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Comparison of Sickle SCAN™ Rapid Diagnostic Test and Cellulose Acetate Electrophoresis with High-Performance Liquid Chromatography for Diagnosing Sickle Cell Disease

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Sickle cell disease (SCD) poses a significant public health challenge in sub-Saharan Africa and central India, where most cases are reported. The Sickle SCAN rapid diagnostic test, designed explicitly for resource-limited settings, is crucial in diagnosing SCD. We studied 147 infants, with the majority (51.7%) aged between 9 and 24 months (52.4% females and 47.6% males). The hemoglobin types were determined using high-performance liquid chromatography (HPLC) with the D-10 instrument from Bio-Rad. Additionally, Sickle SCAN™, a qualitative lateral flow immunoassay, was used to detect the presence of HbA, HbS, and HbC in whole blood samples. Cellulose acetate membrane (CAM) electrophoresis was also performed on the samples. Cellulose acetate membrane (CAM) electrophoresis was also performed on the samples. The Sickle SCAN RDT exhibited a sensitivity of 85.4% and specificity of 90.2%, while the CAM assay showed a sensitivity of 83.3% and specificity of 82.3%. Both methods achieved high positive predictive values (PPV), with the Sickle SCAN RDT at 94.3% and the CAM assay at 89.9%. However, their negative predictive values (NPV) were lower, with the Sickle Scan RDT at 76.7% and the CAM assay at 72.4%. Sickle SCAN's performance in detecting SCD and trait cases was commendable, with diagnostic accuracy on par with HPLC and CAM.

Keywords: Sickle Cell Disease, Sickle SCAN, sensitivity, specificity, positive predictive value, negative predictive value

Introduction

Sickle cell disease (SCD) is an inherited red blood cell disorder. In SCD, abnormal hemoglobin (HbS) causes red blood cells to become rigid and take on a sickle shape. These sickle cells can block blood vessels, leading to pain, infections, and other serious complications. There are different types of SCD, including the most severe form called sickle cell anemia (HbSS) and milder variants like HbSC and HbS beta thalassemia (1,2).

In sub-Saharan Africa and central India, where over 90% of annual sickle cell disease (SCD) births take place, universal implementation of newborn screening programs has been limited due to the significant costs and logistical challenges associated with laboratory diagnostic tests1. These programs aim to identify infants with SCD early on, allowing for timely interventions and improved outcomes. However, the barriers related to resources and infrastructure have hindered widespread adoption in these regions (3). In sub-Saharan Africa and central India, where over 90% of annual sickle cell disease (SCD) births occur, individuals with SCD are often diagnosed only after being hospitalized for severe pain or other life-threatening symptoms. The impact of SCD on mortality, quality of life, and the strain it places on regional healthcare systems has prompted the United Nations General Assembly to recognize it as a public health concern. (4) and a priority non-communicable disease by the World Health Organization (5).

Various techniques can be employed to detect hemoglobin S (HbS) and its variants, but each method has its limitations. Consequently, a combination of these approaches is necessary for an accurate diagnosis. The techniques include microscopic examination of peripheral blood smears and solubility tests. (6,7). The sickling test and alkaline electrophoresis or cellulose acetate electrophoresis also provide a reasonably accurate hemoglobin S

(HbS) diagnosis. However, DNA analysis is necessary to confirm the diagnosis

Early diagnosis of sickle cell disease (SCD), ideally during the neonatal period, is crucial for effective care management. It enables timely parental education and counseling regarding disease complications, immunization, and antibiotic prophylaxis. To enhance care and quality of life for SCD patients, establishing a reliable diagnosis in resource-limited settings is essential(8,9,17). At present, there are three validated gold standard methods for initial neonatal screening and routine diagnosis: High-Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE), and Isoelectric focusing (IEF). These techniques play a crucial role in identifying hemoglobin variants, including hemoglobin S (HbS), in newborns. (10).

