J Community Genet (2011) 2:13–18
DOI 10.1007/s12687-010-0033-x

ORIGINAL ARTICLE

Frequency of sickle cell genotype among the Yorubas in Lagos: implications for the level of awareness and genetic counseling for sickle cell disease in Nigeria

Idowu A. Taiwo · Olufemi A. Oloyede · Ade O. Dosumu

Received: 15 September 2010 / Accepted: 19 December 2010 / Published online: 27 January 2011

Abstract The frequency of sickle cell genotype (HbSHbS) among the Yorubas living in Lagos, Nigeria, was determined. Seven hundred fifteen (715) subjects of different age groups took part in the study after filling consent forms and questionnaires. The haemoglobin genotype of each subject was determined by the usual electophoretic method from blood sample (3.0 ml) obtained from the antecubital vein. Normal and sickle cell haemoglobin genotypes were detected in subjects within the age group 1–50 years such that 366 (73.1%) had HbAHbA genotype, 123(24.5%) had HbAHbS, while 12 (2.4%) had HbSHbS giving genotypic frequencies that were not significantly different from Hardy–Weinberg expectations ($P>0.05$). More than half (58.3%) of the subjects with the HbSHbS were in the 11–30 years age group. The results of this study showed that there is need for more awareness campaign and proper genetic counselling about sickle cell disease in Nigeria. This is particularly so for adolescents since they are likely to constitute the majority of intending couples and, therefore, those that will produce genes that will make up the gene pool for future generations. In situations where pregnancy had occurred before the actual marriage (as is sometimes the case), the importance of proper prenatal diagnosis and genetic counselling for sickle cell disorder cannot be overemphasized.

Keywords Sickle cell genotype · Awareness · Counselling · Yoruba · Nigeria

I. A. Taiwo · O. A. Oloyede (✉)
Department of Cell Biology and Genetics, Faculty of Science,
University of Lagos,
Lagos, Nigeria
e-mail: oloyedeoao@yahoo.com

O. A. Oloyede
Department of Obstetrics and Gynaecology,
Lagos State University Teaching Hospital,
Ikeja, Lagos, Nigeria

A. O. Dosumu
Department of Haematology,
Lagos State University Teaching Hospital,
Ikeja, Lagos, Nigeria

Introduction

Sickle cell anaemia is a serious genetic disease caused by mutation of β-globin gene located on the short arm of human chromosome 11 (Ashley-Koch et al. 2000). The first description of the haemoglobinopathy now known as sickle cell anaemia was by Herrick (1910) when he reported his observation of sickle-shaped erythrocytes in the peripheral blood of a severely anaemic patient. Hahn and Gillespie (1927) later observed that oxygen inhibited the sickling of erythrocytes in this disease entity and that the defect appeared to reside in the haemoglobin molecule rather than red cell membrane. The genetic and the molecular basis of this disorder together with its clinical importance have been well established; sickle cell anaemia results from a substitution mutation at the sixth base position of the β-globin gene leading to replacement of glutamic acid, a polar amino acid with valine, a less polar amino acid (World Health Organization 2006; Daniel and Elizabeth 2000). The normal wild-type allele of the β-globin gene (HbA) gives rise to fully functional beta globin. Over 400 mutant alleles are known to give rise to variant haemoglobins or thalassaemias (Hartwell et al. 2000). Out of these, the allele for sickle cell disease has attracted the greatest attention because of its frequency

and the severity of sickle cell disease especially in malaria-endemic, underdeveloped, and poor countries like Nigeria.

Epidemiological studies have shown that about 300,000 infants are born annually in Africa with major haemoglobin disorders. Out of these, sickle cell anaemia is about 200,000 (World Health Organization 2006). Majority of these cases occur in West Africa with Nigeria having the highest incidence (Akinyanju et al. 1999). Considering the high perinatal, infant and maternal morbidity, and mortality of sickle cell anaemia, the disease places a significant psychological and financial burden on the patients and their families. This situation is complicated by the high level of poverty and illiteracy in the third world countries like Nigeria. In spite of advances in medical technologies and intervention strategies, available data indicate that the prevalence of infant morbidity and mortality from sickle cell anaemia in the third world countries is still relatively high when compared with the developed countries (World Health Organization 2006).

