–50             | 20 (5.00%) | 2 (0.5%) | -     | -     | -     |
| 51–60             | 22 (5.5%) | -     | -     | -     | -     |

Table 2. Frequency and gender distribution of different haemoglobin (Hb) electrophoretic pattern among the study population.

| Hb electrophoretic pattern | Numbers (n) | % frequency | Gender |
|----------------------------|-------------|-------------|--------|
|                            | HbAA | HbAS | HbAC | HbSC | HbSS |
| AA                         | 280  | 70.00 | 93 (76.9%) | 187 (67.02%) |
| AS                         | 93   | 23.25 | 18 (14.9%) | 75 (26.88%) |
| AC                         | 5    | 1.25  | 1 (0.83%) | 4 (1.43%) |
| SC                         | 3    | 0.75  | 1 (0.83%) | 2 (0.72%) |
| SS                         | 19   | 4.75  | 8 (6.61%) | 11 (3.94%) |

template/treatment/management is essential so that appropriate counselling can be provided to couples and families who may be at risk of severe haematological consequences [7]. Knowledge on basic diagnosis, counselling and management of the haemoglobin disorders among doctors, nurses, and social workers particularly in Africa where the disease burden is greatest is limited. Carrier screening and mutation identification are often suboptimal. Carrier screening and mutation identification is the cornerstone prevention program for the haemoglobin disorders in the developed world. The frequencies of these inherited characters have been extensively reported in various populations and ethnic groups in Nigeria [8-11]. However, there has been no known published data on the distribution pattern and frequency of common haemoglobin variants from the North Western region of Nigeria. Therefore, this study is aimed at providing information on the distribution pattern of haemoglobinopathies in Sokoto, Nigeria. Evidenced-based data obtained can be used to formulate policies on diagnosis, prevention and management of haemoglobinopathies in the North West geopolitical zone in particular and Nigeria in general.

Study population

This prospective case study was carried out in the Faculty of Medical Laboratory Science in Usmanu Danfodiyo University in Sokoto, North Western Nigeria. All the participants gave their written, informed consent and were offered pre- and post-test counseling. The aim of this present study was to investigate the prevalence/spectrum of haemoglobin variants, among 400 consecutively recruited subjects of African descent resident in Sokoto in the North West geopolitical zone of Nigeria.

Study area

This present research work was carried out in the cosmopolitan city of Sokoto, in North Western Nigeria. Sokoto State is located in the extreme North Western part of Nigeria near to the confluence of the Sokoto River and the Rima River. With an annual average temperature of 28.3°C (82.9°F), Sokoto is, on the whole, a very hot area. However, maximum daytime temperatures are for most of the year generally under 40°C (104.0°F). The warmest months are February to April when daytime temperatures can exceed 45°C (113.0°F). The rainy season is from June to October during which showers are a daily occurrence. There are two major seasons, wet and dry which are distinct and are characterized by high and low malarial transmission respectively. Report from the 2007 National Population Commission indicated that the state had a population of 3.6 million [12].

Sample collection and methods

Blood samples were collected by venipuncture into ethylene diamine tetracetic acid (EDTA) anticoagulated tubes and used for the determination of abnormal haemoglobin variants. The method described by Brown [13] was used for haemoglobin electrophoresis. A small quantity of haemolysate of venous blood from each of the subjects was placed on a cellulose acetate membrane and carefully introduced into Shandon Electrophoretic tank containing tris-EDTA-borate buffer at pH 8.6. Electrophoretic separation was then allowed to operate for 15–20 minutes at an electro motive force (emf) of 160V. The results were read immediately. Haemolysate from blood samples of known haemoglobin (AA, AS, AC) were run as controls.