Several emerging Point-of-Care (POC) tests for hemoglobin variants offer promising alternatives for reliable and straightforward sickle cell disease (SCD) diagnosis in developing countries. These tests operate based on the solubility difference between hemoglobin A (HbA) and hemoglobin S (HbS). Notably, microfluidic paper-based analytical devices (μPADs) developed by Halcyon Biomedical are among these innovative tools. (12); The Daktari Sickle Cell test, developed by Daktari Diagnostics, relies on differences in red blood cell density within an aqueous multiphase system. In contrast, the Hemotype SC, created by Silver Lake Research Corporation, uses lateral flow immunoassay technology. Additionally, the Sickle SCAN® by BioMedomics is a sandwich format chromatographic immunoassay designed to qualitatively detect hemoglobins HbA, HbS, and HbC in whole blood samples. Notably, the Sickle SCAN® has demonstrated excellent performance in identifying common hemoglobin variants (12,13,16). The research aimed to compare two methods for hemoglobin electrophoresis: the Sickle SCAN® and Cellulose Acetate Membrane. High-Performance Liquid Chromatography (HPLC) served as the gold standard in this comparison.

Materials and Methods

Study Area

The research took place at the Rivers State University Teaching Hospital (RSUTH) in Port Harcourt, Rivers State, Nigeria. Formerly known as Braithwaite Memorial Specialist Hospital (BMSH), RSUTH is a government-owned facility located in Old GRA and has a total capacity of 375 beds.

Sample Size Calculation:

The minimum sample size was calculated using the Cochran standard formula for cross-descriptive studies:

$$n = \frac{Z^2Pq}{d^2}$$

Where,

n= minimum sample size required

Z= Standard normal deviation, set at 1.96, corresponding to a 95% confidence level

P= Proportion of sickle cell disease patients (Patra 2012). = 10% = 0.1

q= 1-P = 1-0.1 = 0.9

d = Level of precision = 0.05

Applying this formula,

$$n = \frac{1.96^2 \times 0.1 \times 0.9}{0.05^2}$$

$$n = \frac{3.8416 \times 0.1 \times 0.9}{0.0025}$$

$$n = \frac{0.345744}{0.0025}$$

n= 138

Attrition= 10/100 × 134 = 13.4

Minimum sample size = 134 + 13.4 = 147.4

Therefore, the minimum sample size for this study = 147

Study Population

The study population consisted of 147 infants (male and female) aged 6 months to 2 years. Participants were recruited from the pediatric and sickle cell clinics and the laboratory department of Rivers State University Teaching Hospital in Port Harcourt, Rivers State, Nigeria.

Ethical Approval; This study received ethical approval from the ethics and research committee of Rivers State University, Port Harcourt, Rivers State, Nigeria.

Informed Consent: All participants voluntarily signed written informed consent forms in their handwriting as proof of their willingness to provide samples for the tests. For infants who could not sign the informed consent form, their parents/guardians signed on their behalf.

Collection of Sample: Two millilitres of whole blood was collected using S-monovette vacutainer syringe. The blood was deposited into an ethylenediaminetetraacetic acid (EDTA) anticoagulated.

Study Design. The study was cross-sectional in nature.

Procedure

The hemoglobin type was determined using two methods: high-performance liquid chromatography (HPLC) with the D-10 instrument from Bio-Rad, and the Sickle SCAN™, a qualitative lateral flow immunoassay. The Sickle SCAN™ detects the presence of hemoglobins HbA, HbS, and HbC in whole blood samples using a sandwich format chromatographic immunoassay approach with colorimetric detector nanoparticles conjugated to antibodies. During the rapid test, 5 µl of blood from either a finger prick or venipuncture was added to a prefilled buffer solution according to the BioMedomics protocol. After mixing by inverting the bottle three times, three drops were discarded, and five other drops were placed into the testing cartridge. The result was read five minutes later.

The recommended storage temperature for the rapid test is between 2°C and 30°C. Additionally, cellulose acetate electrophoresis was performed on samples and controls using standard methods described by Dacie and Lewis (Dacie et al., 2000). High-Performance Liquid Chromatography (HPLC) was carried out using Bio-Rad Laboratories. The chromatographic patterns were evaluated to identify and quantify different hemoglobin variants. Each variant has a characteristic retention time, which is the elapsed time from sample injection to the apex of a hemoglobin peak. "Windows" represent established ranges in which common variants elute using the Variant beta-thalassemia short program. The printed chromatogram displayed all the eluted hemoglobin variants, their retention times, peak areas, and the percentage values of different hemoglobin components. Peaks eluting at retention times not pre-defined were labeled as unknown.

