Assessment of a Rapid Diagnostic Tool for Sickle Cell Hemoglobin S

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ABSTRACT

Sickle cell disease an inherited common blood disorder due to the presence of the abnormal haemoglobin S. This is a prospective study done at Kenyatta National Hospital that describes a point of care method for diagnosis of HbAS, HbSS, HbSC and HbAA using the Sickle SCAN™ test. It determines the sensitivity, specificity in comparison with haemoglobin electrophoreisis.These results validate the use of sickle Scan as a low cost method for screening and diagnosing children and adults with esickle cell disease.

1.0 Introduction

Sickle cell disease an inherited blood disorder due to the presence of the abnormal haemoglobin S [1]. The sickle shaped red blood cells have difficulty passing through small blood vessels, blocking the normal blood flow, damaging tissues, and ultimately leading to many of the manifestations of Sickle cell disease. [2] Additionally, red cells containing mostly hemoglobin S have markedly reduced life span only about 16days compared to 120 days for normal red blood cells [3]

There are several types of Sickle cells conditions with the most common being sickle cell haemoglobin Disease (HbSS), Sickle cell haemoglobin C Disease (HbSC),Sickle cell haemoglobin F (HbSF) and sickle Cell Trait (HbAS). Early diagnosis of Sickle cell Disease is important to initiate prompt management including maintenance therapies such as penicillin prophylaxis, vaccination against pneumococcus bacteria, folic acid supplementation, pain management mediations, blood transfusions and hydroxyl urea[3,4] .

The Hb AS sickle cell trait is not categorized as a type of disease, however, there are ensuing complications in extreme environment such increased atmospheric, high attitudes, low oxygen levels intense athletic competitions, or dehydration) [5].Carriers of Sickle cell trait should be identified to enable caution applied as well as genetic counseling[6].Children under 5 years of age are at increased risk of death from infections and other life-threatening complications of SCA, most of which can be prevented through highly effective and inexpensive prophylaxis [7]

Sickle Cell Anemia(SCA) is normally diagnosed by measuring the HbS content (%HbS) in blood of patients with high-performance liquid chromatography (HPLC), hemoglobin electrophoresis (HE) or isoelectric focusing (IEF) [8]. Universal screening of newborns in the United States and many other developed countries using these highly accurate laboratory methods has enabled early intervention and treatment of SCA, which have significantly reduced SCA-related early childhood mortality and have contributed to the overall improvement in the quality of life and life-span of adults with SCA [9].

1.1 Literature review

Sickle cell disease affects 20-25million of people globally of which 12-15 million live in Africa. The natural distribution covers a broad belt, including the Mediterranean, western, parts of East and central Africa, the Middle East, India and SE Asia .It is estimated that 75-85% are children born in Africa where 50-80% of children born with the disease die before the age of 5 years. In Sub-Saharan Africa, approximately 240,000 children are born with sickle cell. In Kenya, the prevalence varies in regions.

The techniques for the differential diagnosis of a potential haemoglobinopathies are well-established. A clinical history from the patient, blood count and a blood film are essential and haemoglobin electrophoresis should also be mandatory in the initial work-up. Structuraly abnormal haemoglobins with altered charge will be detected by electrophoresis; abnormal haemoglobins with a similar electrophoretic migration to HbA may still be suspected if they are functional consequences e.g. abnormal oxygen affinity or unstable haemoglobins[13]. Several different haemoglobinopathies may be present in the same population due to interactions of several different disorders

1.2 Haemoglobin analysis

1.2.1 Starch gel electrophoresis

Haemoglobin electrophoresis is the main method for the detection of haemoglobin variants. Electrophoretic mobility alone cannot conclusively identify a particular abnormal haemoglobin, but if such information is combined with the

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clinical characteristics and the racial background of the patient it may well lead to an adequate presumptive diagnosis [15].

Electrophoresis is normally carried out at alkaline pH, using one of a number of support media. For large-scale screening purposes, cellulose acetate membranes are probably the most convenient, while many specialist laboratories have retained starch gel as the medium because the combination of its resolving power and larger sample volume improves the detection of minor components.

1.2.2 Cellulose Acetate membrane haemoglobin electrophoresis

This applies the principle that separation of different haemoglobins depends mainly on the charge on the haemoglobin molecule at alkaline pH. This technique enables separation of Hbs; A, F, S and C and useful for screen for sickle haemoglobin and other common haemoglobin variants[16]

1.2.3 Citrate Agar Hemoglobin electrophoresis

The separation of different haemoglobins depends partly on the charge on the haemoglobin molecule at acid pH and partly on its solubility in agar. Citrate agar electrophoresis is used to separate HbS from HbD and HbC from HbE. It also provides clear separation between HbA and HbF.

1.2.4. Metabisulphite - Sickling test

Homozygous sickle cell anaemia can usually be diagnosed from the clinical and haematological picture including changes in red cell morphology. However changes occur in the heterozygotes and the presence of an electrophoretic variant in the position of HbS is not conclusive. The presence of HbS can be confirmed either by a test based on its reduced solubility relative to other haemoglobins or by demonstrating the induction of sickle cell formation when whole blood is deoxygenated. This test preferably be carried out on fresh blood samples; a normal control should be indicated.

