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HemoTypeSC point-of-care testing shows high sensitivity with alkaline cellulose acetate hemoglobin electrophoresis for screening hemoglobin SS and SC genotypes

Samuel Ademola Adegoke [a,*], *Oluwatoyin Ibukun Oladimeji* [b], *Morenike Agnes Akinlosotu* [c], *Alex Ifeoluwa Akinwumi* [b], *Kayode Ademola Matthew* [b]

[a] *Obafemi Awolowo University, Ile-Ife, Nigeria*
[b] *Obafemi Awolowo University Teaching Hospital, Ile-Ife*
[c] *University of Medical Sciences, Ondo, Ondo State, Nigeria*

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ABSTRACT

Introduction: By providing timely actionable results for prompt management, point-of-care testing (POCT) kits have revolutionised medical care for various diseases, ranging from infectious diseases like malaria to genetic disorders, such as sickle cell disease (SCD). They are, however, underutilised in the diagnosis of SCD in developing countries, where the need is greatest.

Objective: The study was aimed at assessing the sensitivity of HemoTypeSC POCT among a cohort of children with SCD, previously diagnosed by Alkaline cellulose acetate hemoglobin electrophoresis (ACAE), with or without high-performance liquid chromatography (HPLC).

Methods: In this descriptive cross-sectional study, HemoTypeSC test was conducted on all participants and its sensitivity was determined by comparing results with those obtained using ACAE. Discordance was verified with HPLC.

Results: One hundred and forty-five children aged one to 19 years were studied. There were 84 males and 61 females (male: female ratio = 1.4: 1). The HemoTypeSC was able to correctly diagnose sickle cell anemia (SCA) and hemoglobin SC in all (100%) of the children tested.

Conclusion: The HemoTypeSC shows high sensitivity in detecting SCA and hemoglobin SC. Hence, it is useful for targeted screening of individuals suspected of having SCD, leading to rapid diagnosis of these hemoglobinopathies, even in resource-constrained settings.

* *Corresponding author at*: Department of Paediatrics and Child Health, Obafemi Awolowo University, PMB 013, Ile-Ife, Nigeria.
E-mail address: adegoke2samade@yahoo.com (S.A. Adegoke).

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Introduction

Despite the high burden of sickle cell disease (SCD) in Nigeria, specific national efforts targeted at stemming the burden do not seem to commensurate with the persistent increase in the prevalence of the disease in the country.[1,2] There is no structured national policy on SCD screening, even in the newborn period. The available diagnostic or screening methods outside the neonatal period are not widespread and results may be inconsistent.[3] Alkaline cellulose acetate hemoglobin electrophoresis (ACAE), the most commonly used method in many settings in Nigeria is prone to error. These errors may result from low expertise, inadequate standards, faulty equipment, fake results and irregular monitoring of laboratories where these tests are carried out.[4] Some other diagnostic methods such as citrate agar electrophoresis, isoelectric focusing (IEF), high-performance liquid chromatography (HPLC), capillary zone electrophoresis and DNA analysis, involve specialized training and expertise, electricity-dependent, and are capital-intensive, hence are not frequently employed in many resource-poor countries.[4,5]

Point-of-care testing (POCT) kits provide timely results needed for prompt management and have revolutionised medical care for various diseases ranging from infection to genetic disorders such as sickle cell disease (SCD).[6–8] An ideal point-of-care test (POCT) for diagnosis of SCD must fulfil the 'ASSURED' criteria that were initially developed by the World Health Organisation for Sexually Transmitted Disease Diagnostics Initiative.[9] The test should be Affordable to the end-users; Sensitive (i.e. has high ability to identify the disease when present), Specific (able to distinguish HbAA or trait {HbAS} from disease {HbSS, HbSC, HbS/β-thalassemia}); User-friendly (simple to perform, easy to interpret and require minimal training); Rapid (results available within minutes); Equipment-free (does not require additional equipment for refrigeration or electricity, hence the device is compact, portable and of low weight); Delivered to those who need it — available in rural and urban communities in areas of high prevalence.

In 2018, a multi-centre, real-world assessment of a low-cost point-of-care testing (POCT) device, HemoTypeSC, was carried out in Nigeria.[10] Authors from 18 primary care centres affiliated to Sickle Cell Support Society of Nigeria screened 1121 newborns and infants using both HemoTypeSC and high-performance liquid chromatography (HPLC). Discordant samples were confirmed by molecular diagnosis. It was reported that, in optimal field conditions, the sensitivity and specificity of HemoTypeSC for SCA were 93.4% and 99.9%, respectively, and its overall accuracy was 99.1%. Authors concluded that POCT device could be scaled up and routinely used across multiple healthcare centres in sub-Saharan Africa for identification and management of vast numbers of individuals with SCD who are currently undiagnosed.[10]