The first approved drug for the treatment of sickle cell anaemia, hydroxyurea, was shown to reduce the number and severity of attacks, but there is some concern that long-term use of this drug may be harmful. Presently, many drugs are available for the treatment of the disease; none of these has provided a total cure for sickle cell anaemia. There are prospects for gene therapy for sickle cell anaemia; however, the technology is still at the experimental stage. Efforts from international, national, and local agencies addressing the problem of sickle cell anaemia include community-based approaches for education, prenatal and postnatal diagnosis, counseling, surveillance, and management (World Health Organization 2006; Akinyanju et al. 1999; Oloyede 2005). In many poor countries of Africa, educating and counseling the public would constitute the most affordable and feasible methods of prevention. The above measures can only be well programmed and executed if there is information on the prevalence and distribution of sickle cell anaemia as well as the genetic structure of the general population with respect to the disease.

Lagos is a cosmopolitan state, and it is the largest and the most populous urban center in Nigeria. Being the commercial nerve center, it attracts different ethnic groups, and this makes it very heterogeneous and suitable for population studies. Among the major ethnic groups in Lagos, Yoruba is the largest group. The present study was carried out to determine the frequency of sickle cell genotype (HbSHbS) among the Yorubas in Lagos, Nigeria. We hope that the results of this study will not only strengthen but stimulate a more purposeful awareness campaign and genetic counselling on sickle cell disorder in Nigeria.

Subjects and methods

Subjects and questionnaires

The subjects were 715 apparently healthy male and female Yorubas (404 male and 311 female) of different ages attending three major clinics in Lagos. The three clinics included general outpatient units in a private and government hospital and one private hospital obstetric unit. After enlightening the subjects about the study, each subject was requested to fill a consent form and a questionnaire. The managements of the two private hospitals approved the patient's participation in the study. The questionnaire was to obtain information on their biosocial characteristics such as age, religion, sex, and occupation. Also collected were data about previous screening tests, presence of sickle cell anaemia in the family, and the decision that may be considered if a pregnancy results in a foetus with sickle cell disease. All the 715 questionnaires were recovered from the respondents. Analysis showed that the questionnaires were filled to a degree that made them usable for the analysis of biosocial characteristics. However, out of the 715 subjects, 501 subjects gave their consent for venepuncture for sickle cell haemoglobin genotype analysis.

Determination of haemoglobin genotype

Venous blood sample (3.0 ml) was obtained from the antecubital vein into ethylenediaminetetraacetic acid (EDTA) bottles for determination of haemoglobin genotypes by the usual electrophoretic method (electrophoretic equipment: model DY-300, USA). A small quantity of blood haemolysate from each subject 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 allowed to run for 15 min at an emf of 160 V. Haemolysates from blood samples of known genotypes (HbAHbA, HbAHbS, and HbSHbS) were run as reference standards.

Data analysis

Data from the questionnaires and the results of haemoglobin genotype analysis were entered into the Epi Info version 6.1 statistical software package to obtain frequency distribution. Actual counts of genotypes were made and the frequency of each allele was determined from the counts. Expected genotypic and allelic frequencies were determined from the observed gene frequencies using the Hardy–Weinberg proportions:

$$P^2 + 2\mathrm{pq} + q^2$$

where p=frequency of the HbA allele and q=frequency of HbS allele. The extent of inbreeding occurring in the population was estimated by inbreeding coefficient (F) using the formula below:

$$F = (2pq - H)/2pq$$

where 2pq is the expected frequency of heterozygotes based on the Hardy–Weinberg law and H is the actual frequency of heterozygotes in the sample. Statistical difference of observed data from Hardy–Weinberg expectations was determined using the χ^2 test, and the discrepancy between the observed and the expected numbers was considered significant when P is less than 0.05.

