PREVALENCE OF SICKLE CELL ANEMIA IN YOUTH BY COST EFFECTIVE STRATEGY ALONG WITH HPLC

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Abstract

This study aimed to determine the prevalence of sickle cell disease among students of Anand People's Medicare Society (APMS) using cost-effective screening methods and confirming the results with high-performance liquid chromatography (HPLC). A total of 395 students from Anand, Gujarat, were screened using the sickling test and solubility test as primary screening methods. Haemoglobinopathies carriers were diagnosed and confirmed using HPLC at the Indian Red Cross Society, State Branch in Ahmedabad. Complete hemogram analysis was performed, and the results were tabulated and analyzed. The results indicated that HPLC exhibited 100% sensitivity, specificity, and accuracy, while the sickling test demonstrated a sensitivity of 66.66%, specificity of 97.90%, and accuracy of 97.21%. The solubility test showed a sensitivity of 53.84%, specificity of 96.85%, and accuracy of 95.44%. HPLC had a 100% positive predictive value (PPV) and negative predictive value (NPV), whereas the sickling test had a PPV of 55.55% and NPV of 99.20%. The solubility test yielded a PPV of 36.84% and NPV of 98.40%. In conclusion, the sickling test and solubility test are cost-effective and easily conducted, with the sickling test providing more reliable results with higher sensitivity and specificity compared to the solubility test. HPLC is considered the gold standard test for screening.

Keywords: Haemoglobinopathies, HPLC, Sickle cell, Sickling test, Solubility test

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Introduction

Abnormal hemoglobin is produced from defective HB genes, which cause anemia and leads to conditions called as "Hemoglobinopathies" (Bajaj and Gupta, 2020). Hemoglobinopathies are groups of genetic blood disorders, it changes structure or synthesis of hemoglobin. Hemoglobinopathies causes many public health issues around the globe. Mainly in the Mediterranean area, in the Middle East and in part of India, Africa and Southeast Asia (Rajput *et al*., 2015).

Sickle cell anemia, thalassaemias and other hemoglobinopathies together are responsible for the largest number of genetic diseases. These genetic diseases are controlled by a single gene that is transmitted from parents to off spring from one generation to another (Datar *et al*., 2015). Around the world about 5% of population carries trait genes for hemoglobin disorders, mainly sickle cell disease and thalassemia (World Health Organization, 2021).

Red blood cell contains major protein which is hemoglobin. Normal major adult hemoglobin is made from 2α and 2β globin chains (Mangla *et al*., 2021). Hemoglobin binds to oxygen and carbon dioxide, then transports it in the body. Abnormal hemoglobin seen in two basic conditions, either decreased production of one of the globin chains e.g., thalassemia or abnormal globin chain e.g., sickle cell diseases (Bajaj and Gupta, 2020).

The sickle cell mutation occurs when negatively charged glutamine at the sixth position of the β-globin chain is replaced by a neutral valine (Mangla *et al*., 2021). The term "sickle cell anemia" should not be used now a day according to international nomenclature because major aspects are vascular obliterations and the organ damage. Sickle cell disease contains all of abnormal hemoglobin S level variations the proportion of hemoglobin S is more than 50%. Sickle cell disease have two conditions these contain Homozygous sickle cell disease (HbSS) and a range of mixed heterozygous hemoglobinopathies (HbS/β-Thalassemia, HbSC disease and other combinations) (Kohne *et al*., 2011).

In the Homozygous (HbSS) condition "sickling" of red blood cell occurs when the hemoglobin S molecules clumps together and from rigid fibers (Rajput *et al*., 2015). Sickle cell disease is one of the most prevalent severe monogenic disorders worldwide. Sickle cell disease is a multisystem disease linked with episodes of acute illness and progressive organ damage (Rees *et al*., 2010).

Among all Hemoglobinopathies most Fatal one is hemoglobin S. Due to lack of oxygen red blood cell changes it shape to sickle cell and it causes vascular obliterations, so infarctions with tissue death can occur in all organ. In severe anemia with viral infections, aplastic crises are seen. Chronic hemolytic anemia can usually be well tolerated. Life-span of patients can be better to 50-60 years if treated well (Kohne *et al*., 2011).