The latter study was conducted among children whose genotypes were unknown, however, the current study focused on assessing the sensitivity of HemoTypeSC among children already diagnosed with SCA or hemoglobin SC. In addition to demonstrating the ability of the kit to be used for a clinical suspicion of sickle cell disease, our study also explores the usefulness of the test kit to distinguish a specific diagnosis of HbSC from HbSS. If the test kit is found to be sensitive in detecting HbSS and HbSC, the two most common hemoglobinopathies in sub-Saharan Africa, it will serve not only as an effective and efficient means of targeted screening to confirm or refute the suspicion of SCD, but a means of differentiating a more severe SCA from less severe HbSC. Thus, we assessed the sensitivity of HemoTypeSC among SCD cohort (aged one to 19 years) who were previously diagnosed by ACAE with or without HPLC, and are already being followed-up in two paediatric hematology clinics in South west Nigeria.

Methods

This cross-sectional descriptive study was carried out from January to March 2020 at the Paediatric SCD clinics of the Wesley Guild Hospital Ilesa unit of Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) and University of Medical Sciences Teaching Hospital, Akure Unit, Ondo State, Nigeria. Both clinics offer comprehensive SCD care.

Study population

These comprised of children and adolescents with SCD already diagnosed with ACAE and have been on routine follow up in the SCD clinics for a variable period ranging from six months to fifteen years. All diagnosed patients with SCD (homozygous or heterozygous SCD) were recruited consecutively as they present in the clinic for routine care. However, those with a history of blood transfusion in the preceding four months and those without consents or assent were excluded.

Ethics

Approval for the study was obtained from the Ethics and Research Committee of OAUTHC (Protocol number: ERC/2017/08/02). Written informed consents and assents were also obtained as appropriate from the parents/ legal guardian and children aged ≥7 years respectively.

Data collection

Sociodemographic information (age, gender and parental socioeconomic status) and age at diagnosis were obtained by direct questioning. Documented hemoglobin genotype results were extracted from the medical charts. In the two hospitals, ACAE is the main diagnostic method for SCD. Occasionally, high performance liquid chromatography (HPLC) is requested to confirm diagnosis or to quantify hemoglobin fractions, especially fetal hemoglobin to commence or monitor those on hydroxyurea therapy. All the tests were performed strictly by standard procedures. HemoType screening was done in the clinic according to the manufacturer's instructions and interpreted based on a reference chart provided by the manufacturer. Hemoglobin genotype results from HemoTypeSC kit were compared with those with ACAE. Patients with discordant results subsequently had HPLC and results from the three methods compared.

Table 1 – Frequencies of hemoglobin genotypes as diagnosed by the three diagnostic methods.

Diagnostic methods	HbSS n (%)	HbSC n (%)	Total n (%)
ACAE	139 (95.9)	6 (4.1)	145 (100)
HemoTypeSC	140 (96.6)	5 (3.4)	145 (100)
HPLC	36 (97.3)	1 (2.7)	37 (100)

ACAE: Alkaline cellulose acetate hemoglobin electrophoresis, HbSS: homozygous hemoglobin S disease, HbSC: heterozygous hemoglobin SC disease, HPLC: high performance liquid chromatography.

HemoTypeSC test kit has the following peculiar properties:

a Affordable: Each kit costs about 1.5USD which is comparable to the cost of ACAE in our facility as at December 2019.
b User-friendly: The test kit did not require elaborate training or special expertise as it is both easy to perform and interpret. For instance, blood samples were collected by fingerpick, after which HemoTypeSC blood sampling device was used to absorb about 1 μl of blood from each patient and then dipped in a container of six drops of sterile water to obtain haemolysate. Testing kit was then introduced into the haemolysate and left to stay for about 10 min, before reading off the result.
c Rapid: The test result for each of the subjects was available within 10 min of sample collection, thus HemoTypeSC provides actionable results within minutes unlike ACAE which requires samples to be pooled with results not been available for some days after sample collection.
d Equipment-free: HemoTypeSC test kit is portable, light and does not require electricity or additional equipment such as electrophoretic tanks which is often needed for ACAE.
e Delivery to those who need it: Unlike ACAE, HemoTypeSC can be readily used both in rural and urban communities. The test kits can potentially empower patients to self-test in the privacy of their homes. In this study, all the tests were done in the clinics.

Results

A total of 145 patients aged one to 19 years with SCD from the two hematology clinics were enrolled in the study. Their mean ± SD age was 8.7 ± 4.6 years. There were 84 males and 61 females (male: female ratio = 1.4: 1). Based on ACAE, 139 (95.9%) of the 145 had homozygous SCD, and six (4.1%) had HbSC genotype (Table 1).