Statistical Analysis

Data was analyzed using Graph Pad Prism 9, and Sensitivity, Specificity, and their corresponding 95% Confidence Intervals were determined. Positive and Negative Predictive values were also calculated. Statistical significance was determined as a p-value of less than 0.05.

Results

Table 1 presents the sociodemographic characteristics of the subjects, highlighting their age distribution and sex. The data is compiled from a sample size of 147 individuals.

The age distribution of the respondents is categorized into three groups: less than 12 months, 12 to 18 months, and 19 to 24 months. Among these, the largest group comprises individuals aged 12 to 18 months, with a frequency of 76 respondents, representing 51.7% of the total sample. This is followed by the 19 to 24-month age group, which includes 41 subjects (27.9%), and the less than 12 months group, which includes 30 subjects (20.4%). The mean age of the subjects is 15.74 months.

Regarding sex distribution, the sample is nearly evenly split between female and male subjects. There are 77 females, accounting for 52.4% of the sample, and 70 males, making up 47.6% of the sample.

In summary, the respondents are predominantly aged between 12 and 18 months, and there is a relatively balanced distribution between female and male respondents. This demographic information provides a comprehensive overview of the sample population used in the study.

Table 1: Sociodemographic characteristics of the subjects

Variable	Frequency (N)	Percentage (%)
Age (Months)		
<12	30	20.4
12 - 18	76	51.7
19 - 24	41	27.9
Total	147	100
Mean	*15.74*	-
Sex		
Female	77	52.4
Male	70	47.6
Total	147	100

The assessment of two haemoglobin genotype assays—Sickle Scan Rapid Diagnostic Test (RDT) and the CAM assay—was conducted using High-Performance Liquid Chromatography (HPLC) as the gold standard. The performance of each assay was evaluated based on sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and statistical significance.

For the Sickle Scan RDT, among 96 individuals identified as AA by HPLC, 82 were correctly identified by the Sickle Scan RDT, resulting in a sensitivity of 85.4% (95% CI: 77 – 91.1). Conversely, out of 51 individuals identified as AS by HPLC, 46 were accurately identified by the Sickle Scan RDT, while 5 were misclassified as AA. This resulted in a specificity of 90.2% (95% CI: 79 – 95.7). The overall performance of the Sickle Scan RDT indicated a PPV

of 94.3% and an NPV of 76.7%, with a p-value of <0.0001, demonstrating statistically significant results.

In comparison, the CAM assay correctly identified 80 out of 96 individuals with the AA genotype (identified by HPLC), leading to a sensitivity of 83.3% (95% CI: 74.6 – 89.4). For the AS genotype, the CAM assay correctly identified 42 out of 51 individuals, with 9 misclassified as AA, resulting in a specificity of 82.3% (95% CI: 69.8 – 90.4). The overall performance of the CAM assay showed a PPV of 89.9% and an NPV of 72.4%, with a p-value of <0.0001, indicating statistical significance.

The Sickle Scan RDT and the CAM assay demonstrated high sensitivity and specificity compared to HPLC, the gold standard. The Sickle Scan RDT slightly outperformed the CAM assay regarding sensitivity and specificity. Both assays exhibited high PPV, indicating that positive results from either test are highly reliable. However, the NPV was lower for both tests, particularly for the CAM assay, suggesting that negative results are less reliable. The highly significant *p*-values (<0.0001) for both tests provided strong evidence against the null hypothesis, confirming the reliability of the tests, as shown in Table 2 below.

Table 2: Assessment of haemoglobin genotype assays with HPLC

Haemoglobin Genotype Assays		**HPLC (Gold Standard)**			**Results for Haemoglobin Genotype Assays**				
		AA (n = 96)	**AS (n = 51)**	**Total**	**Sensitivity (%): 95% CI**	**Specificity (%): 95% CI**	**PPV (%)**	**NPV (%)**	***p*-value**
Sickle Scan RDT	AA	82	5	87	85.4: 77 – 91.1	90.2: 79 – 95.7	94.3	76.7	<0.0001*
	AS	14	46	60					
CAM	AA	80	9	89	83.3: 74.6 – 89.4	82.3: 69.8 – 90.4	89.9	72.4	<0.0001*
	AS	16	42	58					