Result

In this present study, we investigated the prevalence/spectrum of haemoglobin variants among 400 subjects of African descent in Sokoto North Western Nigeria. We evaluated the prevalence based on age groups and observed that HbAA prevalence was highest in the 11–20 years age group while HbAS, HbAC, HbSC and HbSS (35%, 8.5%, 2.25%, 0.75%, 4.25%) prevalence’s was highest among subjects<10 years old. Table 1 show the distribution of the various haemoglobins based on age groups of subjects. Table 2 show the frequency and gender distribution of different forms of haemoglobin (Hb) among the subjects; Hb AA 280(70%); HbAS 93(23.25%); HbAC 5(1.25%); HbSC 3(0.75%) and HbSS 19(4.75%). Of the 400 subjects studied, 121 were males (30.25%) while 279 were females (69.75%). Among the male subjects, 93(67.9%) were HbAA, 18(14.9%) were HbAS; 1(0.83%) were HbAC; 1(0.83%) were HbSC and 8(6.61%) were HbSS. Among the 279 female subjects, 187(67.02%) were HbAA, 75(28.88%) were HbAS; 4(1.43%) were HbAC; 2(0.72%) were HbSC and 11(3.94%) were HbSS. We observed that all subjects with haemoglobin...
SS and SC were less than 20 years of age.

Discussion
In this study, the frequency of HbAA was 70%. This finding is consistent with previous reports in which a prevalence of 80.32% [9] and 69.1% [8] was obtained among students in the Niger Delta of Nigeria. The observed frequency of HbAA is also within the normal range of 55–75% earlier reported for Blacks [14].

The prevalence of HbSS among the black population in the United States, was reported to be 9% and 30–40% generally for Africans [15-16]. The frequency of sickle cell trait (AS) is consistent with prevalence reported for Africa [17]. Sickle haemoglobin (HbS) is the most common and clinically significant haemoglobin structural variant. We observed a prevalence of HbSS of 4.75% among subjects studied. This finding is consistent with other published reports in Nigeria; 3.0% in the South-West region of Nigeria [18], 2% among under-graduate students in Bayelsa State [11] and 3% in Rivers State [19] both in the South-South of Nigeria. Our finding is however at variance with previous report in Kenya, East Africa [20] and among 620 University students in Port Harcourt Nigeria [9] which both obtained a 0% prevalence of HbSS. The zero frequencies observed in these studies, possibly imply that the sickling gene pool is gradually reducing in some African populations due to increased awareness and pre-marital counselling. The low prevalence of HbSS observed in these studies could be attributed to increased awareness of the disease, improved socio-economic conditions, improved pre-marital counselling, environmental and genetic factor which have an overall effect on the sickling gene pool. The zero prevalence may also be attributed to an active program of prenatal diagnosis among pregnant women in Nigeria. By comparison, the prevalence of HbSS among the black population in the United States is reported to be 9% and 30–40% generally for Africans [21-22]. The number of people with homozygous SS in Sokoto, Nigeria is high. The reason for this high prevalence may be due to the absence of carrier testing programs and premarital counseling/testing for prospective couples prior to marriage in a bid to reduce the prevalence of haemoglobinopathies in the area. Sokoto State in particular and Nigeria in general can benefit from universal neonatal screening program. It can be an effective way to diagnose and monitor the trend of haemoglobinopathies in the state. Evidenced-based data from Belgium, a country with universal neonatal screening programme has shown that neonatal screening is an excellent health education tool [23]. The Nigerian government can benefit by implementing a similar program in a bid to improving the healthcare services offered to patients with haemoglobin disorders [24]. There is also a need for a sickle cell disease clinical care programs which should include: infection prophylaxis with penicillin and malarial prophylaxis; family training to identify early, severe, or persistent symptoms and increased awareness of the gravity of malarial crises; the evaluation of the patient’s nutritional status and fluid intake; and education about the importance of regular medical visits.

The frequency of HbAS observed in this present study was 23.25%. This finding is in agreement with prevalence of 20–30% quoted for Nigeria and 20–40% for Africa in general [8,9,25]. Our finding is much higher than a prevalence of 1.15% observed in Bengal, India [26]. Considerable evidence has been provided to show that these traits do confer protection from malaria [27]. Evidence exists on the protective role against clinical Plasmodium falciparum malaria [28-30].