Results of hemoglobin genotype by ACAE and HemoTypeSC were the same for 144 of the 145 (99.3%) patients. The only patient with discordant results was a seven-year-old boy whose ACAE was reported as HbSC but read HbSS with HemoTypeSC™. The HPLC result of this boy was HbSS, consistent with HemoTypeSC™ result.

Only 37 (25.5%) of the 145 patients had documented HPLC results. In all the 37 patients, there was 100% consistency in the hemoglobin genotype results by both HemoTypeSC and HPLC.

Discussion

This matrix confirmation study with two comparator assays: ACAE and HPLC, tested the analytic characteristics of the HemoTypeSC in children with prior diagnosis of SCD. The study adds to the growing evidence that emergent, novel, inexpensive, SCD-POCTs have validated analytic characteristics and field-tested performance comparable to laboratory methods. POCT kits seem to be the way to go in screening and diagnosis and of a wide range of disorders including SCD. This is because it provides real-time actionable results within minutes, which will improve patient care.[11] Thus, the kits help in making rapid diagnosis and immediate management of children in whom healthcare workers suspect a diagnosis of SCD, allowing for prompt communication of results and timely referral for follow-up care. Also, most POCT systems for SCD are portable and not dependent on electricity or highly skilled personnel which make them choice in developing countries for prompt diagnosis.[11] At about 6 weeks of age, there is decrease in HbF with relative concentration of approximately 60% in babies such that POCT can be useful, especially in climes where other newborn screening programs are not readily available and or affordable.[12] HemoTypeSC device is low cost (less than 2 USD), demonstrates high sensitivity and specificity, requires <1 μL of blood by finger prick or heel prick, does not need instruments or a power source, user-friendly (simple to perform with minimal training), rapid (total time-to-result of less than 20 min) and does not require refrigerated storage of samples.[13,14]

From our study, we have been able to demonstrate the high sensitivity of HemoTypeSC in detecting SCD correctly in 100% of our patients, when compared to the commonly employed ACAE in Nigeria. The kit also gave a result similar to HPLC in a 7-year-old boy initially diagnosed to have HbSC with ACAE. This may be a pointer to a better performance of the kit compared to ACAE. Our finding is in keeping with a previous multicentre study in Nigeria which reports the specificity and sensitivity of HemoTypeSC as 99.9% and 93.4% respectively under standard field circumstances.[10] Similarly, a multicentre study involving 1559 participants in India reported the sensitivity and specificity of HemoTypeSC as 98.1% and 99.1% respectively for all hemoglobin phenotypes.[15] These studies highlight the accuracy of HemoTypeSC in diagnosing SCD.

Unlike previous studies that examined the diagnostic accuracy of the HemoTypeSC among individuals with unknown genotypes, our study assessed it sensitivity among children already diagnosed with SCA or hemoglobin SC.[10,12,15,16] It thereby explored the usefulness of the kit for targeted screening of individuals clinically suspected to have SCD based on history and or presence of specific external stigmata.

A major advantage of HemoTypeSC POCT kit over other commonly used methods of SCD diagnosis is that it requires only a small drop of blood for diagnosis.[11] This is especially important for individuals with SCD who are often

anaemic even when in steady-state. Also, the test does not require professional knowledge and skills to conduct.[11] Hence, community health workers can conduct the test and readily diagnose SCD at the grassroots. This would make a tremendous impact on early diagnosis of SCD, which is a major positive prognostic factor among individuals with SCD.[17]

One main drawback of the HemoTypeSC is that although it can readily detect Hb A, C and S, other compound heterozygous hemoglobinopathies such as HbS-thalassaemia, SO-Arab, SD-Punjab among others cannot be detected by this POCT kit. Though its limit of detection was estimated as 1.3% for HbC, 2.7% for HbA and 3.3% for HbS, it cannot readily identify HbF and HbA2.[12] Also, HbSβ+-thal and HbSβ0-thal were detected as HbAS and HbSS, respectively.[12] This calls for further innovative research into developing equally sensitive and specific POCT kits that can readily diagnose a more encompassing range of sickle cell disorders.

One major limitation of this present study is that only a few of our study cohort had HPLC, a gold standard method for hemoglobin quantification, hence comparison of HemoTypeSC with HPLC was not done for all study participants. This is due to its limited availability and high cost which buttresses the need for a low-cost and readily available screening tool for SCD such as HemoTypeSC.

