ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Efficacy of High Speed Super Solubility Test In Detection Of Sickle Cell Diseases at Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India.

Chandrahas Prasad[1], Hemant Narayan Rai[2], and Shashi Bhushan Singh[3*].

[1]Associate Professor, Department of Laboratory Medicine,[2]Associate Professor ,Department of Cardiology, [3]Lecturer Cum Statistician, Department of Preventive and Social Medicine, Rajendra Institute of Medical Sciences (RIMS), Ranchi, Jharkhand, India.

ABSTRACT

Sickle cell anemia (SCA) is a genetic disorder characterized by severe hemolytic anemia and shorter life span. Diagnosis is an important aspect in the management of this disease. To assess the efficacy of Super Speed Sickle Solubility test in the diagnosis of SCA in population attending this medical institute. A total of 63 patients were screened in 2013 . All the positive samples were assayed by solubility test simultaneously analysed on high-performance liquid chromatography (HPLC) 'BIO-RAD' Variant II analyzer for the confirmation along with the distinction of SCA (heterozygous) and sickle cell disease (homozygous). Out of all 63 patients screened, 29 were found to be positive with HPLC as well as with solubility test too. A total of 10 samples was diagnosed as SCA (heterozygous) , 12 samples were diagnosed as sickle cell homozygous and 7 cases as Sickle Thal with HPLC and Solubility tests .In case of Sickle cell Homozygous, Solubility test was found to be fairly effective with 75.0 % and 80.85% sensitivity and specificity respectively, with the predictive value of positive test 57.14% and a predictive value of negative test 90.47 % along with an Accuracy of 79.37 % . It was observed that in case of Sickle Cell Trait, the sensitivity specificity, positive predictive and negative predictive values were 76.92 % , 97.5 %, 57.14% and 92.8% respectively . We found that Solubility test in Sickle Thal Trait revealed sensitivity of 70.0 %, specificity 73.58 %, positive predictive value 33.33 %, negative predictive value 92.85 % along with an Accuracy of 73.0 %. This study concludes that solubility test is better for mass screening and does not need any microscope. It is also cost effective test for early detection of disease and for timely intervention to minimize morbidity and mortility.

Keywords: Sickle Cell Anaemia, High Speed Super Sickle Test , HPLC, Efficacy, Tribal zone.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The most common clinical presentation of SCD is the periodic painful crises, when sickle erythrocytes hinder capillary blood flow causing intense pain in the chest, abdomen, arms and legs. The duration, frequency and intensity of pain episodes varies from patient to patient, in most severe cases prompting patients to seek emergency treatment for pain. The existing laboratory-based techniques for objectively confirming suspected SCD in patients presenting with pain and requesting pain medication may require days and/or be completely unavailable in resource-limited settings. The ability to diagnose SCD at the point-of-care within minutes using a low-cost, portable device will substantially improve patient care by enabling more accurate differential diagnosis in patients presenting vague common symptoms (such as abdominal pain) and by allowing healthcare workers to choose the correct course of treatment for these patients [1,2].

The clinical manifestations of SCD include jaundice, elevated susceptibility to infection, delayed growth and development, acute chest syndrome, splenic sequestration, stroke, avascular necrosis of the femoral or humeral head, pulmonary hypertension, chronic hemolytic anemia and episodic pain in the chest, stomach, arms and legs [3].

Worldwide, SCD affects about 30 million people of African, Mediterranean, and Middle Eastern descent [4].

Sickle cell disease is a hereditary blood disorders due to defective haemoglobin structure. There is replacement of valine with glutamic acid at 6^{th} position of beta chain of haemoglobin. Prevalence of sickle cell disorder is high among tribal population. A simple rapid and reliable test is necessary to screen a various number of populations attending this institute . We found solubility test to be effective and reliable in diagnosis of sickle cell disorder [5].

MATERIALS AND METHODS

The present study was carried out in the Department of Labotatory Medicine, RIMS, Ranchi. For this, we did a screening of specially referred patients attending the outpatient department (OPD),patients admitted in wards in the year 2013. A total blood sample of 63 patients attending OPD and admitted in wards were examined. Samples were taken from suspected patients of Hemoglobinopathies having anaemia, spleenomegaly, joint pains etc.

