Sickle-Cell Disease in Nigerian Children: Parental Knowledge and Laboratory Results

Stephen K. Obaro, Yvonne Daniel, Juliana O. Lawson, Wei-Wei Hsu, John Dada, Uduak Essen, Khalid Ibrahim, Adebayo Akindele, Kevin Brooks, Grace Olanipekun, Theresa Ajose, Claire E. Stewart, Baba P.D. Inusa

Division of Pediatric Infectious Diseases, University of Nebraska Medical Center, Omaha, Nebr.; Department of Statistics, Kansas State University, Manhattan, Kans., and Michigan State University, East Lansing, Mich., USA; International Foundation Against Infectious Diseases in Nigeria (IFAIN), Fantsuam Foundation, Kafanchan, Nigeria; Viapath, Guy’s Hospital, Evelina Children Hospital, Guy’s and St Thomas’ NHS Trust, London, UK; Epidemiology Research Unit, University of The West Indies, West Indies, Jamaica

Abstract

Background: Sickle-cell disease (SCD) is the most common inherited genetic disorder in sub-Saharan Africa, and it is associated with early mortality and lifelong morbidity. Early diagnosis is essential for instituting appropriate care and preventive therapy. Objective: To compare parental knowledge or perception of their offspring’s hemoglobin phenotype prior to testing and actual validated laboratory test results. Methods: In a prospective community-based survey, we assessed parental knowledge of their children’s hemoglobin phenotype and corroborated this with the results from a laboratory confirmatory test determined by high-performance liquid chromatography. Results: We screened 10,126 children aged less than 5 years. A total of 163 (1.6%) parents indicated that their offspring had been previously tested and had knowledge of the child’s hemoglobin genotype. However, 51 (31.2%) of 163 parents of children who had been previously tested did not know the result of their offspring’s test, and 18 (35.3%) of these 51 children were found to have SCD. Of those who claimed previous knowledge, 25 (15.3%) of 163 reported incorrect results. Overall, we identified 272 (2.76%) new cases from 9,963 children who had not been previously tested.

Conclusion: There is the need to promote public awareness about SCD and the benefit of early diagnosis, quality assurance in laboratory diagnosis and institution of sustainable patient care pathways.

Introduction

Sickle-cell disease (SCD) is caused by an autosomal recessive disorder of hemoglobin (Hb S) due to the substitution of glutamic acid by valine in position six of the beta globin chain. SCD is a constellation of disorders...
with either homozygous state (sickle-cell anemia) or Hb S in combination with another abnormal allele such as C (lysine replaces glutamic acid) or beta thalassemia trait (deletion or reduced expression of the beta allele) [1]. The central phenomenon is the paracrystal formation of affected hemoglobin in the deoxygenated state, resulting in microvascular obstruction, ischemia and tissue infarction. This leads to a complex cascade of hypoxic reactions including hemolysis and the release of reactive oxidative species and cytokines, which results in multisystemic complications. The deformation of the red blood cells’ structure (‘sickle cell’) accounts for vaso-occlusive complications such as acute painful crisis and acute chest syndrome. On the contrary, hemolysis is believed to be responsible for the other complications such as overt stroke, priapism and pulmonary hypertension. The twin contributions of vaso-occlusion and hemolysis in SCD are therefore acknowledged as the main features of the disorder [2]. Although much is known about the basic pathophysiology of this condition, mortality and morbidity remain high in developing countries [3]. Mortality is highest during the first 5 years of life, with approximately 50% of deaths occurring during the second 6 months of life [4]. These deaths have been largely attributed to infections and acute splenic sequestration. The types of infections are malaria and bacterial infections, but the relative significance of these remains a subject of debate. This uncertainty has continued to delay implementation of appropriate preventive measures. The World Health Organization has recently declared SCD to be a public health problem [5]. While limited observational studies in north central Nigeria of about four decades ago suggest that only about 2% of children born with this condition survive beyond the age of 5 years [6], contemporary survival patterns are not known. In most developed countries, newborn screening programs are routine and diagnosis is followed by prompt referral to comprehensive clinics for routine preventive care [7, 8]. This approach of early diagnosis with preventive care has resulted in increased survival in these settings.