HemoTypeSC shows high sensitivity in detecting sickle cell anemia and hemoglobin SC when compared to commonly employed ACAE in most resource-limited settings. It also has other advantages as it fulfils the 'ASSURED' criteria described by the World Health Organization for POCT kits.[9] It is, however, limited in detecting other hemoglobinopathies, thus necessitating further research into expanding its usefulness in the diagnosis of a wider range of hemoglobinopathies. While the science of analytic validity of SCD-POCT is settled, future studies on the usefulness of HemoTypeSC could focus on viable options for effective implementation of sustainable POCT programs for SCD. The mere availability of a test does not necessarily lead to successful outcomes as lessons from use of POCTs for Malaria and HIV have demonstrated.[18,19] More data that could help improve optimization of SCD-POCT across healthcare settings are needed.

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Conflicts of interest

The authors declare no conflicts of interest.

Authors contribution

Study conception and design — SAA; Data collection — All authors; Data Analysis - SAA; Initial drafting of the article — SAA, OIO; Critical revision for important intellectual content — All authors; and Final approval of the version to be published — All authors.

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REFERENCES

1. Galadanci N, Wudil BJ, Balogun TM, Ogunrinde GO, Akinsulie A, Hasan-Hanga F, et al. Current sickle cell disease management practices in Nigeria. Int Health. 2014;6(1):23–8.
2. Diakwu-Akinwumi IN, Abubakar SB, Adegoke SA, Adeleke S, Adewoye O, Adeyemo T, et al. Blood transfusion services for patients with sickle cell disease in Nigeria. Int Health. 2016;8(5):330–5.
3. Lobitz S, Telfer P, Cela E, Allaf B, Angastiniotis M, Johansson C, et al. Newborn screening for sickle cell disease in Europe: recommendations from a Pan-European Consensus Conference. Br J Haematol. 2018;183:648–60.
4. Kotila TR. Guidelines for the diagnosis of the haemoglobinopathies in Nigeria. Ann Ib Postgrad Med. 2010;8(1):25–9.
5. Yang X, Kanter J, Piety NZ, Benton MS, Vignes SM, Shevkoplyas SS. A simple, rapid, low-cost diagnostic test for sickle cell disease. Lab Chip. 2013;13:1464–7.
6. Yager P, Domingo GJ, Gerdes J. Point-of-care diagnostics for global health. Annu Rev Biomed Eng. 2008;10:107–44.
7. Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. Clin Microbiol Infect. 2010;16:1062–9.
8. Pai NP, Vadnais C, Denkinger C, Engel N, Pai M. Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. PLOS Med. 2012;9(9):e1001306.
9. Kettler H, White K, Hawkes S. Mapping the landscape of diagnostics for sexually transmitted infections. Key findings and recommendations. WHO/TDR; 2004.
10. Nnodu O, Isa H, Nwegbu M, Ohiaeri C, Adegoke S, Chianumba R, et al. HemoTypeSC, a low-cost point-of-care testing device for sickle cell disease: promises and challenges. Blood Cells Mol Dis. 2019;78:22–8.
11. Nelson R. New sickle cell test can transform screening. Available at https://www.medscape.com/viewarticle/906278. [Access4th edn April 2020].
12. Alapan Y, Fraiwan A, Kucukal E, Hasan MN, Ung R, Kim M, et al. Emerging point-of-care technologies for sickle cell disease screening and monitoring. Expert Rev Devices. 2016;13(12):1073–93.
13. McGann PT, Hoppe C. The pressing need for point-of-care diagnostics for sickle cell disease: a review of current and future technologies. Blood Cells Mol Dis. 2017;67:104–13.
14. Quinn CT, Paniagua MC, DiNello RK, Panchal A, Geisberg M. A rapid, inexpensive and disposable point-of-care blood test for sickle cell disease using novel, highly specific monoclonal antibodies. Br J Haematol. 2016;175(4):724–32.
15. Mukherjee MB, Colah RB, Mehta PR, Shinde N, Jain D, Desai S, et al. Multicenter evaluation of HemoTypeSC as a point-of-care sickle cell disease rapid diagnostic test for newborns and adults across India. Am J Clin Path. 2020;153:82–7.
16. Steele C, Sinski A, Asibey J, Hardy-Dessources MD, Elana G, Brennan C, et al. Point-of-care screening for sickle cell disease in low-resource settings: a multi-center evaluation of HemoTypeSC, a novel rapid test. Am J Hematol. 2019;94(1):39–45.

17. Headings VE. Sickle cell disease in childhood. Strategies for early diagnosis. Am J Pediatr Hematol Oncol. 1982;4(1):67–71.
18. Palmer T, Aiyenigba AO, Bates I, Okyere DD, Tagbor H, Ampofo GD. Improving the effectiveness of point of care tests for malaria and anemia: a qualitative study across three Ghanaian antenatal clinics. BMC Health Serv Res. 2020;20:444.
19. World Health Organisation. Improving the quality of HIV-related point-of-care testing: ensuring the reliability and accuracy of test results. WHO/CDC; 2015. p. 1–90. Available at https://www.who.int. [cited 2020 August 20].