

Evaluation of Hb α RBC product as a screening method in sickle cell Anemia

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Abstract

Background: India has been ranked the second worst affected country in terms of predicted SCD births. In developed countries, new-born screening programs have been established to ensure early diagnosis, but in most developing countries, screening is not performed, and the diagnosis is often delayed. Aim of present study was to analyse the effectiveness of Hb α RBC product as a screening tool. **Methods:** In the present study, blood from 200 patients suspected to have Sickle cell hemoglobinopathies was analysed and subjected it to Sickling screening test. All positive cases were subjected to HPLC to separate constituent haemoglobins and CBC analysis was done to check RBC indices. Hb α RBC product was analysed. **Results:** Analysis of Hb α RBC product revealed that there was a statistically significant difference in values between controls and disease patients. Hb α RBC product had good sensitivity and specificity in predicting sickle cell disease. **Conclusions:** Hb α RBC<45 was sensitive and specific predictor of sickle cell disease and can be used as a screening tool. Patients with this finding should be further subjected to specific tests like Hb electrophoresis for confirmation.

Keywords: Sickle cell anemia, Hb α RBC, Sickle cell disease

Introduction

Sickle cell disease (SCD) is an autosomal recessive disorder [1] resulting from single base A>T mutation in the triplet encoding the sixth residue of the β -globin chain, leading to a substitution of valine for glutamic acid and the abnormal haemoglobin S (HbS). The disease is expressed when Haemoglobin S (HbSS) is inherited from both parents, the homozygous patient or HbSS suffers from sickle cell anaemia, while the heterozygous child or Haemoglobin AS (HbAS) is a carrier of a sickle cell trait [2]. Individuals with SCD experience considerable morbidity. Without effective treatment, the most severe cases can be fatal within the first few years of life.

The primary pathophysiology is based on formation of long fibres within the Red Blood Cells (RBCs) causing a distorted sickle shape which eventually leads to haemolysis and vaso-occlusion. There are several events that may trigger vaso-occlusion. Recent work has shown the importance of red cell dehydration, abnormal adhesion of RBCs to the vascular endothelium, inflammatory events,

and activation of all the cells in the vessel and abnormalities of nitric oxide metabolism in the pathophysiology of this multi-organ disease. [3]

India has been ranked the second worst affected country in terms of predicted SCD births [4]. In India, SCD is distributed geographically in the central and western regions [5,6]. As per an Indian Council of Medical Research survey, about 20% of children with SCD expired by age of two and 30% of children with SCD among the tribal community die before they reach adulthood [7,8]. Particularly in Gujarat, studies indicate a higher percentage of Sickle cell disease prevalence versus sickle cell trait [9].

In developed countries, new-born screening programs have been established to ensure early diagnosis, but in most developing countries, screening is not performed, and the diagnosis is often delayed. Aim of present study was to analyse the effectiveness of Hb α RBC product [10] as a screening tool.

Methods

Setting- This study was done in a tertiary care hospital in Western Indian city of Vadodara, Gujarat on an outpatient basis.

Duration- Study was carried out for a period of two years from 2017 to 2018.

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Sampling method and size- The study population consisted primarily of tribals from Central Gujarat and southern Madhya Pradesh. Venous blood (2ML) was collected in EDTA bulb under aseptic conditions from 200 patients suspected of having sickle cell hemoglobinopathies (i.e. patients suffering from anemia, joint pains, weakness, abdominal pain etc.).

Inclusion and exclusion criteria- 2ml venous blood in EDTA bulb was subjected to sickling test. 151 patients showed sickling test positive and were selected as cases. Also, blood was collected from the 40 normal healthy individuals and subjected it to the same tests as above. They were sickling test negative and were selected as controls. Cases and controls further underwent a CBC examination and HPLC to separate constituent haemoglobins.

The following criterial were used to identify hemoglobinopathies on HPLC patterns [10] (Table 1).

Table-1 Criteria used for differentiating HPLC patterns

Hemoglobin	Disease
A>S	Sickle cell trait, sickle alpha-thalassemia
S, F and no A	Sickle cell disease, Sickle-beta thalassemia
S> A and F	Sickle – beta thalassemia
A>C	HbC trait
C, F and no A	HbC disease, HbC-beta thalassemia
C>A	HbC – beta thalassemia

49 patients who were negative by sickling test were excluded from the study.

Data collection procedure- Data was collected from patients case files from medical records section.

