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HemoTypeSC™ Point-of-care Testing as a Screening Tool for Sickle Cell Disease among Newborns in Ilesa, Nigeria

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Abstract

Background: The prohibitive costs and inefficient health systems are major factors that hinder the successful and sustainable implementation of newborn screening (NBS) for sickle cell disease in many countries in sub-Saharan Africa. Cheaper point-of-care testing from previous studies is effective for screening across age groups, including neonates.

Objective: To determine the diagnostic accuracy of HemoTypeSC™ as a screening tool for sickle cell disease among the newborn population at Primary Health Care facilities in Ilesa, Osun State, southwest Nigeria.

Methods: This was a community-based cross-sectional study. Blood samples of 304 newborn babies were collected for haemoglobin phenotype determination by both high-performance liquid chromatography (HPLC) and HemoTypeSC™ testing. The performance of HemoTypeSC™ was compared with the gold standard (HPLC) to determine its diagnostic accuracy.

Result: The overall diagnostic accuracies of the HemoTypeSC™ kit for Hb AA, Hb AS, Hb AC, Hb SC and Hb SS phenotypes were 98.0%, 99.0%, 100.0%, 100.0% and 99.3%, respectively.

Conclusion: The HemoTypeSC™ kit can be used reliably as a rapid diagnostic tool for screening newborns for SCD due of its high overall diagnostic accuracy.

Key words: Haemoglobin phenotypes, Haemoglobinopathy, HemoTypeSC™, Newborn Screening.

Introduction

The prohibitive cost of screening for sickle cell disease (SCD), especially during the newborn period, is a major challenge of newborn screening programs in Sub-Saharan Africa. The most readily available method for haemoglobin phenotype determination in Nigeria is cellulose acetate electrophoresis in alkaline medium, which is not an efficient screening tool for neonates because of their relatively high foetal haemoglobin (HbF) level. [1, 2]

Diagnostic tools for newborn screening for haemoglobin phenotypes are not readily available; when available, they are expensive, difficult to maintain, electricity-dependent, and require significant expertise, with longer turnaround times.[3] Despite the huge burden of SCD in the sub-Saharan African region, non-communicable diseases such as SCD are given less priority, while emphasis is more on communicable diseases.[4] Early diagnosis through newborn screening (NBS) is necessary for the survival of affected children. Therefore, devices

that can achieve this purpose will be desirable in our locale. One of such is a point-of-care testing (POCT) device, HemoTypeSC™, which is a qualitative enzyme-linked immunosorbent assay (ELISA) based kit. This device works on the principle of monoclonal antibodies (mAbs), which differentiate between haemoglobin A, C and S with less than 1% cross-reactivity.[5] It also has the advantages of user friendliness, quick turnaround time and is not electricity-dependent.[6] Multicentre studies by Steel *et al*. and Nnodu *et al*. were conducted among subjects across age groups from children to adults. The studies showed sensitivity and specificity above 90%. However, a cursory look at the accuracy of this tool exclusively among the newborn population is important as this age group has the highest levels of HbF. [7, 8] Therefore, this study aimed to validate the accuracy of HemoTypeSC among the newborns seen at the Primary Health Care facilities of Ilesa, Osun State, Nigeria, using HPLC as the gold standard.

Methods

Study design

This was a descriptive, cross-sectional, community-based study.

Study location

This study was conducted at eight selected primary health centres (PHC) within Ilesa East and West Local Government Areas (LGA) of Osun State, southwest Nigeria. [9] Using data from the previous year of the study, the average number of babies immunised with Bacille Calmette–Guérin (BCG) per month were 608 and 317 for Ilesa West and East LGAs, respectively and these were used to determine the proportionate samples for the study.

The subjects were recruited consecutively at their first immunisation visits. Subjects recruited from each PHC were proportionately selected based on the number of babies patronising these facilities for immunisation; 200 subjects from Ilesa West LGA and 104 from Ilesa East LGA.

Ethical consideration

The study was approved by the Ethics and Research Committee of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife (ERC/2019/08/03). Permission for the study was also obtained from the Primary Health Care Development Agency in Ilesa East and West LGAs, Osun State. Signed written informed consents were obtained from the parents before participating in the study.