Results

Table 1 shows that when age is characterized by decades, the largest number (276; 38.6%) of the respondents was between 21 and 30 years, while the age decade with the lowest frequency was 61–70 years (19; 2.7%). No respondent was found to be above 70 years. Further analysis of the questionnaire also revealed that majority of the subjects were Christians (570; 79.7%) while 145 (20.3%) were Muslims. Males constituted 43.5% and females constituted 56.5% of the study population. In terms of education, 330 (46.2%) subjects did not go beyond the secondary school education.

Table 1 Biosocial characteristics of respondents

Characteristics	Responses (n=715)	Frequency (%)
Age		
1–10	28	3.9
11–20	112	15.7
21–30	276	38.6
31–40	169	23.6
41–50	79	11.0
51–60	32	4.5
61–70	19	2.7
>70	–	–
Sex		
Male	404	56.5
Female	311	43.5
Religion		
Islam	145	20.3
Christianity	570	79.7
Others	–	–
Educational Level		
Nil	28	3.9
Primary	40	5.6
Secondary	262	36.6
Tertiary	385	53.8

Table 2 shows the actual counts of the three different genotypes and the two alleles responsible for them, while Table 3 shows the genotypic and the allelic frequencies derived from the count. It can be seen that the genotype with the highest frequency was HbAHbA (366; 73.1%), while HbSHbS was of the lowest frequency (12; 2.4%). The allelic frequencies of the HbA and the HbS alleles as obtained from actual counts were computed to be 0.853 and 0.147, respectively (see Table 3). Chi-square analysis revealed that there was no significant difference (P=0.908) between the observed and Hardy–Weinberg expectations (Table 4). The coefficient of inbreeding (F) of the population as estimated from the sample was found to be 0.061. As can be observed in Table 5, individuals with HbAHbA were well represented in the age groups with peak incidence at the age group 21–30 years. The frequency of HbSHbS genotype was generally low among the subjects when compared to the frequency of the other two genotypes.

The attitude of the subjects toward sickle cell disorder and the consistency of the present results with previous screening were also examined. Out of the 501 blood samples screened for the sickle cell haemoglobin, 48 (6.7%) were not consistent with the previous results. Concerning the general attitude of the respondents to sickle cell disorder, all the 715 questionnaires were analyzed. The majority (578; 80.8%) considered premarital diagnosis of haemoglobin genotype worthwhile, while only 4 (0.56%) considered it not important. When subjects were asked for their attitudes if both partners were found to be carriers, 289 (41.2%) would opt for prenatal diagnosis in the event of pregnancy, 281 (39.30%) would stop the relationship, and 145 (20.28%) would leave to fate. Five hundred twenty-eight (74.15%) were aware of prenatal screening for sickle cell disorder.

Discussions

Human haemoglobin polymorphism with respect to sickle cell disease is of considerable interest in population genetics and clinical medicine. Heterozygotes are easily detectable using the common electrophoretic method. This makes it easy to determine the gene frequency for population genetic and statistical analysis. Although the sample is small and hospital-based, the cosmopolitan nature of Lagos and the heterogeneous characteristic of the sample make it representative of the Yorubas, the most predominant ethnic population in southwestern Nigeria. In this respect, it is noteworthy that Lagos population has

Table 2 The actual counts of genotype and alleles in the sample

		HbAHbA	HbAHbS	HbSHbS	Total	
Number of Individuals		366	123	12	501	
Number of Alleles:	Hb^A	732	123	-	855	1002
	Hb^S	-	123	24	147	

experienced extensive gene flow due to rural–urban migration especially of the Yorubas who move to Lagos in hundreds of thousands in search of better opportunities. Unlike previous study by Bakare et al. (2006) which was carried out in Ogbomosho, a smaller and a more homogenous community, the present study was carried out in Lagos, a cosmopolitan hetrogenous community and the largest urban centre in Nigeria. In spite of this, the overall distribution of haemoglobin genotype in our study population was similar to that obtained from previous studies (Bakare et al. 2006; Zaccheaus 2006).These studies suggest that the Nigerian population, like other populations that are found in the malaria belt, is highly polymorphic for sickle cell haemoglobin variant. This is in contrast to the results carried out in many populations in the north and fewer in the south of the endemic malaria zone where the population is mainly composed of HbAHbA homozygote (WHO 2006).

