ORIGINAL RESEARCH PAPER

Pathology

STUDY OF SICKLE CELL DISEASE IN A TERTIARY CENTRE IN CENTRAL INDIA.

KEY WORDS:
.Haemoglobinopathies, Sickle cell disease, Solubility test, Sickling test

Dr. Ch. Deepak Singh MBBS,MS,M.Ch (Paed. Surgery), Assistant Professor, Department of Surgery, JNIMS, Imphal, Manipur, India.

Dr. Khundrakpam Sunitarani Devi MBBS,DCP, PGT, Department of Pathology, JNIMS, Imphal, Manipur, India.

ABSTRACT

Hemoglobinopathies are disorders affecting the structure, function or production of hemoglobin. Sickle cell anemia is the most common heritable hematologic disease affecting humans.

Objectives: 1).To screen Sickle cell disorder in the age group of up to 18yrs in rural population in and around Sawangi (Meghe), Wardha. 2).To establish the role of Solubility test as screening test for detection of Sickle cell disease. 3). To compare sensitivity and specificity of Solubility test with that of Sickling test.

Material and methods: The study was carried out at the department of Pathology, Acharya Vinoba Bhave Rural Hospital, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha over a period of six months. Four hundred and forty patients up to 18yrs of age were subjected to both Solubility test and Sickling test.

Results: Out of 440 cases, both Solubility and Sickling test were positive in 40 cases (9%). Out of this 40 positive cases 28 cases (70%) were male and 12 cases (30%) were female. Number of positive cases were more i.e 16 cases (40%) in the 0-6 age groups.

Conclusion: Prevalance rate of sickle cell anemia is 9%. The ratio of male to female is 2.3:1. Maximum cases were recorded in the age group of 0-6 age groups. Sensitivity and specificity of both the test is found to be 100%.

INTRODUCTION

Of all the genetic disorders to which man is known to be liable, there is probably no other that presents a collection of problems and challenges quite comparable to sickle cell disease and related disorders, because of its extensive distribution, problem created by its chronicity, and its resistance to therapy. It is a genetic abnormality whose control and cure still elude clinicians, research workers, and social scientists. Impact of this disease is now being felt all over the Indian Subcontinent and in many parts of Asia. **(Weatherall DJ, 2001)**[1]

Hemoglobinopathies are disorders affecting the structure, function or production of hemoglobin. Sickle cell anemia is the most common heritable heamatologic disease affecting humans. In sickle haemoglobinopathy, the mutation substitutes Thymine for Adenine in 6th position of beta globin chain.

Herric James (1910)[2] of United States reported elongated sickle shaped red blood cells in young Black student. In 1940,Sherman observed that the cells in sickle cell anemia were birefreigent**.(Wintrobe MM,1969)**[3].**Pauling L (1949)**[4] described the molecular basis of the disease to be due to one abnormal hemoglobin called Hb S, which has slow electrophoretic mobility as compared to normal hemoglobin Hb A.

Prior to 1950, nothing was known about the existence of sickle cell anemia in the Indian Subcontinent. In 1951, for the first time high prevalence was reported among some tribal population groups from South India (Nilgiri hill region).

In India the prevalence of sickle cell gene varies from 0-44% in different tribal and some scheduled caste population. The frequency distribution of Hb S varies between:

0-18.5% in North Eastern Zone
0-33.5% in Western Zone
22.5-44.4% in Central Zone
1-40% in Southern Zone.

Sickle cell anemia in paediatric age group causes high incidence of morbidity and mortality.Maximum number of cases of sickle cell anemia were seen in age group of 0-10 yrs**(Kulkarni,2000)**[5]. In study of **Kamble(2000)**[6],63% of the patients of sickle cell disease were less than 5years.

AIMS AND OBJECTIVES

- To screen Sickle cell disorder in the age group of upto 18 yrs in rural population in and around Sawangi (Meghe), Wardha, Maharasthra, India.
- To establish the role of Solubility test as screening test for detection of Sickle cell disease.
- To compare sensitivity and specificity of Solubility test with that of Sickling test.

MATERIALS AND METHODS

Approval for this study was obtained from our Institutional Ethics Committee. This was a retrospective study conducted at Department of Pathology , Acharya Vinoba Bhave Rural Hospital, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha from January 2009 to June 2009 over a period of six months.

