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Sickle Cell Disease Screening in Northern Nigeria: The Co-Existence of B-Thalassemia Inheritance

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Abstract

Background: Despite the high burden of Sickle Cell Disease (SCD) in Nigeria, the underlying haemoglobinopathy profile remains uncertain. Although a number of urbanised areas have pilot hospital-based newborn screening programmes, the impact of the disease in rural areas is unknown.

Methods: From January 2010 to December 2011 this community-based study screened children aged 0-60 months in 29 randomly selected local communities of three adjoining northern Nigerian states - Abuja, Kaduna and Katsina. For infants 0-6 months, blood spots were used and for infants 7-60 months EDTA blood samples were analysed using high performance liquid chromatography (HPLC). 31 selected sample with high Hb A2 (3.5-7.4%) were further analysed using molecular diagnosis to ascertain the presence of the Beta Thalassemia gene.

Findings of 10,001 infants and children screened, 269 (2.69%) had a SCD diagnosis; 90% of which were HbSS (n=243), 5% HbSC (n=13), 3% with high A2 > 6% (possible S with existence β thalassaemia (n=9) and 1% HbSD (n=2). 74% of infants screened were HbAA (n=7,391). 2,341 (23%) were carriers; 96% HbAS (n=2,236), 2% HbAC (n=51), 1% HbAD (n=25) and 1% HbABeta-thal (n=22). HbSβo was confirmed by molecular analysis from the 31 selected samples.

Conclusion: Early infant diagnosis of SCD in Northern reports an incidence of 1.72%, Homozygous SS accounts for over 90% of cases; double heterozygous SC is very low (4%). The presence of beta (β) thalassemia co-inheritance is now confirmed using molecular analysis. Community and family counselling and educational material in Northern Nigeria must include the risk of beta thalasemia inheritance.

Keywords: Sickle cell disease; Beta thalassaemia; Nigeria

Introduction

Sickle cell disease (SCD) is the most common life threatening genetic disorder worldwide [1]. Much is known about the basic pathophysiology of SCD and the benefits of early intervention, and mortality from SCD has decreased to <1% in the Western world with a life expectancy of 53-60 years [2,3]. However 80% of SCD cases are in sub Saharan African where it has been reported that 50-90% of children born with HbSS will not reach their fifth birthday and SCD accounts for up to 20% of neonatal mortality [4,5]. In 2006, the World Health Organisation declared SCD to be a problem of major public health significance and a burden that must be addressed if recent improvements in overall child survival are to be consolidated [6].

Despite growing prevalence worldwide, the burden on Sub Saharan Africa is expected to increase to 88% of cases by 2050 [7,8]. No global data regarding the precise numbers of children born with SCD and their haemoglobinopathy profile exist because, in contrast to Western countries, newborn screening for SCD is not available in most low-income countries with the highest predicted burdens. Nigeria is known to bear the highest burden of SCD in the world and therefore the country is in urgent need of policies for prevention and management of SCD [7,9]. In Nigeria recent small-scale epidemiological studies have shown not only a high frequency of HbS but high prevalence of other associated haemoglobin genetic mutations like β-Thalassaemia, prompting the need for larger epidemiological research [10,11]. As new potentially curative therapies emerge and growing evidence for prevention strategies builds, it is of paramount importance that the burden of SCD in resource-poor countries is recognised to direct interventions and improve child survival.

In this study we present the results of the first 10,000 infants screened for SCD in North-West and North-Central Nigeria to determine the frequency of the sickle cell gene in infants and the prevalence in children less than five years of age, the coexistence of β-Thalassemia and the use of HbA2 as a discriminator for S/ β-Thalassaemia.

Methods

Data collection

Children aged less than 5 years of age in Kaduna and Katsina states (North-West zone) and the Federal Capital Territory (North Central) of Nigeria were offered free screening for SCD during planned screening sessions. These sessions were held alongside existing community outreach clinics in all two states (Kaduna and Katsina) and the Federal Capital from June 2010 to March 2011. The selection of the communities for screening was based on the states' primary health care schedule for the distribution of 'treated mosquito net' to families with under five aged children. This was done within the primary health setting after gaining written consent from parents and caregivers. Seven (7) of the twenty three (23) local government areas of Kaduna sate were chosen for easy access to the central laboratory in Abuja and three (3) primary care centres in Katsina state which were running during the time the N-253 UNDP project team visited Katsina state. Both Kaduna and Katsina states have estimated annual birth rate of about 240,000 babies and a total population of six million people each.