In order to obtain data on the contemporary survival patterns in children affected by SCD, we have recently conducted a community survey across 17 communities in central/Northwestern Nigeria. During this period, we gathered information on parental knowledge of the hemoglobin phenotype of their children, and the results of this survey are presented and discussed in this study. The detailed report of the distribution of phenotypes across age groups is the subject of another report.

Materials and Methods

Children aged less than 5 years across several communities in the Kaduna and Katsina states (North-West zone) and the Federal Capital Territory of Nigeria were offered free screening for sickle-cell anemia during June 2010 to March 2011. The program was part of an ongoing research project designed to establish a sustainable cohort of children with sickle-cell hemoglobinopathy for the purpose of providing comprehensive health care. Following pretest counseling, informed consent for testing was obtained from an accompanying parent or guardian. A short questionnaire was then administered to obtain basic biodata and history of parental knowledge of the child’s previous test results if they had been previously tested. Children with a history of blood transfusion in the previous 3 months were excluded from enrollment. Of all eligible infants actually investigated, none was excluded because of a blood transfusion history, and this is quite uncommon in these communities. In children aged less than 6 months, blood was obtained from a heel prick onto a filter paper. In older children, blood was obtained by venipuncture into an EDTA vacutainer. All specimens were transported to the processing laboratory at the Zankli Medical Center, Abuja, Nigeria, on the same day of collection.

The hemoglobin phenotype was determined by high-performance liquid chromatography (HPLC) using a Biorad Variant Classic (Biorad, Hercules, Calif., USA). Newborn blood spot samples were assayed using reagents designed for newborn screening, the sickle-cell short program and liquid capillary samples with the beta thalassemia short program and reagents. The instrument and the running program were operated in accordance with the manufacturer’s recommendations and using proprietary controls (Biorad). Hemoglobins were identified by their relative retention times with preassigned peak identification windows. Standard diagnostic algorithms were followed; however, second-line confirmatory testing was not available.

The Institutional Review Boards of the Federal Capital Territory, the Kaduna and Katsina states, Nigeria, and the Michigan State University, East Lansing, Mich., USA, approved this study.

Results

We screened 10,126 children aged less than 5 years across 29 communities in the Federal Capital Territory, the Kaduna and Katsina states of Nigeria. In table 1, we present a summary of the results of the screening in relation to the results provided by the parents or the guardians of the children aged less than 1 year, while table 2 shows a summary of laboratory results in relation to the results provided by the parents or the guardians of children aged 1–5 years. Overall, in 9,028 subjects, the parents or the guardians reported that the child had not been previously tested for SCD. Of these, 6,677 (73.9%) had normal hemoglobin (Hb AA), while 2,031 (22.5%) and 243 (2.7%) had sickle-cell trait (Hb AS) and sickle-cell anemia (Hb SS), respectively. There were 4 children with Hb SC and 84 children with other hemoglobinopathies. There was pa-

Obaro et al.
rental knowledge in 163 (1.7%) children whose parents/guardians reported that their child had been previously tested. However, the parents/guardians of 51 of these infants could not recall ever receiving the result of the test, while 9 of 163 (5.5%) indicated that they were told that the result was normal (Hb AA), 11 (7.5%) had sickle-cell trait, 91 (58.8%) sickle-cell anemia (Hb SS), and there was 1 (0.6%) child with Hb SC. We compared this information with the results from the laboratory using HPLC. Table 3 shows a summary of the distribution of laboratory results of subjects who were tested at less than 1 year of age, while table 4 summarizes the distribution of laboratory results in children aged 1–5 years. Of the 163 children who had been previously tested for SCD, 25 (15.3%) had an incorrect result, and 51 parents did not know or were unable to recall the results of the test. In 9 subjects who reported a normal hemoglobin, 2 of 9 (22.2%) had SCD. In 11 children who reported sickle-cell trait, 4 (36%) had SCD. Of 91 children who reported SCD, 8 (8.8%) had Hb AA and 4 (4.4%) had sickle-cell trait.
Of those with no previous testing, 2.8% (283 of 10,260) had SCD, while 3.0% of those who could not recall whether the child was previously tested or not (36 of 1,214) had SCD. The parental report of the test results had the highest concordance for children with a previous report of Hb SS.