Study population, including inclusion and exclusion criteria

The study population comprised all babies presenting at the selected PHCs during the neonatal period for immunisation. Apparently healthy neonates whose parents consented to the test were included. At the same time, babies who had been transfused with blood, regardless of the time since birth, were excluded from this study, as blood transfusion with a different haemoglobin phenotype may yield a false result.

Sample size determination and sampling techniques

The minimum number (n) of babies required for the study was calculated using Leslie Fisher's formula to estimate the sample size in prevalence studies. [10]

$n = z^2 p (1-p) / d^2$

z is the critical value for a 95% confidence interval and equals 1.96.

p is the estimated prevalence of sickle cell disease among neonates in a previous study by Odunvbun *et al*. in Benin City, Nigeria, which reported a prevalence of 3.0%. [11]

d is the absolute sampling error tolerated for this study; it was set to 2.0%.

$n = \underline{1.96^2 x\ 0.03\ (1 - 0.03)}\ /0.02^2 = 279.476 \approx 280$

A total of three hundred and four apparently healthy newborns were recruited consecutively into the study.

Data collection methods

Data proforma detailing the sociodemographic information (age, sex, ethnicity, and socioeconomic class of the patients) and the place of delivery was used. Blood samples were drawn from the babies by heel-prick under aseptic conditions. One blood sample (1.5 uL) was drawn into the Hemotype SC device, and the second blood sample was drawn into the Whatman™ 903 filter card and air-dried. [12] The dried Whatman paper was placed in an air-tight nylon envelope containing desiccants and then stored in a refrigerator at 4°C for not more than seven to ten days before final processing.[11] These tissue samples were analysed using the High-Performance Liquid Chromatography machine (Bio-Rad VARIANT™ nbs) according to the manufacturer's specification.

About 1.5 µL of blood was collected onto the absorbent pad of the blood collection device, which was then inserted into a test tube containing six drops of water. The tube was swirled to ensure the blood moved from the absorbent pad of the collection device into the water. The Hemotype SC™ testing strip was inserted into the tube, and the test strip result was visually interpreted after 10 minutes. This procedure was performed and interpreted according to the manufacturer's guide, and the results of the haemoglobin phenotype were immediately documented in the proforma.[13] The HemotypeSC™ kit was the test instrument, while the HPLC was the gold standard.

Quality Assurance

The primary investigator and the research assistants were trained for a day on how to use the kit and interpret results using the manufacturer's guide. Every result was read and interpreted by the primary investigator and another trained personnel. The Bio-Rad vNBS HPLC device includes a reagent kit comprising calibrators and control samples that are run each day before analysis of test samples. The kits used were within their shelf lives and stored at room temperature. The average ambient temperature in the study area ranged from 17°C to 36°C during the study period (the kit recommended ambient temperatures are up to 40°C).

Feedback and follow-up

The mothers of babies diagnosed with sickle cell disease were contacted and counselled on the need for follow-up care at the haematology clinic of the Wesley Guild Hospital, Ilesa. Of the six infants diagnosed with the disease, three were eventually recruited into the haematology clinic of our facility.

Data management

Data was analysed using the Statistical Product and Service Solutions (SPSS) version 20.0 (IBM IL, Chicago, USA). Appropriate descriptive and inferential statistics were used to analyse variables. Tests of accuracy for HemoTypeSC™ were calculated relative to HPLC, including sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy. [14]

The following definitions were used:

A true positive (TP) result was defined as a blood sample positive for a haemoglobin phenotype using both HemoTypeSC™ and HPLC.

A true negative (TN) result was defined as a blood sample negative for a haemoglobin phenotype using both HemoTypeSC™ and HPLC.

A false negative (FN) result was defined as a blood sample negative for a haemoglobin phenotype using HemoTypeSC™ but positive using HPLC.

A false positive (FP) result was defined as a blood sample positive for a haemoglobin phenotype using HemoTypeSC™ but negative using HPLC.

Sensitivity: TP/(TP+FN)
Specificity: TN/(TN+FP)
Positive Predictive Value (PPV): TP/(TP+FP)
Negative Predictive Value (NPV): TN/(TN+FN)

Results

Three hundred and four subjects were recruited into the study, with a male-to-female ratio of 1.3:1. Their ages ranged from 2 to 672 hours, with a median (interquartile range) age of 96 hours (45.2-192.0 hours). One hundred and sixty-five (54.3%) babies were delivered in government-owned health facilities (hospitals and maternities), and 17.4% in private hospitals. In comparison, 28.3% of the babies were delivered outside formal health facilities (at homes, mission houses, and at traditional birth attendants' places). The majority (93.4%) of the babies were of Yoruba ethnicity, while the rest of the babies were from Igbo, Hausa and other minor ethnic groups.

