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Prevalence of some haemoglobin variants among students of Usmanu Danfodiyo University, Sokoto, North Western Nigeria

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Abstract

Haemoglobinopathies are global public health problem and is predominant in the Mediterranean, African and Asian regions. This aim of this present study is to determine the distribution of some haemoglobin variants among students of African descent attending Usmanu Danfodiyo University in Sokoto North Western, Nigeria. Ethylenediamine tetracetic acid anticoagulated blood was used for the determination of haemoglobin electrophoresis. A total of two hundred and fifty (250) apparently healthy students of African descent attending Usmanu Danfodiyo University in Sokoto North Western Nigeria aged 18-35 years and mean age 26 ± 2.0 years made up of 145 male (58.0%) and 105 female (42.0%) constituted the subjects in this present study. Haemoglobin electrophoresis pattern indicated that 176 (70.4%) were HbAA, 60 (24.0%) were HbAS, 2 (0.8%) were HbSS, and 12 (4.8%) were HbAC respectively. The prevalence of HbAS, HbSS and HbAC was higher among male students (13.6%, 0.8% and 2.8%) compared to female students (10.4%, 0% and 2%) respectively. Data derived from this study will help policy makers make evidenced –based decisions on pre-marital screening, genetic counseling, neonatal genetic testing for haemoglobinopathies, carrier screening, mutation identification screening and in the management of cases of disputed parentage. It can also help in the formulation of genetic counseling policies to help prospective couples make informed decisions in an effort to reduce the sickling gene pool in North Western Nigeria. We recommend that a sickle cell disease clinical care program that includes prophylaxis with penicillin to control infection, malarial prophylaxis; family training to identify early, severe, or persistent symptoms and increase awareness of the gravity of malarial crises, the evaluation of the patient's nutritional status, fluid intake and education about the importance of regular medical

visits should be implemented.

1. Introduction

Haemoglobinopathies is a global public health problem and is predominant in the Mediterranean, South East Asia , sub-Saharan Africa, West Pacific region and among migrant population in Europe [1-2]. There is concern by the World Health Organization (WHO) that haemoglobinopathies are a growing public health problem in 71% of 229 countries, mostly in low and middle income countries[3]. Haemoglobinopathies are fast becoming a global public health challenge and the most common rare disease (RD) of genetic origin in Europe [4-5]. An estimated 300,000 children are born each year with a haemoglobinopathy with a significant number occurring in developing low to middle income countries particularly in Africa and Asia [6]. Some haemoglobin variants such as sickle-cell anaemia cause pathologic diseases and are considered haemoglobinopathies.

Haemoglobinopathies are inherited disorders of haemoglobin. They are the most common gene disorders with 7% of the world's population being carriers and an estimated 300,000 children born with sickle cell disease (SCD) worldwide every year [7]. Other variants (AC, CC and SC) do not produce any detectable pathology and are thus considered non-pathological variants [8]. The sickling disorders include the heterozygous 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 a single amonio acid substitution of valine for glutamic acid at position 6 in the β – chain [9]. Sickle haemoglobin (HbS) is the most clinically significant haemoglobin structural variant [10]. Due to poor implementation of genetic counseling and suboptimal carrier screening and mutation identification in endemic countries, it is feared that the global economic burden of the haemoglobinopathies on public health will increase over the coming decades [11-12].

Early diagnosis and characterization of the haemoglobinopathies is expedient to facilitate prompt and appropriate counseling of intending couples and families who may be at risk. Knowledge on basic diagnosis, counseling and management of the haemoglobin disorders among healthcare professional in endemic countries particularly in Africa where the disease burden is greatest is suboptimal [13]. Carrier screening and mutation identification are often unavailable and when available, it is often suboptimal [14]. Carrier screening and mutation identification is the cornerstones of prevention program for haemoglobin disorders in the developed world [15]. The frequencies of these inherited characters have been extensively reported in various populations and ethnic groups in Nigeria [16-21]. However, there has been no known published data on the distribution pattern and frequency of common haemoglobin variants among University Students in North Western region of Nigeria. Therefore, this present study is aimed at providing information on the distribution pattern of haemoglobinopathies among registered students of African descent attending Usmanu Danfodiyo University in Sokoto, North Western Nigeria. Evidenced-based data obtained from this case study can potentially be used to formulate policies on the diagnosis, prevention and management of haemoglobinopathies in the North West geopolitical zone in particular and Nigeria in general.