Discussion

SCD is well recognized in Nigeria and has a descriptive terminology in virtually all languages and dialects. Given that this is the most common genetic disorder in this population, the proportion of parents who have had their children tested for this disorder is surprisingly small. Of grave concern is that 75 (47%) parents/guardians of previously tested children were either given the wrong information or were never informed of their offspring’s test results. These parents may be left with false assurance or a missed diagnosis. It is conceivable that some parents/guardians were in denial of the child’s result and needed more reassurance of the disorder. There are several possible explanations for this observation. The lack of portable, verifiable documentation makes the interpretation of this observation open to speculation: (i) inadequate recall of the results as these were only communicated verbally to the parents; (ii) parental denial of the results communicated to them, especially in communities where the myth about SCD is still prevalent, and (iii) the test results may not have been performed correctly and were wrong anyway.

The fact that only 13% of children aged less than 5 years had previously been tested for SCD is evidence that screening is not available to the vast majority of infants in Nigeria, notwithstanding that SCD is the most common genetic disorder in sub-Saharan Africa and particularly Nigeria. It underlines the need for more affordable testing which is urgently required to encourage newborn SCD screening in a low-income setting. This must be followed with the provision of appropriate counseling and treatment centers for those diagnosed with SCD.

The World Health Organization has recently recognized SCD as a major public health problem and has issued a number of communique’s about the need for national and international governments to provide support for the care of individuals that are affected by this disorder. However, this call to action will not achieve its intended goal if the level of general knowledge and awareness of the condition in the population remains poor. SCD is estimated to be the sixth leading cause of death in children aged less than 5 years in Nigeria [7]. While intervention programs have been implemented for several other conditions, such as HIV/AIDS, polio and malaria eradication, no focused program has been established for the commonest inherited genetic disorder, which may exacerbate the rate or morbidity and mortality of the aforementioned disorders and other diseases.

The use of HPLC for hemoglobinopathy screening is novel in this setting where standard alkaline gel electrophoresis has been the standard. The widespread use of unstained paper electrophoresis in isolation of any other testing is inherently prone to error, and the results are not amenable to long-term storage or reference. The alkaline electrophoresis technique is not suitable for newborn screening, as it lacks the required sensitivity. HPLC was used because it is easier to operate and train staff to use with little support and limited training time. Post-training results are more robust and reproducible. The other methods, especially isoelectric focusing, were considered and may in fact be adopted as a second-line procedure for future implementation of screening in the country.

Table 4. Self-reported testing for SCD by laboratory test results in children aged between 1 and 5 years

<table>
<thead>
<tr>
<th>Laboratory results: genotype, n</th>
<th>Total, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (28.57%)</td>
<td>3 (42.86%)</td>
</tr>
<tr>
<td>AS (33.33%)</td>
<td>3 (33.33%)</td>
</tr>
<tr>
<td>SS (8.33%)</td>
<td>4 (4.76%)</td>
</tr>
<tr>
<td>SC (0.00%)</td>
<td>0 (0.00%)</td>
</tr>
<tr>
<td>Don’t know (60.53%)</td>
<td>5 (13.16%)</td>
</tr>
<tr>
<td>Total</td>
<td>35 (25.18%)</td>
</tr>
</tbody>
</table>
The HPLC method requires less man power, time for setup and attention during the analysis. HPLC is semi-quantitative, and the HPLC machines installed at that time allowed us to perform newborn screening and collect adult samples by switching kits and therefore fulfilled other requirements of our study. It is not possible to quantitate A2 with an isoelectric focusing platform. Other electrophoretic techniques do not reliably detect low levels of Hb A and S and cannot be reliably used on blood spots. HPLC provides a more sensitive, robust and reproducible approach with the ability to retain records which can be scanned and referred for second opinion.