1.2.5 Sickle cell solubility test

Sickle cell tests depend on the decreased solubility HbS in conditions of reduced oxygen concentration. The mixture of HbS in a reducing solution will give a turbid appearance, whereas haemoglobins with normal solubility will give a clear solution [18]. It is important that both negative and positive controls are indicated with each test are later checked using haemoglobin electrophoresis.

1.3. Objectives

1.3.1 General

To determine the detection of Haemoglobin S using Sickle Cell Scan.

1.3.2 Specific Objectives

.Determine the HbAS,Hb SS, HbSF and the Known HbAA and to correlate age, gender, geographic origin and ethnic/tribal origin of those tested.

2.0 Materials and methods

This was a prospective cross-sectional study. Patients were consecutively recruited in consulting wards and haematology clinics.The Study was conducted in Kenyatta National Hospital haematology clinic and laboratory. Haematology clinic is a specialist clinic held once a week .On the average there are between 20-30 Sickle cell patients attended to every clinic. The haematology laboratory has a dedicated subsection for haemoglobin analysis. This section is equipped with tools and competent staff to analyse for sickle cell using Peripheral blood film, Sickling test and

haemoglobin electrophoresis using Cellulose acetate paper (CAPE) electrophoresis.

The sample size was achieved through convenient sampling method; the minimum recommended for this (validation and comparative studies R) is 50.The inclusion criteria was clinical diagnosis of sickle cell with results of haemoglobin electrophoresis showing; HbAS, HbSS or HbSF or a Positive sickling test. Those who did not consent or assent, had no definite laboratory and clinical diagnostic records and also patients with other known causes of haemolytic anemias were excluded

2.1 Clinical Methods

The participants included all the patients seen at the KNH recruited consecutively till the number 50 is attained. Permission to conduct the study was sought from Kenyatta national hospital and university of Nairobi scientific and ethical review committee. Patient records were identified by the diagnoses that appear in the hospital's records. The patients selected were those whose records were bearing sickle cell diagnosis as disease, with HbSS , other states HbAS , HbAC or HbSF by haemoglobin electrophoresis performed in a standard horizontal tank (Kohn Model U77, Shandon Southern Instruments Ltd., Camberley, Surrey, UK) on 78 mm X 150 mm cellulose acetate membranes (Shandon Celagram), with a Vokam power pack (Shandon Southern). A form and an instruction manual developed was used as a tool to guide extraction of information on the patients and their admissions from the patient records. Demographic covariates included age, gender, ethnicity and geographical are of birth. The independent clinical covariates evaluated were Hb phenotype, diagnosis of clinical sickle cell manifests such as; event, crisis, syndrome and number of packed red blood cell transfusions

2.2 The design of the rapid diagnostic tool

The sickle scan designated, The Sickle SCAN™ test (BioMedomics, Inc., Durham, NC, USA), uses a lateral flow qualitative immunoassay to identify abnormalities in haemoglobin A, S and C. The test specifically confirms the presence of HbAS, HbSS, HbSS, HbSC, HbSβ^0, and HbSβ^+genotypes.It comes as a kit with immunoassay, a capillary sampler and a diluted pretreatment buffer.

2.3 Specimen collection, preparation and analysis

The participants were identified and subjected to the sickle scan test following the manufacturer's guidelines. The results were interpreted accordingly as per the purposes of this study. Specimen was obtained using finger stick, heel stick, or venipuncture whole blood samples for those who needed other routine tests. The manufacturer's instructions were adhered to in performing the test. The Sickle SCAN limit of detection for haemoglobins A, S, and C is determined to be 40%, 1%, and 2% respectively. The display of results was expected on a total of four possible detection lines, with the control (Ctrl) line appearing after sample has diffused through the cartridge. The presence of haemoglobin variants A, S and C greater than the limit-of-detection was indicated by the presence of a blue line in that region

The blood counts were performed by standard method at the laboratory. Leucocyte counts corrected for the presence of nucleated red blood cells. Reticulocyte count performed by mixing blood with supravital stain and manual counting in blood slide. The investigators looked at all blood films, red blood cell morphology and WBC differential count results as required for continuous care of the patient.

The results were recorded against the previous haemoglobin electrophoresis obtained by using Cellulose acetate paper (CAPE) electrophoresis method.

2.4. Quality assurance

The study was conducted by well trained personnel during recruitment, specimen handling and analysis of the results. Each Sickle SCAN™ test device has a built-in control. It is of note that performance of Sickle scan has not been established for sickle cell patients with beta-thalassemia; however those with other abnormal haemoglobins were excluded during clinical screening. The control used was for a known case of hereditary elliptocytosis to confirm negative results for other congenital haemolytic disorders.