Study design

Hospital based retrospective study.

Study setting

The study was conducted at Department of Pathology, Acharya Vinobha Bhave Rural Hospital, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha.

Study period

The study was carried out over a period of six months from 1st January 2009 to 30th June, 2009.

Sample size:

Four hundred and forty (440) patients whose requisition of sickling given by the clinician irrespective of sex.

Methodology:

- Solubility test
- Sickling test

Solubility Test:

- Screening test for detection of sickle cell disease
- Small amount of blood (20 µL) is added to a solution (2 ml) that contains high phosphate buffer, a reducing agent (sodium dithionite) and saponin.
- Red cells are hemolyzed and HBS if present, is reduced by dithionite.

PRINCIPLE: HbS in RBC_s in presence of high phosphate buffer,precipitate in reduced state giving cloudy appearance to solution.

REAGENTS :
Stock Solution(Phosphate buffer: p^H7.1)
1.KH_2PO_4 (Potassium Dihydrogen Phosphate) - 33.78g
2.K_2HPO_4 (Dipotassium Hydrogen Phosphate) - 59.33g
3.White Saponin - 2.5g
4.Distilled Water - 250 ml

1.Stock Solution:
Dissolve KH_2PO_4 and K_2HPO_4 in 125 ml of distilled water each. Mix both reagents and then add White Saponin. Thus 250 ml of stock solution is ready.

2.Working Solution:
Stock Solution (Phosphate buffer) -10 ml
Sodium Dithionate -100 mg
Thus 10 ml working solution is ready.

SAMPLE:
Whole blood/EDTA blood/Double Oxalate blood.

EQUIPMENTS:
Test tubes, Reader scale,Pipette-20µl.

METHOD:
Take 3 test tubes and label Test, Positive control, Negative control. Add 2ml of Working Buffer Solution in each test tube. Then add 20µl of Blood Sample in each. Mix well and wait for 5 minutes at room temperature.

INTERPRETATION:
-A reader scale is held at the back of the tube. In negative test lines will be closely seen since HB A is soluble in phosphate buffer, while lines will not be seen in Positive test due to formation of polymers of HBS. Positive results is also obtained with HBS Travis and HBC Harlem. The solution remains clear in presence of HbA, HbF, HbC, HbD, and HbO- Arab.

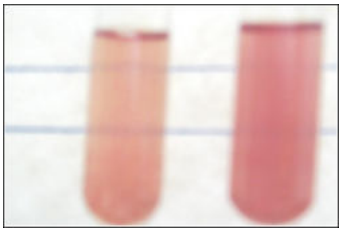
Causes of False Negative test
- Use of old or outdated reagents
- Low concentration of HBS as in young infants or in severe anemia
- Following blood transfusion

Causes of False Positive test
- Paraproteinaemia
- Hyperlipidaemia
- Polycythaemia
- Leucocytosis

LIMITATIONS OF TEST:
Falsely positive test may be due to polycythemic blood, interference by some forms of hyperglobulinemia and a variety of abnormal hemoglobin. Positive test should be confirmed by Hemoglobin Electrophoresis.

Falsely negative test may be due to inadequate quantities of blood from anemic patients. High concentration of Hb F or quantities of HbS too small to detect is present.

This test should not be performed on infants until they are about 6 months old because children with sickle cell will not produce significant amounts of HbS until several months after birth.



Negative **Positive**

SOLUBILITY TEST

b. Sickling test
- When red cells containing HbS are subjected to deoxygation, they become sickle shaped while cells that do not contain HbS remain normal
- Certain reducing chemical agents such as 2% sodium metabisulfite or sodium dithionite can deprive red cells of oxygen.

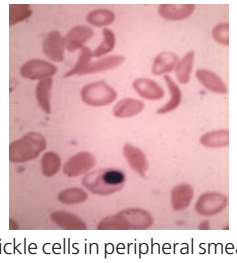
Method:
Blood and reducing agent are mixed on glass slide and a cover slip is placed over it that is sealed with petroleum jelly paraffin wax mixture. Amount of HbS in red cells and degree of deoxygenation influence the speed and extent of sickling.

