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)

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: Prevalence 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%

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 Vinoba 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, in presence of high phosphate buffer, precipitate in reduced state giving cloudy appearance to solution.
REAGENTS:
- Stock Solution (Phosphate buffer: pH 7.1)
  1. KH₂PO₄ (Potassium Dihydrogen Phosphate) - 33.78g
  2. KH₂PO₄ (Dipotassium Hydrogen Phosphate) - 59.33g
  3. White Saponin - 2.5g
  4. Distilled Water - 250 ml

1. Stock Solution:
   Dissolve KH₂PO₄ and KH₂PO₄ 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.

Sickle cells in peripheral smear Sickle cells in wet preparation

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

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>
<thead>
<tr>
<th>Test</th>
<th>No. of positive cases</th>
<th>Incidence out of 440</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility</td>
<td>40</td>
<td>9%</td>
</tr>
<tr>
<td>Sickling test</td>
<td>40</td>
<td>9%</td>
</tr>
</tbody>
</table>

Graph 1: Prevalence of sickle cell anemia in the study group
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)

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) 2, Nalbandian (1971) 3, Rosner (1972) 4, Bornes (1972) 5, 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) 6 prevalence of sickle trait was found 11.1%. In study by Balgir RS (1988) 7 and Balgir RS (1996) 8, the prevalence of sickle cell gene in Maharashtra was found to be 1-31.4%.

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.

CONCLUSIONS

In the present study, the prevalence of Sickle cell anemia in age group up to 18 years is found to be 9% suggesting a high prevalence 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%.

COMPARATIVE STUDY OF SOLUBILITY TEST AND SICKLING TEST:

<table>
<thead>
<tr>
<th>STUDY</th>
<th>PREVALENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kar BC (1986) 6</td>
<td>11.1%</td>
</tr>
<tr>
<td>Balgir RS (1988) 7</td>
<td>1-31.4%</td>
</tr>
<tr>
<td>Balgir RS (1996) 8</td>
<td></td>
</tr>
<tr>
<td>Kamble M (2000) 9</td>
<td>5.7%</td>
</tr>
<tr>
<td>Abhyankar et al (2000) 10</td>
<td>12%</td>
</tr>
<tr>
<td>Gupta (2006) 11</td>
<td>18-33%</td>
</tr>
</tbody>
</table>

In study done by Kamble M (2000) 9 showed prevalence of sickle cell disorder to be 5.7%. According to Abhyankar et al (2000) 10, prevalence of the disease was found to be 12%. In study done by Gupta (2006) 11, the prevalence of disease was noted to be 18-33%.

In the present study, age-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.

In the present study, 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.

In the present study, age-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-2

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of positive cases</th>
<th>% out of 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>28</td>
<td>70%</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>30%</td>
</tr>
</tbody>
</table>

TABLE–3

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of Positive cases</th>
<th>% out of 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>16</td>
<td>40%</td>
</tr>
<tr>
<td>7-12</td>
<td>15</td>
<td>37.50%</td>
</tr>
<tr>
<td>13-18</td>
<td>9</td>
<td>22.50%</td>
</tr>
</tbody>
</table>

TABLE-4

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility test</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Sickling Test</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STUDY</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hicks EJ (1973) 12</td>
<td>98.9%</td>
<td>100%</td>
</tr>
<tr>
<td>Chasen ST (1999) 13</td>
<td>88.9%</td>
<td>79.4%</td>
</tr>
<tr>
<td>Surve (2000) 14</td>
<td>93.8%</td>
<td>100%</td>
</tr>
<tr>
<td>Present study</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

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

References:
2) Herrick JB. Peculiar elongated and Sickled shaped red blood corpuscles in case of severe anemia. Arch Intern Med 1910; 6:517
3) Wintrobe MM. Anemia, Serendipity and Science. JAMA 1969; 210:318