2.5. Statistical analysis

Data was entered in to a computer database and transferred to the Statistical Package for Social Sciences (SPSS) software version 17 for analysis. The sensitivity, specificity and accuracy of the Sickle scan test were calculated using CAPE hemoglobin electrophoresis as the gold standard. Positive predictive values (PPV) and negative predictive values (NPV) of each method were assessed and the correlation was done by kappa statistics, the concordance by use of chi-square.

3.0. Results

During the study period between August 2016 and April 2017, fifty participants whose results had been obtained by haemoglobin electrophoresis were evaluated and blood samples analyzed with Sickle SCAN™ .Haemoglobin electrophoresis (CAPE Shandon southern) was the gold standard . The age of the participants ranged from 1year to 43years with a median age of 8 years and interquartile range 3.5-12 years. The females were 58% (29/50) and the male 42 %(21/50). In terms of geographical distribution ,26.5% were from Siaya,18.4% Kisumu, 16.3%Kakamega , 12.2% Busia and Kisii **Siz**

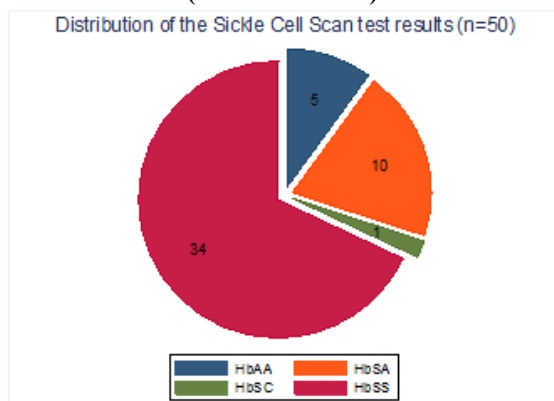**Figure 1. The display of results on Sickle scan (Sickle SCAN™).**

Figure 2. The proportion of the various haemoglobins detected.

The representation of haemoglobin types were HbSS ,HbSA ,HbAC and HbAS. Out of the 50 patients screened, 34 (68%) were found to have sickle disease, 10 (20%) were sickle cell trait carriers, 1(2%) had HbSC and only 5 (10%) had normal hemoglobin (HbAS). These Sickle cell scan results were 100% similar to the test results obtained using the electrophoresis analyzer

Table 1. The Haemoglobin phenotypes identified by the different methods.

Phenotype	Sickle SCAN™ results	Cape-Electrophoresis
HbSS	35	35
HbAS	10	10
HbSC	1	1
HbAC	1	1
HbAA(control samples of patient with hereditary eliptocytosis)	3	3
Total	50	50

The performance of Sickle scan against the CAPE electrophoresis was evaluated using Cramer's V test to assess the level of agreement between the two tests in detection of haemoglobin S.

Table 2. Comparison of cape electrophoresis results and sickle scan test.

		Electrophoresis analyser			
		HbAA	HbAS	HbSC	HbSS
Sickle scan kit	HbAA	5	0	0	0
	HbAS	0	10	0	0
	HbSC	0	0	1	0
	HbSS	0	0	0	34

The test showed 100% agreement (Cramer's V=1.000)

4.0 Discussion and conclusions

The aim of this study was to assess the utility of sickle scan in the detection of abnormal haemoglobin S. It is capable of distinguishing HbSS, HbAS and HbSC. The comparison of the test with results of haemoglobin electrophoresis shows 100% agreement with Cramer's V test. Sickle SCAN™ performance compared to haemoglobin electrophoresis based diagnosis in the initial validation at the developmental stage shows a sensitivity>99% and specificity>99%.The device uses lateral flow immunoassay technology which displays results within five minutes that is interpreted visually. It is simple, rapid and does not require technicalities.The documented potential uses of sickle scan include newborn screening for early diagnosis, sickle trait screening for informed parenteral planning and rigorous blood screening for donors in blood banks[19] .

The hemoglobin HbAS reflects carrier state. However, patients transfused within 60days display HbAS and that is important consideration while using this device as a primary tool for screening and diagnosis. Therefore, the transfusions history is important for proper interpretation of the results[20].

The most common clinically symptomatic haemoglobin abnormality in this study was haemoglobin S. These results reflect the various scenarios in the clinical set up for informed decisions. The current management challenges in sickle cell disease include early diagnosis, screening and management. Early diagnosis is appropriate for timely intervention in aspects of prevention and management in accordance to the guidelines. Given the geographical distribution of the participants who largely come from the rural areas, large scale screening using the conventional methods is impractical

because of high costs involved in the infrastructure and access[21].

The way forward is developing a comprehensive sickle cell program that incorporates early screening and intervention. The drawback has been inaccessible laboratory diagnosis. The documented reliability of the results using this sickle SCAN test for early screening is after five weeks of birth[22].For screening purposes in the endemic regions, this should be incorporated in children immunization schedules at four months so that the affected can be recruited in a comprehensive sickle cell program.There is no reliable data to reflect on the burden of the disease in Kenya because of inaccessible diagnostic services. Future work to be done includes documentation to create a sickle cell registry for reliable database to ascertain the disease burden for future planning and management.

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