Pattern and Factors Associated with Hemoglobin Genotype Testing among Children Attending a University Teaching Hospital in Lagos, Nigeria

Samuel Olufemi Akodu, Elizabeth Aruma Disu, Olisamedua Fidelis Njokanma

Department of Paediatrics, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

Abstract

Background: Sickle cell disorders are chronic debilitating genetic disorders affecting the red cells. Sickle cell disorders were originally found in the tropics and subtropics but are now common worldwide due to migration of people from tropical to temperate zones. **Objective:** The objective was to describe pattern and factors associated with hemoglobin (Hb) genotype testing among children attending a University Teaching Hospital in Lagos, Nigeria. **Methodology:** The study was conducted at the General Children Outpatient Clinics of Lagos State University Teaching Hospital, Ikeja, Lagos in South west Nigeria. It is a cross-sectional study using research administered questionnaire to obtain information from caregivers. **Results:** A total of 202 subjects aged 6 months to 15 years were conveniently recruited. Overall, the Hb genotype uptake rate was 17.8%. The overall prevalence of Hb disorders was 25.8%. One-ninth of the subjects with known Hb genotype status at commencement of the study had their Hb genotype status confirmed before the age of 1 year. First birth order and upper social stratum were significantly associated with younger age at Hb genotype uptake. **Conclusion:** Fewer children had Hb genotype uptake during infancy and this underscores the need for early Hb genotype testing of infants. This screening can be during the prenatal, neonatal or at most in infancy during immunization, and infant welfare clinics visit.

Key words: Genotype, hemoglobin, survival analysis, uptake

Introduction

Genotyping is the process of determining differences in the genetic make-up of an individual by examining the individual's DNA sequence using biological assays and comparing it to another individual's sequence or a reference sequence.[1] It is interesting to note that error in assigning genotype to individuals do exist as individual who thought they had no problem with their genotype end up with different genotype result when the test was repeat most especially after marriage.[1] The reasons advanced for these errors in genotyping range from suboptimal laboratory conditions, file confusion, laboratory methodology and mis-reporting.[1] The occurrence of this error in assigning genotype if it occurs in parents it may make them have children with sickle cell anemia (SCA) unwillingly, with serious consequences bordering on strained relationship between the parents, with the children bearing the brunt.

Hemoglobin genotype testing can be performed during the prenatal, natal, and postnatal periods. The importance of hemoglobin (Hb) genotype determination for abnormal Hb is to permits establishing the presence of hemoglobinopathies in the population. The status of Hb variants in a community has been described as an indicator of health awareness of its population.[2] Knowledge and care of whether one is a carrier or a sickler or has normal Hb is therefore the responsibility of the person and the entire community.

Defects in Hb genes can produce abnormal Hb which leads to hemoglobinopathies. This hereditary disorder contributes the equivalent of 3.4% mortality in children aged under five worldwide or 6.4% in Africa.[3] It is noted that at least 5.2% of the world population carry a significant trait.[3] About 85% of sickle cell disorders and over 70% of all affected births occur in Africa.[3] The prevalence of SCA in Nigeria ranges from 0.4% to 3% affecting about 20 per thousand newborns.[3]

Consequent upon early diagnosis of SCA many complications can be prevented with early diagnosis and treatment.[4-6] Early diagnosis of SCA is important as early commencement of appropriate standardized comprehensive care can reduce morbidity and mortality.[7] Despite having the highest burden of sickle cell disease in the world,[8] Nigeria does not have a

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Address for correspondence: Dr. Samuel Olufemi Akodu, Department of Paediatrics, Lagos State University Teaching Hospital, P.O Box 11950, G.P.O. Ikeja, Lagos, Nigeria. E-mail: femiakodu@hotmail.com

routine Hb genotype screening program and in the majority of cases the Hb genotype testing is done when medical care is sought or as part of school entry medical examination. In a study conducted by Chukwu *et al*.[9] among children with confirmed Hb genotype aged 11 months to 18 years, it was observed that none of the children presented for routine screening rather diagnosis was made after symptoms and complications have developed.