We observed a prevalence of HbAC and HbSC in 1.25 and 0.75% of subjects studied. Our finding is consistent with a 2.0% SC obtained in the South-West region of Nigeria [17-18]. Our study is also consistent with a 1% prevalence of HbC reported in a previous study in Ecuador, a tropical Latin-American country with an important presence of afro-descendants [32]. Haemoglobin C (HbC) is one of the commonest structural haemoglobin variants in human populations. Although HbC causes mild clinical complications, its diagnosis and genetic counselling are important to prevent inheritance with other haemoglobinopathies [33]. Haemoglobin C (HbC) is a structural variant of normal haemoglobin (HbA) caused by an amino acid substitution at position 6 of the β-globin chain (β6Glu-Lys) [34]. Homozygosity (CC) causes clinically mild haemolytic anaemia, due to the reduced solubility of the red blood cells which can lead to crystal formation [35]. Hbc is mainly of clinical significance when inherited in combination with HbS (sickle-haemoglobin C disease), causing chronic haemolytic anaemia and intermittent sickle cell crises, slightly less severe or frequent than in homozygous Hbs patients (SS), and when co-inherited with β-thalassaemia (haemoglobin C-β thalassaemia), causing moderate haemolytic anaemia with splenomegaly.

In this present study, we observed that all subjects with haemoglobin SS and SC were less than 20 years of age. Despite being the most prevalent genetic disease in Africa and associated with serious health and socioeconomic impacts, Sickle cell disease is largely neglected [36]. SCD ultimately results in multiple organ failure and premature death, occurring mostly in children under five years and adolescents [37]. The overall mortality associated with sickle cell disease is increased and life expectancy decreased when compared to the general population [38-39]. Painful vaso-occlusive events are the most common complication experienced by children and adults with sickle cell disease. Evidence-based care of patients with sickle cell disease particularly in the developed world is largely supportive with hydroxyurea widely used to modify the disease pathogenesis. Other supportive therapies include effective transfusion support, pneumococcal vaccination, antibiotic, steroids, effective pain management with opioids and Non-steroidal anti-inflammatory drugs (NSAIDS) as well as fluid replacement. A significant of patients with sickle cell anaemia survived beyond the fifth decade in
the developed world [40]. A significant number of sickle cell disease patients in developing countries are not as fortunate as their counterparts in the developed countries. Sickle cell anaemia survival to adulthood in Africa was reported to be 10-15% in the first decade of life, with the death rate of about 5% during subsequent decades. Large portion that died have shown no overt chronic organ failure but died during acute episodes of pain, infections, acute chest syndrome, stroke and anaemic crises [41-42]. There are several reasons for high mortality seen among patients with HBSS and SC particularly in resource-limited settings compared to developed economies; unaffordability of disease modifying agents such as hydroxyurecarbamide (hydroxyurea), antibiotics such as phenoxymethylpenicillin and cefotaxime, pneumococcal vaccination, suboptimal access to adequate and safe red cell transfusion support, lack of access to iron chelating agents like deferoxamine, the presence of other compounding tropical diseases (malaria, tuberculosis and HIV) and suboptimal neonatal diagnosis and genetic counselling [43]. There is the need to develop long-term partnerships between SCD clinicians and researchers in developed and developing income countries in order to build the capacity and improve clinical care offered to SCD patients in developing countries [44].

Conclusion
A significant percentages of haemoglobinopathies is prevalent in Sokoto, North Western Nigeria. Knowledge of the prevalence and distribution of haemoglobinopathies among any population is useful in healthcare planning, appropriate allocation of resources, justification of the need for appropriate pre-marital and prenatal diagnosis and genetic counselling policy as an avoidance strategy to reduce the incidence of haemoglobinopathies in Sokoto, Nigeria.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
YA Study design. FPU, ACE, YM and AW Recruitment and counselling of subjects. ZII, BMS, IPI, DI and FA Laboratory analysis. IAB and OI Statistical analysis. EKU, TCA, OE Manuscript reporting.

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