There is limited data available on epidemiology of sickle cell disease, according to data it is more common in sub-Saharan Africa, people who are carrier of HbAS has natural protection against severe plasmodium falciparum malaria (Mangla *et al*., 2021).

Best treatment available is bone marrow transplantation, but it is costly. So, prevention is the best and cheapest strategy we have, so population screening, genetic counseling and prenatal diagnosis is very important (Patel *et al*., 2012).

The prevalence of HbS is high in tribal and non-tribal population where, Pre-marriage, Antenatal, Preconception, Preoperative, New born screening is essential (Ghosh *et al*., 2014). Around 30cr people have sickle cell trait and approximately 64 lakh people have sickle cell disease around the world. Every year 3 lakh children are born with sickle cell disease. Now sickle cell disease is having an impact on other areas of world such as north and south America and Europe due to migration. Some studies show number of children born with sickle cell disease is expected to grow by 30% from 2010 to 2050 (Novartis AG, 2021).

The presence of HbS can be detect by sickling test and hemoglobin solubility test it is phenotypic screening techniques not 100% sensitive and specific and cannot identify exact phenotype/genotype. To detect phenotype of sickle cell disease Hb electrophoresis is used but it has limitation should not be performed on infants until at least 6-month-old or recipients of allogeneic blood transfusion. These primary screening tests are not enough to make proper diagnosis (Rajput *et al*., 2015). HPLC is used routinely now days, Isoelectric focusing, Genetic testing are also being used. Innovative techniques are also available (Arishi *et al*., 2021). In this study diagnosis is done by sickling test and then conformed by HPLC.

Materials and Methods

This study was carried out at the department of paramedical, Anand People's Medicare Society, Anand Gujarat by the Indian red cross society, Ahmedabad, Gujarat. A total 395 students were participated. The aim of the study was explained to the students. The inform consent form was given to

the students. which included the socio-demographic information about the students, history of anemia, history of blood transfusion and other relevant history.

The basic knowledge of sickle cell disease, thalassemia and other hemoglobinopathies was provided through counselling, IEC (Information by Electronics and Communication), material supplied by Indian Red Cross society Gujarat Branch and the posters and video CD containing information of sickle cell disease, thalassemia and hemoglobinopathies was also presented. An informed consent was taken.

For Analysis of samples sickling test and solubility test are used for primary screening. For sickling test a blood drop is mixed with chemical reducing agents, such as sodium metabisulfite in equal amount and sealed between a cover slip and a slide, the decline in oxygen tension due to oxidative processes in the blood cells. This rapidly reduces oxyhemoglobin to reduced hemoglobin, then this will be accelerated sickling (Figure 1 and Figure 2).