The main objective of this study was to describe pattern and factors associated with Hb genotype testing among children attending a University Teaching Hospital in Lagos, Nigeria. In this report, we intended to evaluate the uptake of Hb genotype testing among a high-risk zone like Lagos. It is hope that the information derive from this study could assist to stimulate a more purposeful health education campaign about routine Hb genotype testing in Nigeria.

Methodology

The cross-sectional study was conducted between October and December 2009 among apparently healthy children attending the Outpatient clinics of the Department of Paediatrics of Lagos State University Teaching Hospital, Ikeja, Lagos in South west Nigeria that is a major referral center serving the whole of Lagos State.

Approval for the study was obtained from the Ethics Committee of the Lagos State University Teaching Hospital. Consecutive apparently healthy children who came for follow-up consultation after recovery from acute illness acute illness like malaria, and pneumonia were recruited. The studied sample size was 202 children whose parents consented to participate in the study. Therefore, all the eligible children were conveniently recruited, except those who did not give consent.

The Hb genotype was determined using cellulose acetate paper in alkaline electrophoresis combined with sickling test. Those whose Hb genotype was known had their status confirmed while those who are yet to know their Hb genotype had the opportunity of Hb genotype testing.

Data collection was by quantitative means with the aid of pretested structured questionnaires administered by the investigators. Questions covered demographic details with age at Hb genotype testing. Social classification was done using the scheme proposed by Oyedeji[10] The system defines five socioeconomic classes, I to V, in descending order of privilege based on the occupational and educational levels of parents. Classes I and II were grouped together as upper social stratum while classes IV and V were grouped together as lower social strata; class III was considered middle stratum.

The data analysis was by Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA) version 17.0. Survival analysis was employed with those subjects with unknown genotype status on the date of interview considered as censored cases and their age at Hb genotype testing recorded and treated as censored data, as it was not known when they would determine their Hb genotype. They contribute valuable information and should not be omitted from the analysis. It would also be wrong to treat the observed time at censoring as age at Hb genotype determination. Life table and the Kaplan–Meier method[11] was used to subdivide the period of observation into smaller time intervals and for each interval, all who had been observed at least that long were used to calculate the probability of a terminal event occurring in that interval. The life table technique allows us to consider children with unknown Hb genotype status at the time of interview, and also to know the proportion of children that know their Hb genotype by the end of each month of their life. Hence, it allows a longitudinal approach to the cross-sectional data collected. Log-rank test was also applied to compare survival experiences of groups.

Age at Hb genotype testing indicator was expressed as a dichotomous variable with category "1" for subjects with known Hb genotype status prior to commencement of study and category "0" for those with unknown Hb genotype status at survey period. This variable was examined against a set of independent variables (maternal and child characteristics) in order to determine the factors associated with age at Hb genotype testing. Log rank tests were used to assess the significance of factors associated with age at Hb genotype estimation and those with $P < 0.05$ were considered significant.

Results

Characteristics of the study population

A total of 202 children were recruited: 150 with Hb genotype AA, 29 with sickle cell trait and 19 with SCA. The remaining four subjects had HbAC. The age, socioeconomic class and gender distribution of the study patients are given in Table 1. Overall, the age of the subjects ranged from 7 months to 15 years, with a mean of 53.42 ± 45.01 months.

Frequency of hemoglobin variants among study subjects

The results of the analysis of the Hb genotyping reports are presented in Table 2. About three-fifth of the subjects of age

Table 1: Characteristics of study subjects

Characteristics	Male	Female	Total
Age group (months)			
0-<12	9 (40.9)	13 (59.1)	22
12-<36	43 (59.7)	29 (40.3)	72
36-<60	22 (50.0)	22 (50.0)	44
60-<120	16 (44.5)	20 (55.5)	36
120-180	12 (42.9)	16 (57.1)	28
Total	102 (50.5)	100 (49.5)	202
Socioeconomic class			
I	20 (55.6)	16 (44.4)	36
II	36 (49.3)	37 (50.7)	73
III	36 (54.6)	30 (45.4)	66
IV	10 (38.5)	16 (61.5)	26
V	0 (0.0)	1 (100.0)	1