In the present study, the data although extremely limited, suggest a high frequency of HbSS among the youths. However, the number of HbSS obtained in this study is too low for a categorical statement on the distribution of HbSS among the youth. More epidemiological studies with a considerably larger sample size are needed to clarify this. If confirmed, the implications might be serious because youths are the most likely group to constitute the gene pool from which alleles of the offspring for the next generation will be constituted. The low inbreeding coefficient obtained in the study and the genotypic frequencies which were not significantly different from Hardy–Weinberg frequencies may indicate absence of assortative mating with respect to HbA/HbS gene locus among the Yorubas in Lagos. This may imply inadequacy of genetic counselling and the need for more aggressive awareness campaign aimed at reducing the incidence of sickle cell disease and other haemoglobin disorders. If one reflects on this situation in Lagos, a metropolitan mega city, the situation in other parts of the country especially in the rural areas are likely to be worse.

There were more subjects within the reproductive age group and over two-thirds below 50 years. This may be accounted for by the source of subject recruitment. Being hospital based, the majority of those who attend are middle aged females and children. Literacy level is relatively high compared to other places in Nigeria. Many people received tertiary or at least secondary education. It is pertinent to note that education has a major influence in access and utilization of healthcare facilities in southwestern Nigeria (Salako et al. 2006).

Of special significance was the finding that the Hardy–Weinberg equilibrium was not disturbed in the study population. The occurrence of HbSS was low in agreement with the results of other studies (Bakare et al. 2006; Zaccheaus 2006). The implication of this is that the frequency of sickle cell haemoglobin allele might be gradually reducing in the population. Several factors may contribute to this: first, the high level of education in the study population could positively influence awareness of the condition and improve socioeconomic levels might have led to a better utilization of reproductive health options such as the use of contraception in AS/AS

Table 3 The observed and the expected genotypic and allelic frequencies

	Alleles		Genotypes		
	HbAHbS		HbAHbAHbAHbSHbSHbS		
Observed	0.853	0.147	0.731	0.246	0.024
Expected	0.845	0.155	0.714	0.262	0.024

Table 4 Discrepancy between observed number of individuals in different genotypic classes and Hardy–Weinberg expectations

	HbAHbA	HbAHbS	HbSHbS
Observed	366	123	12
Expected	364.5	125.6	10.8
		χ^2=0.193;	P=0.908

Table 5 Age group and genotype distribution

Age group	Genotypes		
	HbAHbA (%)	HbAHbS (%)	HbSHbS (%)
1–10	6 (1.2)	8 (1.6)	3 (0.6)
11–20	40 (8.0)	23 (4.6)	5 (1.0)
21–30	183 (36.5)	26 (5.2)	2 (0.4)
31–40	105 (30.0)	25 (5.0)	1 (0.2)
41–50	23 (4.6)	24 (4.8)	1 (0.2)
51–60	6 (1.2)	11 (2.2)	–
61–70	3 (0.6)	6 (1.2)	–
71 and above			
Total	366 (73.1)	123 (24.6)	12 (2.3)

marriages. Secondly, it is possible that the availability and utilization of preventive options such as antenatal chorionic villus sampling or amniocentesis for early pregnancy diagnosis could make parents opt for abortion of affected fetuses (Oloyede 2005). Previous study has shown that the majority of those who utilize such services are in Lagos, being a centre of excellence for the service (Lewis 1970). Thirdly, there is an increasing societal discouragement of marital union of heterozygous (HbAHbS) individuals as a way of reducing the incidence of sickle cell disease. Moreover, as implied by the low inbreeding coefficient obtained in the study, Yorubas are strictly outbreeding ethnic group that strongly discourage consanguineous matings. The implication of such practice is that autozygousity or homozygosity by common descent for HbS allele would be rare or absent in the population.

It is of interest that despite the seriousness of the sickle cell anaemia and the selective pressure against HbSHbS genotype, the disease is still present in many populations especially in Nigeria and other tropical countries where malaria is endemic (Moormann et al. 2003; WHO 2006). This observation had been attributed to balancing selection referred to as heterozygote superiority, whereby heterozygotes are at a selective advantage over the two homozygotes. Apparently, the abnormal haemoglobin mixture in the heterozygote provides an unfavourable environment for the growth and survival of the malaria parasite in the red cell. Natural selection cannot therefore eliminate the detrimental allele from the population because of its beneficial effects in the heterozygote state.