This was a feasibility study for the introduction of high performance liquid chromatography (HPLC) method of SCD diagnosis towards establishing a newborn screening in Nigeria. Full ethical approval was granted in Kaduna and Katsina states and the Federal Capital territory.

Following pre-test counselling, informed consent for testing was obtained from the parent or carer of child before testing. A short questionnaire was then administered to obtain basic demographic information and parental knowledge of their child's previous haemoglobinopathy screening result, if applicable. In children aged less than 6 months blood was obtained from a heel prick unto a Guthrie card. In older children blood was obtained by venepuncture into EDTA vacutainers. Specimen collection was facilitated by Fantsuam Foundation Community project, and all specimens were transported to the processing laboratory at Zankli Medical Center, Abuja on the same day of sample collection.

Laboratory testing

The use of alkaline gel electrophoresis (paper chromatography) is widely practiced for SCD screening and diagnosis in developing countries. Due to poor sensitivity, this technique is not suitable for newborn screening. In addition the widespread use of unstained paper electrophoresis in isolation of any other testing is inherently prone to error. Furthermore there are no permanent records of results or reference plots for quality assurance. HPLC is a reliable tool for newborn SCD screening. HPLC is a more sensitive, robust and reproducible approach with the ability to retain records. Results can also be scanned and referred for second opinions. An integral part of this study was the installation, in Abuja (Federal Capital Territory) Nigeria, June 2010, of a HPLC instrument. Prior to the installation, haemoglobinopathy screening was carried out using only unstained paper electrophoresis. Minor bands were difficult to visualise and the proportions of haemoglobins were not measured Therefore awareness of both α- and β-Thalassaemia was low as was the implications of coinheritance of β-Thalassaemia with haemoglobin (Hb) S.

The blood specimens were analysed for hemoglobin phenotype using high performance liquid chromatography (HPLC), a Bio-Rad Variant Classic (Biorad Hercules CA). Blood spot samples were analysed using newborn sickle cell screening reagents and whole blood samples using β-Thalassaemia short reagents, the latter allowing accurate Hb A2 and Hb F measurement. Samples were processed on a Biorad™ Classic in accordance with manufacturer's recommendations and using proprietary controls (instrument and reagents, Biorad, Hercules, CA). Reporting algorithms were defined for both reagent sets. Using the β-Thalassaemia reagents, Hb S/β-Thalassaemia was considered when Hb S was predominant, Hb A absent or significantly reduced and the Hb A2 >6. The information given by the parent or guardian in the aforementioned questionnaire was corroborated with the results obtained from HPLC testing performed as part of the screening programme. To validate the algorithm 31 samples with varying HbA2% (3.5-7.4%) levels, were selected for further beta gene sequencing [12] and/or PCR analysis for the 7 common alpha gene deletions (3.7kb, 4.2kb, SEA, MED, THAI, FILL, 20.5kb and triplicated alpha gene locus, anti 3.7kb), [13,14].

Statistical Analysis

The statistical analyses were conducted in STATA version 11.2 (Stata-Corp LP, College Station, Texas) and Prism GraphPad (GraphPad Software, Inc., San Diego, California). Haemoglobin profile was stratified by 6 months age intervals and characterised by the following phenotypes: HbAA, Hb AS, Hb AC, HbAD, HbAβ, HbA/HPFH, HbSS, HbSC, HbSβ, HbSD, HbSO, HbCC. Differences between SCD prevalence among females and males as well as age groups were tested using the Pearson's Chi2 ($\chi 2$) test.

Results

Across 29 communities in the Federal Capital Territory, Kaduna and Katsina states of Nigeria, we screened 10,001 children. Of the total sample, 4949 (49.49%) were female and 5045 (50.44%) were male. The median age of the 10,001 children screened was 1.54 years and the mean age was 1.88 years (SD 1.53); of these, 2737 (27.4%) children were screened between the age of 0 and 6 months. (Table 1) shows age and sex demographics of all children stratified by 6 months age intervals (i.e. 0-6 months, 7-12 months, 13-18 months etc.). 22 (0.22%) children were initially classified as HbAA, but had HbA2 above 4%; these were relabelled as HbAβ. 31 (0.31%) children were shown to have HbSβ previously on chromatography; however, 22 of them had HbA2 < 6% and were relabelled as HbSS.

