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VALIDITY OF THE SICKLING TEST & PERIPHERAL BLOOD SMEAR METHOD IN CENTRAL INDIA'S TRIBAL POPULATION FOR SICKLE CELL DISEASE SCREENING

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Abstract

Background: One of the most prominent hereditary monogenic diseases is sickle cell disease, responsible for around 70 % of the major hemoglobinopathies in the world. The sickle cell genes are primarily seen in India's tribal population, many of whom live in remote hilly areas. In developing countries like India, there are affordable methods of varying reliability, ease of application & affordable costs for screening for Sickle Cell Disease. Solubility & sickling test & peripheral smear of blood include these.

Materials & Methods: The sickling test was performed using the meta-bisulphate sodium method. Light microscopy (x100) examined thin blood smears, stained with Giemsa stain. Hb electrophoresis was performed at pH 9.2 using the cellulose acetate membrane method.

Results: The sickling test had 75.34 percent sensitivity & 88.25 percent specificity. It had 68.58 percent & 91.37 percent positive & negative predictive values, respectively. With a kappa score of 0.68, the diagnostic precision of the sickling test was 85 per cent.

Conclusion: For Sickle Cell Disease patients in low resource settings & using Hb electrophoresis as a confirmatory tool, the Sickling test will therefore be the most recommended test for screening.

Keywords: HB electrophoresis, Peripheral blood smear (PBS), Sickling test, tribal population.

Introduction

One of the most prominent hereditary monogenic diseases is sickle cell disease, responsible for around 70% of the major hemoglobinopathies in the world. HbS is an uncommon form of normal adult HbA, inherited as an autosomal mendalian recessive feature. Heterozygous people (AS) are typically asymptomatic & homozygous (SS) people with acute & chronic problems suffering from Sickle Cell Disease[1,2]. In low & middle income countries, the vast majority of newborns with sickle cell anemia occur, with approximately 2.5 lakh births per year in African countries & 30 thousand per year in South-East Asian countries. Most of those affected, die within the first few years of life without early diagnosis & treatment. It has been estimated that 44 thousand children are born every year with sickle cell anemia in India, which is the third highest birth rate in the world after Nigeria & Congo, with recorded excess mortality reaching up to 92 percent[3,4]. The sickle cell genes are primarily seen in the Indian tribal population, many of which live in rural, hilly regions[5]. In high-resource nations, however, advances in early diagnosis & improved preventive care treatment have led to a significant change in childhood survival & a rise in the average life span of individuals affected. In some developed countries, methods like Hb electrophoresis, iso-electric focusing, high performance liquid chromatography & polymerase chain reaction are used to test for hemoglobinopathies[6,7]. In resource-constrained countries, such as India, this is impractical because of the high cost. However, in developed countries, there are other affordable methods of varying effectiveness, ease of use & affordable costs for screening for Sickle Cell Disease[8,9]. This include the procedure of solubility & sickling test & PBS. Therefore, the sensitivity, accuracy, positive predictive value, & ease of implementation of the sickling test & PBS method were evaluated in this reasearch.

Materials & Methods

This was a cross sectional, laboratory-based research carried out for a period of two years in the Department of Pathology of a tertiary healthcare centre in Tribal region of Central India. 300 samples from the age group of 0 to 40 years is chosen. Using the sickling test & peripheral blood smear procedure, the samples were examined. The statistical approach adapted for research was the odds ratio & Cohen's kappa score value was the significance standard. Sickling experiments are conducted using meta-bisulphate sodium.

Results

Among the 300 blood samples used in this analysis, 216 samples of HbAA were detected by Hb electrophoresis. 76 HbAS, 8 HbSS. By the sickling test form, out of 300 blood samples, 78 cases are positive (Table-1).

Table 1: Comparing tests

Variable	Sickling test	PBS	Hb electrophoresis
True-negative	202	206	216
False-positive	28	22	0
True-positive	50	38	84
False-negative	20	34	0

Notably, the sickling test had 75.34 percent sensitivity & 88.25 percent specificity. It had positive & negative predictive values of 68.58 percent & 91.37 percent. With a kappa score of 0.68, the degree agreement (diagnostic accuracy) between the sickling process & the gold standard Hb Electrophoresis was 85 percent (Table-2).

Table 2: Sickling test Vs Hb Electrophoresis

Parameter	Estimate
Specificity	88.25 %
Sensitivity	75.34 %
Negative Predictive Value	91.37 %
Positive Predictive Value	68.58 %
Likelihood ratio of a negative test	0.54
Likelihood ratio of a positive test	6.29
Odds ratio	23.18
Diagnostic accuracy	85 %
Cohen's kappa value	0.68

By the PBS process, out of 300 blood tests, 60 cases are positive. Notably, the sickling test had 57.45 percent sensitivity & 91.14 percent specificity. It had positive & negative predictive values of 68.38 percent & 86.88 percent. The sickling process & gold standard Hb Electrophoresis have level agreement (diagnostic accuracy) was 82.14 percent with a kappa score of 0.566 percent (Table-3).