1.1. Study Area

The selected area for this study is Usmanu Danfodiyo University Teaching Hospital (UDUTH) which is located in Wamakko Local Government within Sokoto Metropolitan city in Sokoto State. Sokoto State is located in the extreme Northwest of Nigeria, near the confluence of the Sokoto River and Rima River. With an annual average temperature of 28.3^0c ($82.9\ ^0$F). Sokoto is, on the whole, a very hot area. However, maximum day time temperatures are for most of the year generally under 40 ^{0}C (104.0 ^{0}F). The warmest months are February to April when daytime temperatures can exceed 45 ^{0}C (113.0 ^{0}F). The rainy season is from May 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 [22].

1.2. Study Setting

The study was conducted in the Faculty of Medical Laboratory Science of Usmanu Danfodiyo University in collaboration with Haematology Department of Usmanu Danfodiyo University Teaching Hospital.

2. Sample Collection and Methods

Blood samples were collected by venipuncture into ethylenediamine tetracetic acid (EDTA) and used for haemoglobin electrophoresis determination. The method described by Brown [23] was used for haemoglobin electrophoresis. A small quantity of haemolysate of venous blood from each of the subjects was placed on the cellulose acetate membrane and carefully introduced into the electrophoretic tank containing Tris - EDTA - Borate buffer at pH 8.9. The electrophoresis was then allowed to run for 15 – 20 minutes at an electro motive force (emf) of 160 V. The results were read immediately. Haemolysate from blood samples of known haemoglobin (AA, AS, AC) were run as controls. At alkaline pH, haemoglobin is a negatively charged protein and when subjected to electrophoresis will migrate toward the anode. Structural variants that have a change in the charge on the surface of the molecule at alkaline pH will separate from Hb A. This is based on the presence of different amino acid group composition on the globlin chain

which carries different charge and travels differently across a cellulose acetate support when an electric current applied.

2.1. Statistical Analysis

The data collected was recorded on an Excel spreadsheet and later subjected to statistical analysis using a statistical software SPSS version 18.0. Statistical analysis included descriptive statistics of mean and bivariate analysis of t- test and chi- square. Correlation was compared using linear regression analysis. Differences were considered significant when $p \leq 0.05$.

2.2. Eligibility Criteria

All consenting, consecutively recruited legal adults (≥ 18 years), confirmed students of Usmanu Danfodiyo University Sokoto without a recent history of red cell transfusion constituted the subjects of this study.

2.3. Exclusion Criteria

The following students of Usmanu Danfodiyo University Sokoto who did not meet the inclusion criteria were excluded from the study; non-adult students < 18 years, non - consenting students and students who have had a red cell transfusion in the 4 months.

2.4. Informed Consent

Verbal informed consent was obtained from all students participating in this study, together with socio-demographic information. Ethical clearance was sought from the ethical committee of Usmanu Danfodiyo University (UDUS), Sokoto North Western, Nigeria.

3. Result

A total of two hundred and fifty students (250) apparently healthy students of African descent attending Usmanu Danfodiyo University in Sokoto North Western aged 18-35 years and mean age 26 ± 2.0 years made up of 145 males (58.0%) and 105 female (42.0%) constituted the subjects in this case study. Haemoglobin electrophoresis was carried out on red cell samples of each subject. The Hb electrophoretic pattern indicated that 176 (70.4%) were HbAA, 60 (24.0%) were HbAS, 2 (0.8%) were HbSS, and 12 (4.8%) were HbAC respectively. Figure 1 show the distribution of Haemoglobin Variants among Students.