The total incidence of SCD in infants 0-6 months was 1.72% (n=47), with HbSS accounting for 96% (n=45) of SCD incidence and HbS/HbC and HbS/Hbβ-thalassaemia accounting for 2% of the SCD incidence each. In the total sample assessed, 7,391 (73.90%) were HbAA, 2,236 (22.36%) were HbAS, 51 (0.51%) were HbAC, 25 (0.25%) were HbA variants, 22 (0.22%) were β-thalassaemia carriers, 243 (2.43%) were HbSS, 13 (0.13%) were HbSC, 2 (0.02%) were HbSD, 1 (0.01%) was HbSO, 9 (0.09%) were HbS/β-thalassaemia, based on HbA2 > 5.0% and 1 (0.01%) as HbCC (Table 2 based on HPLC chromatography). The total prevalence of SCD was 2.68%. HbSS accounted for 90.67% of the SCD prevalence, with HbS/β-thalassaemia accounting for 3.36% of the SCD prevalence.

Age	0-6	07-12	13-18	19-24	25-30	31-36	37-42	43-38	49-54	55-60	Total
M: F	1390: 1343	566: 567	552: 510	322: 274	454: 476	275: 266	513: 478	287: 272	463: 487	223: 276	5,045: 4,949
n (%)	2737 (27.37)	1134 (11.34)	1062 (10.62)	596 (5.96)	931 (9.31)	541 (5.41)	991 (9.91)	559 (5.59)	950 (9.50)	500 (5.00)	10,001 (100)
n (%)=Data; M=Male; F=Female											

Table 1: Demographic characteristics of 10,001 children screened.

Phenotype	Number by age in months (%)										
	0-6	07-12	13-18	19-24	25-30	31-36	37-42	43-48	49-54	55-60	Total
AA	2056 (75.12)	852 (75.13)	768 (72.32)	440 (73.83)	667 (71.64)	404 (74.68)	744 (75.08)	391 (69.95)	695 (73.16)	374 (74.80)	7391 (73.90)
AS	597 (21.81)	246 (21.69)	255 (24.01)	131 (21.98)	226 (24.27)	110 (20.33)	202 (20.38)	144 (25.76)	216 (22.74)	109 (21.80)	2236 (22.36)
AC	18 (0.66)	5 (0.44)	3 (0.28)	3 (0.50)	4 (0.43)	2 (0.37)	6 (0.61)	3 (0.54)	6 (0.63)	1 (0.20)	51 (0.51)
Variant AD or AO	15 (0.55)	2 (0.18)	1 (0.09)	2 (0.34)	2 (0.21)	2 (0.37)	0	1 (0.18)	0	0	25 (0.25)
ABeta	3 (0.11)	3 (0.26)	3 (0.28)	3 (0.50)	2 (0.21)	2 (0.37)	1 (0.10)	2 (0.36)	1 (0.11)	2 (0.40)	22 (0.22)
A/HPFH	1 (0.04)	0	1 (0.09)	1 (0.17)	1 (0.11)	1 (0.18)	1 (0.10)	1 (0.18)	0	0	7 (0.07)
SS	45 (1.64)	22 (1.94)	30 (2.82)	16 (2.68)	23 (2.47)	17 (3.14)	31 (3.13)	15 (2.68)	31 (3.26)	13 (2.60)	243 (2.43)
SC	1 (0.04)	1 (0.09)	0	0	4 (0.43)	2 (0.37)	3 (0.30)	1 (0.18)	0	1 (0.20)	13 (0.13)
S/Beta*	1 (0.04)	2 (0.18)	0	0	2 (0.21)	1 (0.18)	3 (0.30)	0	0	0	9 (0.09)
SD	0	0	1 (0.09)	0	0	0	0	1 (0.18)	0	0	2 (0.02)
SO	0	0	0	0	0	0	0	0	1 (0.11)	0	1 (0.01)
CC	0	1 (0.09)	0	0	0	0	0	0	0	0	1 (0.01)
Total	2737 (100)	1134 (100)	1062 (100)	596 (100)	931 (100)	541 (100)	991 (100)	559 (100)	950 (100)	500 (100)	10,001 (100)
*The diagnosis of Sβ based on HbA2 > 6.0%											

Table 2: Haemoglobinopathy profile of all 10,001 children.

The result of further molecular analysis of 31 samples with HbA2; 3.5-7.4

1. 5/31 with Hb A2of 3.5-4.9%, and 2 with Hb A present (23 and 25%), were confirmed Hb SS, 4 negative for alpha thalassaemia deletions and 1 heterozygous for the alpha 3.7kb deletion.