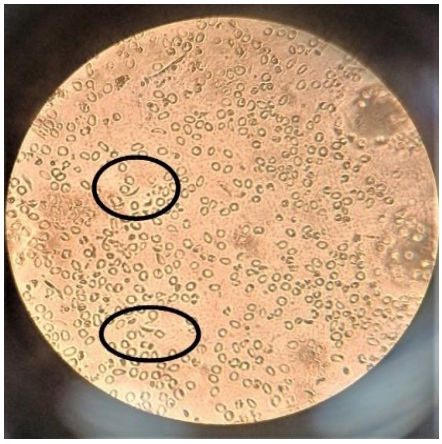

Figure 1. Shows RBC of patient with sickle cell

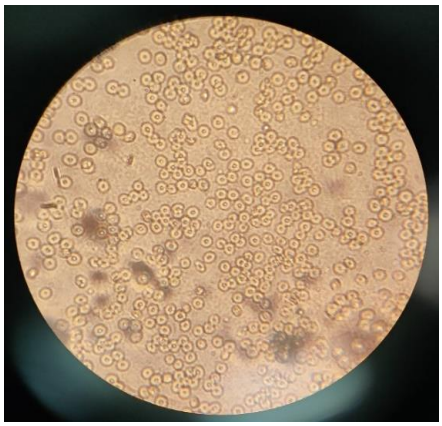

Figure 2. Shows normal RBC

In solubility test we use dithionate qualitative method, Hb-S precipitates in higher molar buffered medium in presence of Dithionate and detergent leaving non-sickling haemoglobin soluble which is free from turbidity. Whereas Dithionate reduced HBS get precipitated and form translucent in the test system. 2 mL Working reagent mixed with one drop of blood. Mix well and wait for 10 minutes at room temperature. Samples having very low haemoglobin mixed 2 drops of blood. Mix the tubes and observe a Negative test as transparent to see the background in Rapid Sickle Cell Reader rack. Whereas the background is not visible in positive sample (HbAS or SS) given as above (Figure 3 and Figure 4) (Obeagu *et al*., 2015).

Complete blood count is performed to analysis of hematological parameter by Sysmex K-4500. HPLC is used as confirmatory test for this study using the Variants Hemoglobin Testing System (Bio-Rad Variants II).

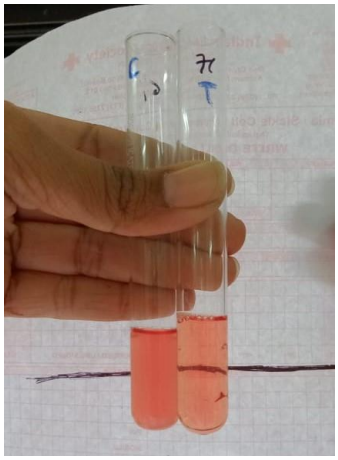

Figure 3. Negative solubility test results

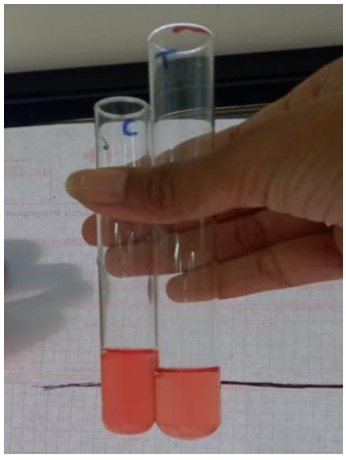

Figure 4. Possitive solubility test results

Results and Discussion

Mean±SD value of hematological parameters of student detected for sickle cell trait, sickle cell trait (with co-existence / co-inheritance of one or more α thalassemia gene), suggestive of compound heterozygous for sickle cell and β thalassemia / sickle cell disease shows anemia is more prevalent in compound heterozygous for sickle cell and β thalassemia / sickle cell disease compare to sickle cell trait (with co-existence / co-inheritance of one or more α thalassemia gene). Sickle cell trait shows normal haematological parameters (Table 1).

Table 2 shows prevalence of sickle cell cast wise. High Prevalence of suggestive of sickle cell trait (With co-existence / co-inheritance of one or more alpha thalassemia gene) was found in schedule tribe (ST) where in general cast and SEBC shows less prevalence than ST. Compound heterozygous for sickle cell and β- thalassemia, sickle cell disease is also found in ST.

Distribution of sickle cell in non-Trible district and Trible district given in Table 3. It shows prevalence of sickle cell, sickle cell trait (With co-existence / co-inheritance of one or more alpha thalassemia gene) and Compound heterozygous for sickle cell and β- thalassemia / sickle cell disease is in Trible district compare to non-Trible district

Table 1. Hematological parameter of sickle cell trait and sickle cell disease

Parameter	Suggestive of sickle cell trait	Suggestive of sickle cell trait (with co-existence/co-inhertitance of one or more alpha thalassemia genes)	Suggestive of (a) compond heterozygous for sickle cell and beta thalassemia (b) sickle cell disease
HB (g/dl)	14.28	12.2±1.4	9.4±0.96
HCT	44.32	41.6±4.7	33.11±4.9
MCV(fl)	94	79.5±7.4	72.5±2.12
MCH(pg)	30.16	23.4±2.6	20.63±0.26
MCHC (%)	32.22	29.37±0.76	29.44±1.35
RBC($\times 10^6/\mu l$)	4.74	5.2±0.4	4.56±0.52
WBC	5.45	7.9±1.59	7.95±2
PLATELET	323	300.2±45.45	264±56.5