Sickling is usually evident after 30 minutes, if it is not then the slide is re-examined after allowing it to stand overnight The sickled cells have minimum of two pointed projections.

False Negative test: Causes
- Inactive, outdated reagents (incomplete reduction of oxygen tension)
- Blood samples containing low proportion of HbS (some cases of sickle cell trait)
- Improper sealing of coverslip

Causes of False Positive test:
- High concentration of sodium metabisulphite
- Carry over from positive sample due to inadequate washing of pipette.
- Mistaking crenated red cells for sickled cells.

Limitations of Sickling Test:
- This test simply detects presence of HbS and does not differentiate sickle cell anemia from sickle cell trait or other sickling syndromes.
- This test cannot be used for mass screening as an experienced microscopist is required for interpretation



Sickle cells in peripheral smear

Sickle cells in wet preparation

OBSERVATIONS
- The hospital based cross sectional study of detection of Hb S gene with Solubility test and Sickling test was carried out at the department of Pathology, Acharya Vinoba Bhave Rural Hospital, Jawarharlal Nehru Medical College, Sawangi (Meghe), Wardha, over a period of six months. Four hundred and forty patients up to 18 years of age were subjected to both Solubility test and Sickling test.

TABLE-1
- **Prevalance of sickle cell anemia in the study group:**

Test	No. of positive cases	Incidence out of 440
Solubility	40	9%
Sickling test	40	9%

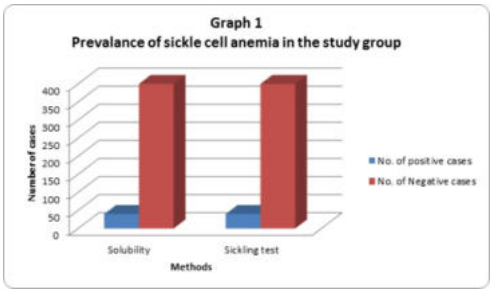


TABLE-2

- **Sex – wise distribution of sickle cell anemia in the study group:**

Sex	No. of positive cases	% out of 40
Male	28	70%
Female	12	30%

GRAPH-2

- **Sex-wise distribution of sickle cell anemia in the study group: Out of 40 cases 28 cases(70%) were male and 12 cases (30%) were female.**

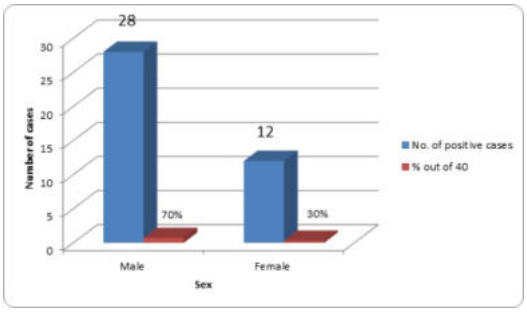


TABLE – 3

- **Age-wise distribution of sickle cell anemia in the study group:**

Age	No. of Positive cases	% out of 40
0 – 6	16	40%
7-12	15	37.50%
13-18	9	22.50%

GRAPH – 3

- **Age – wise distribution of sickle cell anemia in the study group: Out of 40 cases 16 cases (40%) were found in the age group of 0 – 6yrs and 9 cases (22.5%) were found in the age group of 13 -18yrs.**