It is noteworthy that HbC allele was not detected in this study despite the fact that individuals carrying this allele are occasionally encountered clinical practical in Nigeria. Non-detection of HbC allele in the study might be due to its rarity in Nigeria and the small size of the sampled population (n=501). Studies using larger sample size are therefore needed to reveal the actual frequency of HbC allele in the population.

Of particular interest is the findings that 48 (6.7%) of respondents have haemoglobin genotype results that are different from their earlier results in agreement with an earlier study in Nigeria (Oloyede 2009). Inconsistency in haemoglobin genotype could have serious consequence on the population. This is because selective marriages between HbAHbS individuals will continue to occur unknowingly as a result of wrong genotyping. This finding should also stimulate medical scientists to be more alert to laboratory analysis especially in developing countries.

Well-planned awareness campaigns combined with proper genetic and marriage counselling remains a viable option to reduce the incidence of sickle cell disorder. The carrier frequency of one in four (i.e., 25% of the population tested) obtained in this study and the acceptance by the majority of the respondents that the knowledge of haemoglobin genotype and prenatal diagnosis are important, suggest that intensive awareness campaigns and genetic counselling will be welcome and very successful in Nigeria.

Acknowledgement We acknowledge the assistance of laboratory staff of Lagos State University Teaching Hospital and High Rocks Prenatal Diagnosis Centre for the collection of blood samples and analysis.

Conflict of interest The authors declare that they have no conflict of interest.

References

Akinyanju OO, Disu RF, Adewole TA, Otaigbe Al et al (1999) Initiation of prenatal diagnosis of sickle-cell disorders in Africa. Prenat Diagn 19(4):299–304

Ashley-Koch A, Yang Q, Olney RS (2000) Sickle hemoglobin (HbS) allele and sickle cell disease: a HuGE review. Am J Epidemiol 151(9):839–845

Bakare AA, Azeez MA, Agbolade JO (2006) Gene frequencies of ABO and rhesus blood groups and haemoglobin variants in Ogbomoso, South–West, Nigeria. Afr J Biotechnol 5(3):224–229

Daniel LH, Elizabeth WJ (2000) Population genetics and evolution. In: Daniel LH, Elizabeth WJ (eds) Genetics. an analysis of genes and genomes, Chpt. 17, 5th edn. Jones and Bartlett, London, pp 707–747

Hartwell LH, Hood L, Goldberg ML, Reynolds AE, Silver LM, Veres RC (2000) Genetics from genes to genomes, 1st edn. McGraw-Hill, USA, p 820

Lewis RA (1970) Sickle cell: clinical features in West Africans. Ghana University Press, Accra

Moormann AM, Embury PE, Opondo J, Sumba OP, Ouma JH, Kazura JW, John CC (2003) Frequencies of sickle cell trait and glucose —6-dehydrogenase deficiency differ in highland and nearby

lowland malaria—endemic areas of Kenya. Trans R Soc Trop Med Hyg 97(5):513–514

Oloyede OAO (2005) Influence of socio-demographic characteristics on the utilization of chorionic villus sampling service in Nigeria. Trop J Obstet Gynaecol 22(2):116–119

Oloyede OAO (2009) Inconsistency in results of adult haemoglobin genotype and its impact on prenatal diagnosis: lessons from a prenatal diagnosis unit. West Afr J Med 28(1):54–55

Salako AA, Oloyede OAO, Odusoga OL (2006) Factors influencing non utilization of maternity care services in Sagamu, South Western Nigeria. Trop J Obstet Gynaecol 23(1):48–53

World Health Organization (2006) Sickle cell anaemia. Report by the Secretariat. 59th World Health Assembly.11.4

Zaccheaus AJ (2006) Abnormal haemoglobin variants, ABO and Rh blood groups among students of African descent in Port Harcourt, Nigeria. Afr Health Science 6(3):177–181