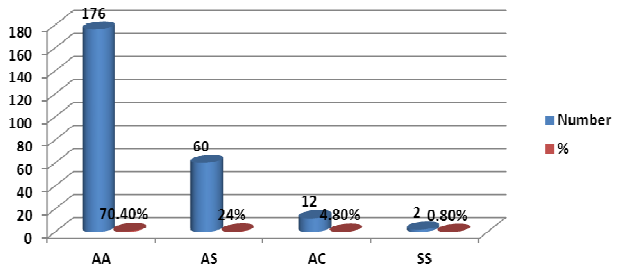

Figure 1. *Distribution of Haemoglobin Variants among Students*

The prevalence of HbAS, HbSS and HbAC was higher among male students (13.6%, 0.8% and 2.8%) compared to female students (10.4%, 0% and 2%) respectively. Table 1 show the distribution of Haemoglobin Variants among the subjects based on gender.

Table 1. *Distribution of Haemoglobin Variants based on gender*

Gender	HbAA(%)	HbAS(%)	HbSS(%)	HbAC(%)	Total
Male	102(40.8%)	34 (13.6%)	2 (0.8%)	7 (2.8%)	145
Female	74(26.9%)	26 (10.4%)	0 (0%)	5 (2%)	105

4. Discussion

Haemoglobinopathies is a growing global public health problem and is predominant in Mediterranean, African and Asian regions. In this study, we observed the haemoglobin electrophoretic pattern AA among 67.7% of students tested. The observed frequency of HbAA in this study is in agreement with previous reports in Nigeria; 68% by Bakare and colleagues [19], 71.02% by Oluwadare and Shonekan [24], 70% by Adeyemo and Soboyemo [25], 66% by Egesie and colleagues [18], 69.1% by Erhabor and colleagues [21], 78.5% by Pennap and colleagues [26], 78% by Adu and colleagues [27], 71.03% by Akhigbe and coworkers [28] and 65.3% by Thomas and colleagues [29]. Similarly a prevalence of 74% and 94% of HbAA was observed in a previous report in lowland and highland areas of Kenya [30].

In this study, we observed the haemoglobin electrophoretic pattern AS among 24% of students tested. Our finding is consistent with previous reports [18, 25, 28, 31-33] which indicated that the distribution of the sickle cell trait (HbAS) is 20-30% in Nigeria. A prevalence of HbAS of 26% and 3% was observed in a previous report in lowland and highland areas of Kenya [30]. The frequency of (AS) was reported as follows: 8%–16% for Black Americans, 8%–10% for White Americans, 6%–15% for Europeans (United Kingdom, Pakistanis and Blacks), 1%–15% for Europeans (Mediterranean), 3%–8% for Caribbean, 7%–8% for Middle Eastern population, 15%–30.5% for Africans, and 40.5% for West Africans and Nigerians [34] . The frequency of HbAS detected in this study (24%) is also consistent with previous reports [21, 26,35] which observed a prevalence of 20%–40% in Africa in general [35]. Haemogloblin AS is thought to offer some protective role against plasmodium falciparium malaria and conclusive evidence of this exist with Haemogloblin S (beta 6Glu-> val) and HbC (beta 6Glu-> lys), both occurring in sub-Saharan Africa [36-38]. However, the mechanism(s) of the protection exerted remain(s) debatable for both haemogloblin variants HbC and HbS

In this study, we observed the haemoglobin electrophoretic pattern AC among 4.8% of students tested. Also none of our cohorts of students were homozygous for haemoglobin C (HbCC) or compound heterozygous (SC). A previous study among students in the Niger Delta of Nigeria by Erhabor and colleagues [21] did not detect any haemoglobin C either as homozygous HbCC, heterozygous HbAC, or compound heterozygous HbSC. However a previous study in the Niger