Data analysis- Continuous variables were presented as mean \pm 2SD. Continuous variables were compared between cases and controls by performing unpaired t test. Categorical variables were compared by performing chi square statistics. P<0.005 was statistically significant. Microsoft excel and GraphPad calculator was used for data analysis.

Ethical consideration & permission- All ethical considerations and permissions were obtained from concerned authorities.

Results

Out of 200 patients suspected of having sickle cell anemia, 151 tested positive by sickling test. Analysis of HPLC patterns revealed the following findings. 58 patients were having sickle cell trait (AS) (38.9%), 83 showed sickle cell disease (SS) (55.7%), 2 were diagnosed to have HbS due to recent transfusion and 8 were sickle beta thalassemia (S β) (5.3%). The latter two categories were not included in the present study.

Both sickle cell trait and sickle cell disease patients had significantly lower levels of Hb as compared with controls. Analysis of Hb in cases versus controls is shown in Table 2.

Table-2: Hb in sickle cell anemia patients versus controls

	N	Mean	SD	pvalue	significance
Control	40.0	13.3	1.4		
SCT	58.0	11.0	4.8	0.0049	yes
SCD	83.0	8.0	1.8	<0.0001	yes

The difference in RBC counts was not statistically significant between controls and both sickle cell trait and sickle cell disease patients. Analysis of RBC count in cases versus controls is shown in Table 3.

Table-3: RBC count in sickle cell anemia patients versus controls

	N	Mean	SD	P value	significance
Control	40	3.67	0.54		
SCT	58	4.19	1.29	0.0181	no
SCD	83	3.29	0.98	0.0239	no

Analysis of Hb*RBC product revealed that difference in Hb*RBC product is statistically significant between controls and sickle cell disease patients. It was not significant in sickle cell trait patients. Details are shown in Table 4.

Table-4: Hb*RBC count in sickle cell anemia patients versus controls

	N	Mean	SD	P value	significance
Control	40	37.98	11.12		
SCT	58	34.74	32.48	0.5459	No
SCD	83	21.77	16.04	0.0001	Yes

Discussion

Sensitivity and specificity of Hb*RBC <45 was analysed to determine whether it can be used as a screening parameter in patients for patients of sickle cell disease. It was observed that it has 93.9% sensitivity and 95% specificity as a screening parameter. SCD is a major public health concern that has great impact on both individuals and society. Mortality associated with this disease is high despite knowledge of the pathophysiology [11,12]. and treatment of the various forms of crisis. Mortality from the disease is highest in the first 5 years of life, with approximately 50% of deaths occurring in the second 6 months of life [13-17].

For most affected children, the parents are usually unaware of the presence of the [13]. In developed countries, newborn screening programs have been established to ensure early diagnosis and thus early enrolment into a comprehensive healthcare program [18-23], but this screening is not performed in India and in most developing countries. If newborn screening programs are started, it is estimated that 85% children with SCD will survive upto 20 years of age with prophylactic penicillin administration [24]. In order to find a suitable and cheap screening method for study population, levels of hemoglobin, RBC count and Hb*RBC product were analysed as a suitable alternative.

Both sickle cell trait and sickle cell disease patients had significantly lower levels of Hb as compared with controls. This correlates with various studies done by Walke et al and Chikhlikar et al [25,26]. The difference in RBC counts was not statistically significant in the present study between controls and both sickle cell trait and sickle cell disease patients. This is in contrast with studies by Chikhlikar et al [26], Pathak et al [27], and Yasmin et al [3]. Analysis of Hb*RBC product revealed that difference in values for controls and trait patients was not statistically significant. There was a statistically significant difference between controls and disease patients. Hb*RBC product had good sensitivity and specificity in predicting sickle cell disease. This was in concordance with study conducted by Valavi et. al [10]. The limitation of this study was simultaneous iron deficiency anemia in many of current patients. Studies on large population would be helpful to support the present observations.

Conclusion

Hb*RBC<45 was sensitive and specific predictor of sickle cell disease and can be used as a screening tool. Patients with this finding should be further subjected to more specific tests like Hb electrophoresis for confirmation.

What this study adds to existing knowledge?

From the present study it can be proposed that a simple CBC test should be done in all newborns. Analysis of their Hb*RBC product can help in knowing the sickle cell disease status and further testing can be initiated in suspected cases. This can prevent long term mortality and morbidity and increase overall survival.

Author's contributions

Dr. Ashish Jawarkar: Data collection and assisting with writing of this manuscript.

Dr. Aakanksha Agrawal: Data analysis and manuscript preparation.

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