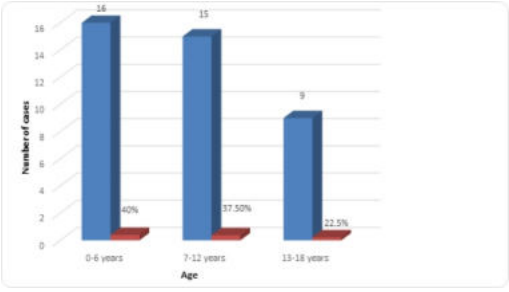


TABLE - 4

- **Sensitivity and specificity of solubility and sickling test :**

Test	Sensitivity	Specificity
Solubility test	100%	100%
Sicking Test	100%	100%

DISCUSSION

- Sickle cell disease is a hemoglobinopathy occurring as a consequence of the presence of sickle hemoglobin. The disease causes significant morbidity and mortality in those affected. . Early diagnosis is therefore essential and is the cornerstone to implementation of prophylactic programmes and successful management of these patients**. (Mabote T)**[7]
- Highest prevalence of Hb S gene is found in tropical Africa and among Blacks. Hb S gene incidence among Blacks is found to be **6-8.5%**, **Loh (1968)**[8]**, Nalbandian (1971)**[9]**, Rosner (1972)**[10]**, Bornes (1972)**[11]
- National prevalence of Hb S gene was found to be **15.1%** in a population survey of 1000 randomized subjects. In India, prevalence rate varies from **0-44%.**
- In study by **Kar BC, (1986)**[12] prevalence of sickle trait was found **11.1%.** In study by **Balgir RS (1988)**[13] and **Balgir RS (1996)**[14]**,** the prevalence of sickle cell gene in Maharashtra was found to be **1-31.4%.**

- **PREVALENCE STUDIED IN DIFFERENT STUDIES**

STUDY	PREVALENCE
Kar BC (1986)[12]	11.1%
Balgir RS (1988)[13] Balgir RS (1996)[14]	1-31.4%
Kamble M (2000)[15]	5.7%
Abhyankar et al (2000)[16]	12%
Gupta (2006)[17]	18-33%

In study done by **Kamble M (2000)**[15] showed prevalence of sickle cell disorder to be **5.7%.** According to **Abhyankar et al (2000)**[16]**,** prevalence of the disease was found to be **12%**.In study done by **Gupta (2006)**[17]**,** the prevalence of disease was noted to be **18-33%.**

In the present study, high prevalence of sickle cell disorder is found which is 9% (40 of 440 cases) in a defined population. As regards to sex distribution of the disorder, in the present study, male is more preponderance to female .

COMPARATIVE STUDY OF SOLUBILITY TEST AND SICKLING TEST:

In India, the Hb S gene is quite prevalent. Clinical manifestations, especially morbidity pattern of sickle cell disease is extremely variable in different population groups. Although modern management provides a better quality of life for those with sickle cell anemia, it does impose a heavy financial and social burden on the entire family.

The objective of a sickle cell screening programmes are to undertake large surveys to determine the prevalence of sickle trait and disease and to identify the high risk groups, which will help the development of a management and control programme for this disorder in the community.

In areas where the sickle gene frequency is very high and facilities are generally limited, a simple, inexpensive preliminary screening test to detect the Hb S gene is needed. Such a test should not require sophisticated equipment, should be easy to implement and would help to select suspected cases for further investigations in Centralized Laboratories where facilities for Hemoglobin Electrophoresis are available.

Sickling test, though used as a screening test is quite tedious and time consuming and requires a trained person to differentiate between sickle and normal RBCs under microscope. Improper sealing of the preparation and contamination of slides by soaps, detergents may give false negative results. In contrast, the Solubility test is rapid, simple and easily done by persons who have specific laboratory training.

COMPARATIVE SENSITIVITY AND SPECIFICITY ANALYSIS OF SOLUBILITY TEST

STUDY	SENSITIVITY	SPECIFICITY
Hicks EJ (1973)[18]	98.9%	100%
Chasen ST (1999)[19]	88.9%	79.4%
Surve (2000)[20]	93.8%	100%
Present study	100%	100%

In the present study ,sensitivity and specificity of both the test is found to be 100%.

CONCLUSIONS

- In the present study, the prevalance of Sickle cell anemia in age group up to 18 years is found to be 9% suggesting a high prevalance of this disorder in this region of Central India.
- In the present study male (70%) were more prevalent than female (30%) in this particular age group.
- Again the particular age group more frequently affected in the present study is 0 - 6 years (40%).
- Sensitivity and specificity of both the Solubility and Sickling test is found to be 100% in this present study.

- The Solubility test is advocated as a rapid, simple, inexpensive preliminary screening test for the detection of sickle cell disorder where the prevalence of sickle cell gene is high as Sickling test required microscopic examination and need expertise persons.
- Solubility test does not require sophisticated equipment, is easy to implement and so helpful to select suspected cases for further investigations in Centralized Laboratories where facilities for Hemoglobin Electrophoresis are available

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