There are few reports on the validation of self-report of SCD in the literature. A recent report from the United States on the discordance between self-report and genetic confirmation of SCD status in African-American adults observed that among a subgroup of 51 subjects who self-reported SCD, only 5.9% (3 of 51) were confirmed with the disease, while 62.7% (32 of 51) had sickle trait, 5.9% (3) had Hb C trait, and 25.5% (13) had no abnormal alleles at all [9]. This is a rather surprising result, coming from an affluent setting where universal newborn screening has been established for several years and much has been done nationally to raise awareness about the knowledge of SCD. In another survey, which was restricted to postpartum African-American women, 25% did not know and 4% incorrectly reported their status. [10]. Similarly, a survey of parents of children with SCD or sickle-cell trait reported significant misunderstanding of the mode of inheritance of SCD, although parents of affected children had a better understanding [11]. Interestingly, from the reports in the United States, there does not appear to be any significant difference by gender or education level in those that misreported SCD status.

Promoting genetic health literacy will require significant attention and innovative public health measures in a population where literacy rates are generally low. Yet education and awareness of this condition offers the only hope for effectively reducing the incidence of SCD.

Our observation in this report highlights the challenge in screening for a disorder when there is no adequate setup to support education, treatment and a robust counseling for affected patients and families. This calls for the need for a well-formed policy for SCD control in the country, a policy that involves all different tiers of health care including primary, secondary and tertiary services. There must be an effective diagnostic program that ensures that the parents receive the true result for their child and also that the correct diagnosis is made. This is required to ensure that the newly diagnosed SCD patient is linked to appropriate care. The institution of newborn screening with a comprehensive follow-up program has been shown to improve outcomes for SCD.

There is a need to promote awareness and provide education on SCD across the population. Improved opportunities for testing either at birth or early infancy will avail opportunities for implementing preventive care and provide better planning for supportive care, reduction in morbidity and improved quality of life.

Studies from developed countries have clearly demonstrated the benefit of early diagnosis on improving the quality of life and survival in SCD [12, 13]. Limited reports from some African countries have also demonstrated the feasibility of this approach [12, 13]. Appropriate preventive care can be instituted through antenatal testing or neonatal screening. In a pilot study, the acceptability of newborn screening was demonstrated by Odunvun et al. [14] in Southwestern Nigeria. Preconception screening has been widely advocated in most Mideastern countries where there is a fair amount of disease burden, and where hemoglobinopathies and consanguineous marriage are common. It remains unknown whether this approach will achieve the same level of acceptability in the more conservative Islamic population in the northern part of Nigeria.

Improvement and monitoring of laboratory diagnostic services are urgently required in order to provide accurate diagnostic test results to patients. In addition, this must be supported by appropriate genetic counseling and referral for care. Availability of written results following testing is strongly advocated as part of the primary health care in this setting, given the prevalence of SCD and the potential impact of this knowledge on patient care.

**Acknowledgement**

This work was supported by funds from the United Nations Development Program, Department of Pediatrics, Michigan State University, East Lansing, Mich., USA, and the Blood Disorders Branch of the Centers for Disease Control and Prevention, Atlanta, Ga., USA. This study was funded by the European Union and UNDP joint initiative; however, the funding agencies had no role in the design or analysis of the results.

**Disclosure Statement**

The authors have no conflicts of interest to disclose.
References

5 Sickle cell anemia. Agenda item 11.4. 59th World Health Assembly, WHA 59.20, 2006.