2. 16/31 with Hb A2 of 5.0-5.9%, were again confirmed Hb SS, 5 negative for alpha thalassaemia deletions and 11 heterozygous for the alpha 3.7kb deletion.

3. 7/31 Hb A2 of 6.0-6.9%, 6 were Hb SS, 1 negative for alpha thalassaemia deletions, 1 heterozygous and 4 homozygous for the alpha 3.7kb deletion. One, Hb A2 6.9%, was a true compound heterozygote for Hb S/ beta zero codon 106/107 (+G) mutation and the remaining

4. 3/31 with Hb A2 of 7.0-7.4%, a review of chromatograms showed poor chromatography due to lack of separation between Hb A2 and Hb S or shoulders to the left of the Hb A2 peak. All were Hb SS and heterozygous for the alpha 3.7kb deletion, with 1 also positive for the triplicated alpha globin gene, anti 3.7kb. As reported in Blood (ASH meeting Abstracts) 2011.118, Abstract 4206; Hb A2 level > 6.0% may improve specificity and reduce the number of false positives [15].

Figure 1 illustrates the SCD haemoglobinopathy profile of children with SCD. In the whole dataset, SCD prevalence was 2.65% (n=131) for females and 2.72% (n=137) for males. The difference was not significant ($\chi2=0.0450$; $p=0.832$). We did not see a downward trend in the SCD prevalence with ascending age groups; in fact a significant difference in SCD prevalence was observed between age group 0-6 months and children above the age of 6 months ($\chi2=13.3866$; $p < 0.001$) with clear increase in SCD prevalence in children above the age of 6 months. This may be attribu e to the fact that more children are likely to get tested for SCD once they start experiencing symptoms due to decrease in HbF.

Discussion

SCD is estimated to be the sixth leading cause of death in children aged less than 5years in Nigeria [16]. Although intervention programs have been implemented for several other conditions such as HIV/AIDS and malaria eradication, there are no established programs for SCD despite the substantial associated morbidity and mortality. To our knowledge this is the first community based study to quantify the prevalence and pattern of SCD in Northern Nigeria.

Studies from developed countries have clearly demonstrated the role of early diagnosis in improving the quality of life and survival in SCD. Appropriate preventive measures can be instituted through antenatal testing or neonatal screening. Pre-conception screening has been widely advocated in Greek Cyprus and Greece with some degree of success. Odunvbun and colleagues in South-Western Nigeria confirmed the acceptability of newborn SCD diagnosis [10]. Similarly there was a high level of acceptability in Northern Nigeria despite the region's perceived conservative, religious profile.

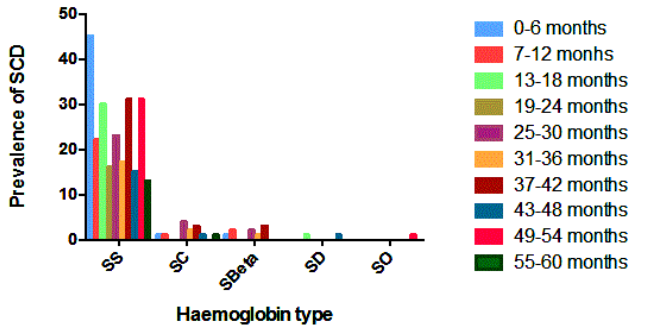

Figure 1: Haemoglobinopathy profile of children with Sickle Cell Disease (SCD).

Two distinct screening activities happened in this case; newborn/early infant (0-6months) using a blood spot for 2737 (Tables 1 and 2) showed an incidence of SS (1.64%); SC (0.04%), AS (21.81%) and AC (0.66%) confirming the fact that the C gene frequency is low (Figure 1) in Northern Nigeria and therefore may be used to inform future screening programme. The results of children using EDTA sample for beta thalassaemia screening indicate that Hb A2 values > 5% were mainly due to co-existing alpha thalassaemia with 76% of those tested, positive for the 3.7kb deletion. Poor chromatography was also a contributor particularly at the higher Hb A2 levels. During the initial stages of the project air conditioning failure caused major temperature fluctuations on overnight runs, this problem was resolved, improving chromatography. One case of S/beta zero thalassaemia was detected confirming the presence in this population. Future aims of the project for children over 6 months of age, include initiating routine Full Blood Count analysis and testing parental samples as a cost effective means of confirming suspected cases.

Conclusion

Newborn Sickle Cell disease in Northern is predominantly due to Homozygous SS (91%). Double heterozygous SC is low (4%) and Sβ is present. The co-inheritance of beta thalassemia gene must be included in health education and patient information sheets.

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