Table 2. Caste wise distribution of sickle cell trait and sickle cell disease

Caste	Suggestive of sickle cell trait	Suggestive of sickle cell trait (with co-existence/co-inheritance of one or more alpha thalassemia genes)	Suggestive of (a) compound heterozygous for sickle cell and beta thalassemia (b) sickle cell disease
		General caste	
Solanki	1	0	0
Rajput	0	1	0
		SEBC	
Koli	0	1	0
		ST	
Bhil	0	2	1
Rathva	0	1	1
Dodiya	0	2	0
Damor	0	1	0
Bhabor	0	1	0
Chaudhari	0	1	0

Table 3. District wise distribution of sickle cell trait and sickle cell disease

District	Suggestive of sickle cell trait	Suggestive of sickle cell trait(with co-existence/co-inhertitance of one or more alpha thalassemia genes)	Suggestive of (a) compond heterozygous for sickle cell and beta thalassemia (b) sickle cell disease
		NON TRIBLE DISTRICT	
ANAND	0	1	0
VADODARA	0	2	0
		TRIBLE DISTRICT	
BHARUCH	0	1	0
CHHOTAUDAIPUR	0	1	1
DAHOD	0	2	0
MAHISAGAR	0	0	1
NARMADA	1	0	0
NAVSARI	0	1	0
SURAT	0	1	0
VALSAD	0	1	0

Variable test result is given in table according to this data sickling test has positive predictive value of 55.55% and negative predictive value of 99.20%. solubility test has positive predictive value of 36.84% and negative predictive value of 98.40%. HPLC has 100% positive and negative predictive value as shown in table 4

In our study we observe that HPLC has 100% sensitivity, specificity and accuracy. In screening of sickle cell disease. While sickling test has 66.66% sensitivity, 97.90% specificity and 97.21% accuracy. Solubility test has 53.84% sensitivity, 96.85% specificity and 95.44% accuracy, Shown in table 5

Table 4. Variable result in different method for screening of sickle cell

Variable	Sickling test	Solubility test	HPLC
True negative	374	370	382
False negative	3	6	0
True positive	10	7	13
False positive	8	12	0
Total	395	395	395

Table 5. Compression of Test result for different method of sickle cell screening

Method	Sensitivity	Specificity	Accuracy
HPLC	100%	100%	100%
SICKLING TEST	66.66%	97.90%	97.21%
SOLUBILITY TEST	53.84%	96.85%	95.44%

Discusion

The study was conducted for assessment of SCD screening among APMS student in this screening 168 male and 227 female students participated it is important to understand prevalence among ethical group and distribution in different region students are from age group 18-30 years.

In present study it observed that 69% female were detected for sickle mutation with high prevalence and 31% male with low prevalence are detected, Yadav *et al.* in their study sickle cell disease in Madhya Pradesh, central India observed high proportion of male patients with SCD (Yadav *et al*., 2016). ahemad *et al* also found high prevalence in male for both carrier and disease compare to female (Ahmad *et al*., 2018). This both studies shows high prevalence in male compare to over finding shows high prevalence in female.

In present study student detected for sickle cell mutation we found majority belong to ST cast. Bhil, Rathva, Dodiya, Damor, Bhabhor, Chaudhari shows 77% prevalence which is higher in tribal population, followed by 15% cases from general caste and 8% cases in SEBC has detected. ahemad *et al* in their findings also observed high prevalence in SC and ST caste (Ahmad *et al*., 2018). We do not find case in SC caste in our study. atul and Vasudha Mujumdar in their study of sickle cell anemia in tribal area of Dahod region Gujrat also concluded 11% sickle cell trait and 0.5% sickle cell disease in tribal (Mujumdar and Mujumdar, 2020).

We found that prevalence of sickle cell is much higher 77% in tribal districts of Gujrat Bharuch, Chhotaudaipur, Dahod, Mahisagar, Narmada, Navsari, Surat, Valsad previously recorded that SCD phenotype is milder in India than the African phenotype. Desai G. *et al* found noticeable cases of sickle cell disease in tribal region of south Gujarat having serious disease manifestations (Desai *et al*., 2016). Reason for this may be illiteracy, consanguineous marriage very common and lack of knowledge about disease, so premarital screening and genetic counseling is best tool to control its gene frequency (Ahmad *et al*., 2018).