Delta by Egesie and colleagues [18] reported a prevalence of 2% and 4%, respectively for HbAC and HbSC. Similarly Nwafor and Banigo [20] obtained AC prevalence of 1% among their cohort of subjects in Bonny Rivers State , Nigeria. A prevalence of 5.26% and 4% HbAC respectively was observed in Ogbomoso and Ibadan in previous reports [28, 32]. A low incidence of sickle haemoglobin AC (0.02%) was observed in Anambra State, Nigeria in a previous report [31]. However a previous report among students in Ladoke Akintola University of Technology, Ogbomoso, Nigeria observed a 0.18% HbCC and 0.80% of HbSC [28]. Also, a previous report in Anambra State, Nigeria indicated HbCC prevalence of 0.01% [32]. Similarly a previous report among the Yoruba ethnic nationality in Ibadan, South Western Nigeria, observed HbSC prevalence of 1.1%[31].

In this study, we observed the haemoglobin electrophoretic pattern SS among 2% of students tested. This finding is consistent with previous reports among undergraduate students in Bayelsa State [18] and also in Rivers State [20], both in the South-South of Nigeria where the prevalence rates for HbSS was 2% and 3% respectively. Our observed prevalence is also consistent with a 1.5% observed by Erhabor and colleagues [21] among students in the Niger Delta of Nigeria. A previous report among students in Ogbomosho, Nigeria observed a HbSS prevalence of 0.54% [28]. Our observed prevalence is however lower than a prevalence of 5.5% and 3.54% respectively observed in a previous report in Anambra State, Nigeria and among the Yoruba ethnic group [31-32]. In another report, the geographical distribution of SS was given as follows: 3%–9% for Black Americans, 1%–8% for White Americans, 3%–7% for Europeans (United Kingdom among Pakistanis and Blacks), 2%–8% for other European countries (Mediterranean), 1%–3% for Caribbean, 1%–3% for Middle East, 1%–10% for Africans. However, a study carried out in Kenya, East Africa observed a zero percent prevalence of HbSS [30]. Similarly, the sickle cell gene in homozygous state (HbSS) was not encountered among students of African descent in Port Harcourt in a previous study [16]. The zero frequencies observed in these studies may imply that the sickling gene pool may gradually be reducing in some African populations, particularly those with an abnormal haemoglobin carrier screening and genetic counseling program for the prevention of haemoglobin disorders. The low prevalence of HbSS in these studies may also be due to increased awareness of the disease, improved socio-economic conditions and other environmental and genetic factor which have an overall effect on the sickling gene pool. The number of people with homozygous SS in most settings in Nigeria and other countries in sub Saharan Africa is high. There are several reasons for the high prevalence of homozygous SS in Sokoto, North Western Nigeria. There is absence of carrier testing programs or pre-marital counseling testing for prospective couple prior to marriage in a bid to reduce the prevalence of haemoglobin disorders. There is no universal neonatal screening program and consanguinity (cousin relationships and marriages) is practiced among the people. Universal neonatal screening program is effective way to diagnose and manage haemogloblinopathies. Experience in Belgium and Greece has shown that universal neonatal screening is an excellent health education tools [15, 39]. Countries in Africa can benefit by implementing similar programs which is pivotal to improving the health care of those affected by haemoglobin disorders.

Management of haemoglobinopathies in developing countries is often challenging resulting in high mortality rate [40-41]. There are several reasons for high mortality seen among patients with HBSS and SC particularly in resource-limited settings in sub Saharan Africa compared to developed economies; unaffordability of disease modifying agents such as hydroxycarbamide (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 counseling [42].

5. Conclusion and Recommendation

There is need to implement a sickle cell disease clinical care programs which include: infection prophylaxis with penicillin and malarial prophylaxis; training for carers to identify early, severe, or persistent symptoms, public enlightenment programme to increase awareness of the gravity of malarial crises, evaluation of the patient's nutritional status and fluid intake and education about the importance of regular medical visits. There is also the need to develop genetic counseling policies to help prospective couples make informed decisions about haemoglobinopathies.

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