PPV= Positive predictive value, NPV= Negative predictive value

Discussion

The study aimed to assess the performance characteristics of three methods—Sickle Scan RDT, Cellulose Acetate Medium (CAM), and High-Performance Liquid Chromatography (HPLC)—for determining hemoglobin genotypes. Results indicate that both Sickle Scan RDT and CAM methods exhibited good sensitivity and specificity in detecting hemoglobin types compared to the gold standard HPLC test. Specifically, the Sickle Scan RDT performed best, with a sensitivity of 85.4% and specificity of 90.2%. This means it correctly identified 85.4% of subjects with the AA genotype and 90.2% without it. The positive predictive value (PPV) was 94.3%, indicating that 94.3% of subjects with a positive test result had the AA genotype. The negative predictive value (NPV) was 76.7%, suggesting that 76.7% of subjects with a negative test result did not have the AA genotype. The CAM method also demonstrated good performance characteristics, with a sensitivity of 83.3% and specificity of 82.3%. Although not as effective as the Sickle Scan RDT, the CAM method still showed high accuracy

The results of this study are consistent with those of previous studies that have evaluated the performance of the Sickle Scan RDT and CAM methods. For example, a study conducted in Mali found that the Sickle Scan RDT had a sensitivity of 100% and specificity of 99.4% for detecting HbAA and HbCC phenotypes (14). Another study in Togo found that the Sickle Scan RDT had a sensitivity of 94.9% and specificity of 99.4% for detecting HbAS and HbAC phenotypes (14). A study conducted in the Democratic Republic of the Congo found that the Sickle Scan RDT had a sensitivity of 96.69% and specificity of 99.43% for detecting HbAA and HbAS phenotypes (15). Another study in Tanzania found that the Sickle Scan RDT had a sensitivity of 98.1% and specificity of 91.1% for detecting HbSS and HbSC phenotypes (15). A study conducted in Gabon has also reported a Sickle Scan sensitivity of 92.25, 100% and 100% for HbSS, HbAS and HbSC respectively while thespecificity exceeded 88% for all phenotypes. (16,17). The conclusion

from these studies indicate that Sickle Scan rapid diagnostic test device is reliable and employed in both epidemiology and diagnostic purposes.

Conclusion

Sickle SCAN's performance in detecting SCD and trait cases was commendable, with diagnostic accuracy on par with HPLC and CAM.

Recommendations:

1. The Sickle Scan RDT and CAM methods can be helpful screening tools for hemoglobin genotyping, especially in resource-limited settings where access to HPLC may be limited. However, confirmatory testing with the gold standard HPLC method is still recommended, particularly for cases with discordant results between the screening tests and clinical suspicion. The results of this study can inform clinical practice and guide the diagnosis and management of hemoglobin disorders.
2. The Sickle Scan RDT should be adopted for initial screening in areas where high-performance liquid chromatography (HPLC) is not feasible due to resource constraints. This method demonstrated good sensitivity and specificity, making it a suitable alternative for early diagnosis and management of haemoglobin disorders. Governments and health organizations should integrate Sickle Scan RDT into national sickle cell disease (SCD) screening programs. This integration will facilitate early detection and intervention, which is necessary for reducing morbidity and mortality associated with SCD.

3.While Sickle Scan RDT and CAM methods are helpful for initial screening, confirmatory testing with HPLC should be available at regional centers to ensure accurate diagnosis and proper management of patients with discordant results. Further research should be conducted to improve the accuracy and usability of Sickle Scan RDT. Studies focusing on different populations and settings can help identify potential limitations and areas for improvement,

4. The study validates the effectiveness of the Sickle Scan RDT and CAM methods as reliable alternatives to HPLC for diagnosing haemoglobin disorders. This is particularly significant for resource-limited settings, where high costs and logistical challenges make HPLC impractical. By demonstrating the high sensitivity and specificity of these diagnostic methods, the research supports their use in early diagnosis and management of SCD.

5. Early detection is critical in implementing timely interventions that can significantly reduce the impact of Sickle cell disease. The findings provide evidence-based recommendations that can inform health policy and program development.

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