Using the High-Performance Liquid Chromatography, the following Hb phenotypes were identified:
HbFA, HbFAS, HbFAC, HbFAD, HbFAE, HbFSC, HbFS, HbFS/β^0-thalassaemia, in 74.3%, 19.0%, 3.3%, 0.7%, 0.7%, 1.0%, 0.7%, 0.3% of the babies, respectively. The prevalence of SCD, as determined by HPLC, was 2.0%. With the HemoTypeSC™ test, the following haemoglobin phenotypes were identified: AA, AS, AC, SC, and SS in 75.6%, 18.8%, 3.3%, 1.0%, and 1.3% of babies, respectively. The prevalence of SCD using the HemoTypeSC™ test was 2.3%. The HemotypeSC did not recognise HbD and E.

Table I: Comparison of HemoTypeSC and HPLC outputs for newborn screening

HPLC	AA	AC	AS	SC	SS	AD	AE	S/β^0thal	Total
HemoType SC Hb Phenotypes									
AA	225	0	1*	0	0	2*	2*	0	230
AC	0	10	0	0	0	0	0	0	10
AS	1*	0	56	0	0	0	0	0	57
SC	0	0	0	3	0	0	0	0	3
SS	0	0	1*	0	2	0	0	1*	4
Total	**226**	**10**	**58**	**3**	**2**	**2**	**2**	**1**	**304**

**Discordant Haemoglobin Phenotype results*

Eight (2.6%) subjects had discordant results (Table I). These included one HbFA, one HbFAS, and another HbFAS, which were read as HbAS, HbSS, and HbAA, respectively, by the HemoTypeSC™ kit. Moreover, two HbFAD, two HbFAE, and one HbS/beta-thalassaemia were identified by the test kit as two HbAA, two HbAA, and HbSS, respectively.

For the haemoglobin phenotypes identified, their overall diagnostic accuracies were 98.0%, 99%, 100%, 100%, 99.3% for AA, AS, AC, SC and SS, respectively. The details of their test of accuracy are presented in Table II. For haemoglobin variant analysis, the accuracy test for haemoglobin S was above 98%, as seen in Table III.

Table II: HemoTypeSC detection analysis by haemoglobin phenotype

Hb Phenotype	Sensitivity: TP/TP+FN (%)	Specificity: TN/TN+FP (%)	PPV: TP/TP+FP (%)	NPV: TN/TN+FN (%)
AA	225/225+1	73/73+5	225/225+5	73/73+5
	99.6	**93.6**	**97.8**	**98.6**
AS	56/56+2	245/245+1	56/56+1	245/245+2
	96.6	**99.6**	**98.2**	**99.2**
AC	10/10+0	294/294+0	10/10+0	294/29+0
	100.0	**100.0**	**100.0**	**100.0**
SC	3/3+0	301/301+0	3/3+0	301/301+0
	100.0	**100.0**	**100.0**	**100.0**
SS	2/2+0	300/300+2	2/2+2	300/300+0
	100.0	**99.3**	**50.0**	**100.0**

Hb - Haemoglobin; TP - True Positive; FN - False Negative; TN - True Negative; FP - False Positive; FN – False Negative; PPV - Positive Predictive Value; NPV - Negative Predictive Value

Table III: HemoTypeSC detection analysis by haemoglobin variants

Hb variant	Sensitivity: TP/TP+FN (%)	Specificity: TN/TN+FP (%)	PPV: TP/TP+FP (%)	NPV: TN/TN+FN (%)
A	297/297+1	6/6+1	297/297+0	6/6+1
	99.7	**85.7**	**100.0**	**85.7**
S	63/63+1	239/239+1	63/63+1	239/239+1
	98.4	**99.6**	**98.4**	**99.6**
C	13/13+0	291/291+0	13/13+0	291/291+0
	100.0	**100.0**	**100.0**	**100.0**

Discussion

Sickle cell disease is the commonest genetic disease in sub-Saharan Africa. However, its features are usually asymptomatic at birth and manifest clinically at varying ages. Newborn screening for SCD with a low-cost, point-of-care testing device offers early enrolment of affected babies into structured care, and early institution of care to reduce the frequency of crises and reduce morbidities and thus mortality in these children.