NB: Values in parenthesis are in percentage of rows total

group 0 to <12 months were found to carry a normal Hb genotype (AA) whilst remaining two-fifth were found to have at least one form of Hb variant except sickle cell trait. Among individuals within the age group 12–36 months, seven-tenth was found to carry the normal Hb whilst one for every 25 was found to carry at least one form of Hb variant. Among individuals of age group 36 to <60 months, three-quarter of the study subjects had normal Hb genotype (AA) whilst one-quarter were found to carry a hemoglobinopathy. Four-fifth of the age group 60 to <120 months carry normal Hb and one-fifth had at least a form of Hb variant except HbAC. Nine-tenth of those aged 10 years and above was found to have normal Hb genotype and one for every 10 were found to possess at least one form of the variant except SCA. The overall prevalence of Hb disorders was 25.8%.

Age at hemoglobin genotype determination

Out of 202 children surveyed, 36 (17.8%) had their Hb genotype status known before survey date (terminal event). The remaining 166 (82.2%) cases were censored cases as they were yet to have Hb genotype determination on the survey date. The overall Hb genotype uptake rate was 17.8%.

Table 3 shows the distribution for age interval at Hb genotype determination using survival analysis. The estimated mean (standard error) age at Hb genotype determination was 129.17 (6.94) months with 95% confidence interval of 115.57–142.76 months. The modal age interval at which the subjects had their Hb genotype performed was 12 to <36 months age interval. One-ninth of the subjects with known Hb genotype status at commencement of the study had their Hb genotype status confirmed before the age of 1 year. Five-ninth of the subjects with known Hb genotype status at commencement of the study had their Hb genotype status confirmed before age 3 years.

Table 2: Frequency of hemoglobin variants among subjects

Age group (months)	AA	AS	AC	SS
0-<12	12 (54.5)	6 (27.3)	0 (0.0)	4 (18.2)
12-<36	50 (69.4)	8 (11.1)	3 (4.2)	11 (15.3)
36-<60	33 (75.0)	8 (18.2)	0 (0.0)	3 (6.8)
60-<120	30 (83.3)	5 (13.9)	0 (0.0)	1 (2.8)
120-180	25 (89.3)	2 (7.1)	1 (3.6)	0 (0.0)
All	150 (74.2)	29 (14.4)	4 (2.0)	19 (9.4)

Table 3: Distribution for age interval at hemoglobin genotype determination

Age interval at hemoglobin determination (months)	Total number	Number that had hemoglobin genotype status known before study	Total number with hemoglobin genotype status unknown at study
0-<12	26	4	22
12-<36	82	16	66
36-<60	39	2	37
60-<120	34	10	24
>120	21	4	17
Overall	202	36	166

Factors associated with age at hemoglobin genotype determination

Table 4 presents the survival analysis to investigate variables associated with age at Hb genotype determination. The estimated mean age at Hb genotype determination was comparable for the female and male subjects ($P = 0.559$). The estimated mean age at Hb genotype determination was significantly lower among subjects of the first birth order ($P = 0.014$). The family size and marital status of parents were not associated with significant differences in estimated mean age at Hb genotype determination ($P > 0.05$). On the contrary, the upper socioeconomic strata was associated with significant lower estimated mean age at Hb genotype determination compared with other socioeconomic strata ($P = 0.049$).