Table 3: Peripheral blood smear method Vs Hb Electrophoresis

Parameter	Estimate
Specificity	91.14 %
Sensitivity	57.45 %
Negative Predictive Value	86.88 %
Positive Predictive Value	68.38 %
Likelihood ratio of a negative test	0.23
Likelihood ratio of a positive test	6.24
Odds ratio	14.14
Diagnostic accuracy	82.14 %
Cohen's kappa value	0.56

Discussion

SCD is a disease that can develop in several different forms and at different stages of childhood. The severity of the disease varies from moderate chronic anaemia to serious acute infections or stroke[10,11]. Because of its heterogeneous existence, healthcare professionals should maintain a high degree of suspicion of prompt testing in areas where no national screening programmes exist. It is a critical health condition in the Indian Subcontinent leading to elevated mortality and morbidity[12]. To reduce SCD mortality and improve the quality of life of patients, educating health care workers and caregivers, reliable point-of-care testing and prophylactic measures together with patient follow-up are all necessary[13,14]. A tetramer consisting of two alpha-like and two beta-like globin chains is hemoglobin (Hb), the abundant oxygen-carrying protein found within red blood cells (RBCs)[15]. Hemoglobinopathies are a diverse category of inherited blood disorders in which a difference in the number, structure, or function of one or more globin chains results in one or more genetic abnormalities. Thalassemias (alpha- and beta-) and sickle cell disease (SCD) are among the most common hemoglobinopathies, however hundreds of globin gene abnormalities have been identified[16]. A good understanding of the genetics and structure of the globin chains and Hb is necessary to diagnose hemoglobinopathies. In order to inform optimal management in affected individuals and to give genetic counseling and reproductive options in carriers, timely and accurate diagnosis of hemoglobinopathies is critical. Three hundred blood samples from the age group of 0 to 40 years were taken in our study, & the male to female ratio was 2.38: 1. In other previous researchs, similar results have been shown[17]. Shrestha A et al[4] described the maximum number of males reported in the 11-20 age group, while the maximum number of females reported in the 0-10 age group. The maximum number of males & females in our sample was 11 - 20 years of age. Our study's sickling test & peripheral blood smear system statistical values were associated with A. L okwai et al[10], respectively. The Sickling test was found to be the most accurate for HbAs identification, although some false positive cases have occurred. Anemia was possibly due to false positive sickling test cases, based on the fact that 22 out of 26 samples that tested false positive had a haemoglobin concentration below 10 gm/dl. The analysis of A.L Okawi et al, Schieninder et al. was in line with this result. In our research, the peripheral blood smear procedure had 22 false positive cases. Because of mechanical artifacts that can be created by high or low temperatures & prolonged drying artifacts, unusual tensile forces during smear preparation, false positive cases were typical in the peripheral blood smear process[18]. True drepanocytes are typically not present in patients with less than 50 percent HbS, so the morphology of RBCs in patients with Sickle Cell Trait (SCT) is reportedly common with few abnormal RBCs. In the detection of previously undiagnosed (sickle cell trait) SCT patients, irregular RBCs support. If patients with sickle cells were under physiological stress, the abnormal RBCs could undergo more sickling & form true drepanocytes. In order to resolve the burden of Sickle Cell Disease among children in backward nations, foreign health care providers & health care agencies face numerous challenges. Focus should be put on diagnostic procedures such as neonatal screening & anti-microbial prophylaxis to spearhead the process[19]. Efforts should also be made to boost morbidity & quality of life, as there are accessible services available in low-income countries. In the past decade, several developments have taken place to develop care options for Sickle Cell Disease. Most of these procedures, however, are costly & reliant on medical

facilities & only a few Sickle Cell Disease patients are able to afford therapies such as gene therapy & transplantation of hematopoietic stem cells. In order to successfully enforce population screening, active initiatives & partnerships need to be deployed on the part of the government. Additionally, to determine current & potential health needs, therapy services & epidemiological studies in Sickle Cell Disease affected countries are important.

Conclusion

The most accurate, cheapest & simplest to conduct was the sickling test. It had a score of high specificity, sensitivity & kappa. High specificity, low sensitivity & high (turn around time) TAT were present in the peripheral smear process. Sickling will therefore be the most suggested screening procedure for Sickle Cell Disease patients in low resource settings & as a confirmatory tool using Hb electrophoresis. The adequate use of testing to detect abnormal hemoglobin, along with the requisite knowledge on the effects of these findings, is both a vulnerability & an advantage for our entire health care system.

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