In this study, the sensitivities, specificities, positive predictive values and negative predictive values of HemoTypeSC™ among newborns for the different haemoglobin phenotypes were above 90%. This shows the ability of this kit to identify specific haemoglobin phenotypes and exclude other phenotypes for which it is not intended.

Though the diagnostic accuracy of HemoTypeSC™ for HbAA phenotype was high, it should be noted that the inherent property in the

HemoTypeSC™ test falsely identifies HbD and HbE as HbA phenotype. This factor needs to be considered when using it for populations where HbD is present, such as in Ibadan, southwest Nigeria and regions with very high prevalence, such as northwest India and the same for HbE, such as countries of South East Asia, where the carrier rate may be up to 60%.[15,16]

The diagnostic accuracy of the HemoTypeSC™ for HbAC and HbSC was 100%. This was similar to the findings in the studies conducted by Nnodu *et al* [8] and Steele *et al.*[7] Since all the HbC were correctly identified, it makes the test an appropriate newborn screening tool in areas with high prevalence of HbC variants, such as Burkina Faso, Ghana, and the western part of Nigeria. [18, 19]

In this study, the overall accuracy of the HemoTypeSC™ test in detecting the HbSS phenotype was 99.3%. However, it had a lower positive predictive value (50%) compared to other parameters of diagnostic accuracy, viz., sensitivity of 100%, specificity of 99.3%, and NPV of 100. This was similar to the findings in the large-scale studies by Nnodu *et al.* [8] and Mukherjee *et al.* [18] who reported PPVs of 56.3% and 85.5%, respectively. In contrast, Steele *et al.* [7] reported a PPV of 100%. The PPV for the HbSS phenotype in this study might have been affected by the small number of subjects identified as HbSS (six babies). Therefore, newborns screened to have the HbSS phenotype using the HemoTypeSC™ test should be further tested with other methods of haemoglobin phenotype analysis to confirm the diagnosis. Moreover, in this study, the accuracy of the tests for HbC and HbS variants was greater than 98% and 100%, respectively. This further makes HemoTypeSC™ an appropriate newborn screening tool for the detection of abnormal haemoglobin variants in populations where they are prevalent.

The proportion of discordant results from this study was 2.6%, which was similar to 2.7% and 2.9% reported by Nnodu *et al.* [8] and Mukherjee *et al.*, [20] respectively. However, this was partly due to the inherent design of the HemoTypeSC™ kit, which does not identify Hb D or Hb E. Therefore, this tool might not be appropriate for populations where HbD and HbE phenotypes are common. This study was conducted at the primary health care facilities where the point-of-care testing was deployed. This was also demonstrated in the work of Nnodu *et al*., who used the immunisation programme platform to screen for sickle cell disease. [21] This is, therefore, a wake-up call to strengthen further the advocacy for newborn screening for sickle cell disease in Nigeria.

Conclusion

Due to the high diagnostic accuracy demonstrated in this study, the user-friendliness, ease of performance, quick turnaround time, and the small volume of blood required, this device can readily be deployed in low-resource settings as a screening tool for sickle cell disease in newborns.

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Abbreviations

DBS: Dried Blood Spot
FMC: Federal Medical Centre
HbF: Foetal Haemoglobin
HbFA: Foetal Haemoglobin with adult haemoglobin

HbFAS: Foetal Haemoglobin with sickle cell trait
HbFAC: Foetal Haemoglobin with adult haemoglobin and haemoglobin C
HbFAD: Foetal Haemoglobin with adult haemoglobin and haemoglobin DHbFAE: Foetal Haemoglobin with adult haemoglobin and haemoglobin E
HbFS: Foetal Haemoglobin with Sickle Cell Anaemia
HbFS/β^0-thalassaemia: Foetal Haemoglobin with Haemoglobin S and Co-existing β^0-thalassaemia
HPLC: High Performance Liquid Chromatography
LGA: Local Government Area
mAb: Monoclonal Antibodies
NBS: Newborn screening
PHC: Primary Health Care
SCA: Sickle cell anaemia
SCD: Sickle cell disease
SSA: sub-Saharan Africa
WHO: World Health Organisation

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