Discussion

This study shows that Hb disorders are common among children attending outpatient clinics in a University Teaching Hospital in Lagos and the finding is therefore of public health significance. The prevalence of the HbAA among individuals does not differ significantly from that carried out in a Nigerian national survey of adults >15 years of age, covering 30 states, in mid-90's.[12] Present study recorded the higher prevalence of SCA than the previous survey reported by Angastiniotis *et al*.[12] This may be due to difference in the survey design we employed. Present data was obtained from a tertiary level hospital which also serves as a referral center and regularly holds a sickle cell clinic. We may therefore have overestimated the actual prevalence. Our study, however, recorded lower frequencies for HbAS but recorded almost the same estimates for HbAC than the previous study.[12]

In the current study, the proportion of study subjects who had performed Hb genotype testing before the survey interview was low. Cost may have a significant contribution to the observed low uptake rate. It costs an average of seven dollars to have Hb genotype testing done in our facility as at the time of reporting of the current study. This will be an impossible request in a country where most of her population lives below 1 dollar per day. This poor Hb genotype uptake did not spare even the sickle disorders population as Chukwu *et al*.[9] reported that none of the studied children with SCA aged 11 months to 18 years presented for routine screening rather diagnosis was made after symptoms and complications have developed. Hb genotype testing is a key strategic entry point to prevention, treatment, care and support services for children with Hb disorders. This low uptake ultimately will result in majority of affected child with Hb disorders being missed. This further reinforced the need for a routine Hb genotype testing within our setting.

The estimated mean age at Hb genotype determination was high in the current study (129.17 months). There is however no data available locally or elsewhere on the mean age of Hb genotype determination. Nearly one-ninth of the subjects

Table 4: Survival analysis of factors associated with age at hemoglobin genotype determination

Characteristic	Number of terminal events	Number of censored cases	Mean age at hemoglobin determination (months) SE (95% CI)	Log-rank test for comparison of group
Gender				
Male	16	86	124.8 8.8 (107.6-142.0)	0.559
Female	20	80	129.5 9.2 (111.5-147.6)	
Position of birth				
1	21	50	111.6 10.5 (91.0-132.2)	0.014
>1	15	116	140.3 9.3 (122.0-158.6)	
Socioeconomic strata				
Upper	26	83	120.3 9.3 (102.1-138.5)	0.049
Others	10	75	132.7 8.9 (115.1-150.2)	
Family size				
<5	18	93	122.6 11.4 (100.3-144.9)	0.417
≥5	18	73	133.9	
Marital status of parents				
Married	34	163	130.4 7.0 (116.7-144.1)	0.139
Others	2	3	55.2 15.2 (25.4-85.0)	

CI: Confidence interval, SE: Standard error

with known Hb genotype status at commencement of the study had their Hb genotype status confirmed before the age of 1 year. It is plausible that a number of factors, particularly social, and health circumstances could have contributed to delayed age at Hb genotype estimation of some of our children. Early determination of Hb genotype status may in turn depend on health-seeking attitude of caregivers among other factors. In any case, the establishment of a routine screening program which could be tied to child welfare services like immunization would significantly reduce the number of children with unknown Hb genotype status beyond infancy. The major limitation of our report is that we do not know the circumstances under which the subjects who reported known Hb genotype status at survey time carried out the testing. It is noteworthy that among sickle cell disorder population, similar delayed Hb determination beyond infancy was observed.[9,13]

The present study showed that the mean age at Hb genotype determination was significantly higher among subjects with birth order two or more. These findings indicate the importance accorded to first born as they are given better care and others are neglected. Some authors[14] have demonstrated a decrease in maternal attentive after the birth of a first child.

The study also revealed that an upper socioeconomic stratum was associated with younger age at Hb genotype determination. It is logic to argue that upper socioeconomic stratum implies that there is better access to health care by such families, as well as a privilege to health information. This finding is in agreement with a study done by Akodu *et al*.[13] to evaluate age at diagnosis of SCA in Lagos.

The public health significance of our study is that it has supplied data that could be used for planning and as a guide for allocation of resources for care of patients with Hb disorders. Overall the uptake of Hb genotype testing was very low among the study subjects. To mitigate this problem, the least that could be done is to make Hb genotype testing free and compulsory for all children within the population. This screening can be during the prenatal, neonatal or at most in infancy during immunization and infant welfare clinics visit.

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