In homozygous condition of sickle mutation severe anemia commonly observed it's severity depend on the co-inherited β-thalassemia mutation. If β-thalassemia gene is co-inherited with the sickle gene, resulting in Sβ-thalassemia its severity is comparable to homozygous sickle genotype. But if β+-thalassemia gene is co-inherited with the sickle gene, resulting in Sβ+-thalassemia it results in wide range of clinical severity so it important to identify this individual in this study 10 student found with suggestive of sickle cell trait (with co-existence/co-inheritance of one or more alpha thalassemia genes) (Yadav *et al*., 2016).

In our study Sensitivity, specificity and accuracy of HPLC is 100% while sickling test has 66.66% sensitivity, 97.90% specificity and 97.21% accuracy, Solubility test has 53.84% sensitivity,96.85% specificity and 95.44% accuracy, Jain R. *et al* In their study observed 96.8% sensitivity and 29.6% specificity in solubility test (Jain and Saxena, 2020). Delux V. godghate and Sarika more in their study also observed 58.82% sensitivity, 95.49% specificity and 91.33% accuracy in sickling test, in solubility test they observed 48.39% Sensitivity, 89.96% specificity and 85.67% accuracy (Delux and Sarika, 2018).

We observed that HPLC has 100% positive predictive value (PPV) and 100% negative predictive value (NPV), sickling test has 55.55%

PPV and 99.20% NPV, in solubility test PPV is 36.84% and NPV is 98.40%. Jain R. *et al* observed 87.9% PPV and 64% NPV in their study (Jain and Saxena, 2020). Delux V. godghate and Sarika more also observed 62.50% PPV and 94.78% NPV in sickling test, 35.17% PPV and 93.80% NPV in solubility test (Delux and Sarika, 2018).

Conclusion

The present study provides information on the prevalence of sickle cell trait and other hemoglobinopathies in the screened population, which can help in planning and implementing appropriate public health interventions, such as genetic counseling, carrier screening, and newborn screening programs. By assessing the sensitivity, specificity, and predictive value of the sickling test and solubility test as primary screening tools, and the role of HPLC as a confirmatory test. This can help in refining screening algorithms and improving the accuracy and efficiency of screening programs and determine the most appropriate and cost-effective screening strategies for different populations and settings.

This data can also help in the early detection and diagnosis of hemoglobinopathies, which can prevent complications and improve outcomes for affected individuals. Early identification of carriers and affected individuals can also enable targeted interventions, such as prophylactic treatment and close monitoring, to prevent or manage complications associated with hemoglobinopathies, such as sickle cell crisis, stroke, and organ damage.

The study could also help in identifying the strengths and limitations of each test and in developing algorithms for combining and interpreting the results of different tests to improve the accuracy and efficiency of screening programs.

The study could be unique in terms of its focus on a specific population or geographical region, which may have unique genetic, environmental, and social factors that affect the prevalence and clinical presentation of hemoglobinopathies. This could lead to tailored interventions and policies that address the specific needs of the population and improve health outcomes.

Overall, a study that measures the sensitivity, specificity, and accuracy of sickling test, solubility test, and HPLC, and conducts an epidemiological study could contribute to advancing the field of hemoglobinopathies diagnosis and screening, and improving public health outcomes for affected individuals and populations.

Limitation

Sickling test and Solubility test are only a screening method. This method is not a substitute for High-Performance Liquid Chromatography (HPLC).

Recent blood transfusions may alter the results. Samples with hypergammaglobulinemia may show the false-positive result which can be avoided by using saline washed cells instead of whole blood.

Samples with haemoglobin more than 19gm/dl and higher fetal haemoglobin lead to a false-positive result. Very anaemic patient or sample with less RBC may lead to false-negative observation.

Conflict of interest: Nil

Ethical considerations

The Ethics board of H M Patel Center for Medical care & education, Karamsad, faculty of medicine approved this study. Written informed consent was obtained from all students involved in the study.

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