SOLUBILITY TEST

(Super Speed Sickle test)

The normal hemoglobin is red and when they release oxygen they maintain their shape, which is round, but the sickle hemoglobin sometimes is sickle-shaped when oxygen is released. There are different kinds of hemoglobin depending on the type of gene inherited from each parent. The most common gene is Hemoglobin A often written as HbA. In sickle cell anemia, a point mutation (GAG to GTG) in the B- chain at codon position 6 results in the encoding of a valine instead of normal glutamine. The resulting abnormal B-chains combine with normal B-chains to form an abnormal hemoglobin S (HbS). HbS is poorly soluble in low oxygen tension situations forming a gel and polymerizing into fibrilary structures .This distorts the red blood structure causing them to become rigid and sickled.

RBCs are exposed to a hemolytic agent (e.g. saponin) to release Hb into the phosphate buffer solution containing a strong reducing agent (e.g. sodium hydrosulfite). Under these conditions, HbS precipitates (unlike HbA which remains dissolved in solution) – the resulting turbidity of the solution is easily detected through simple visual observation [6].

The HbS solubility assay is relatively fast (5-15 minutes), requires minimal reagents and can be easily performed at the point of care. This test has very limited use for definitive diagnosis of SCD, however, because it can only detect the presence of HbS, but can't differentiate between the sickle cell trait (HbA / HbS) and sickle cell disease (homozygous HbS / HbS) [7,8] without the help of HPLC.

ISSN: 0975-8585

Finally, SCD can be diagnosed through direct microscopic observation of the morphology of the patient's RBCs in a peripheral blood smear preparation [9,10] as a cross examination.

Individuals with sickle cell anemia (Homozygous S/S) may have early mortality with vascular occlusions of multiple organ system, severe hemolytic anemia and hypoxia [11]. Individuals with sickle cell trait (Heterozygous A/S) are usually asymptomatic. However, under certain conditions of reduced oxygen tension such as hypoxia during anesthesia, flight in poorly pressurized airplanes, severe pneumonia, these individuals can experience a sickle cell crisis [12].

Hi-Super Speed Sickle Test is based on the solubility difference between HbS and HbA in Solubility Test Reagent. When red cells are introduced into such a solution, they lyse immediately [13]. The hemoglobin released from the lysed red cells, is reduced by reagent mixture. This reaction causes precipitation of HbS leading to the turbidity of the reaction mixture. However, HbA, as well as other hemoglobins are soluble, leading to clarity in the reaction mixture. This test is a simple and stable screening test and so the samples tested positive should be confirmed by HPLC so as to reduce the chances of False Positives [14].

Reagent

Phosphate buffer (pH 7.1) was the reagent used.

Stock solution using 270.24 g of potassium dihydrogen phosphate, 474.64 g of dipotassium hydrogen phosphate, 20 g saponin and 2 litres distilled water. The ingredients are mixed properly and the resultant solution was filtered. From this stock solution working solution is prepared.

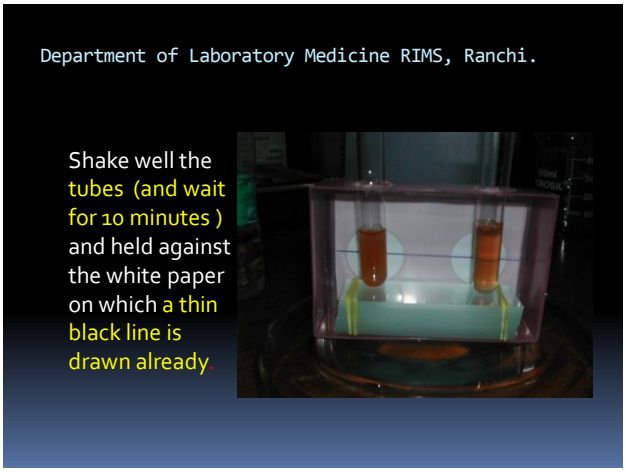
Working solution

A measure of 100 mg of sodium dithionite is added to 10.0 ml of phosphate buffer stock solution to prepare the working solution.

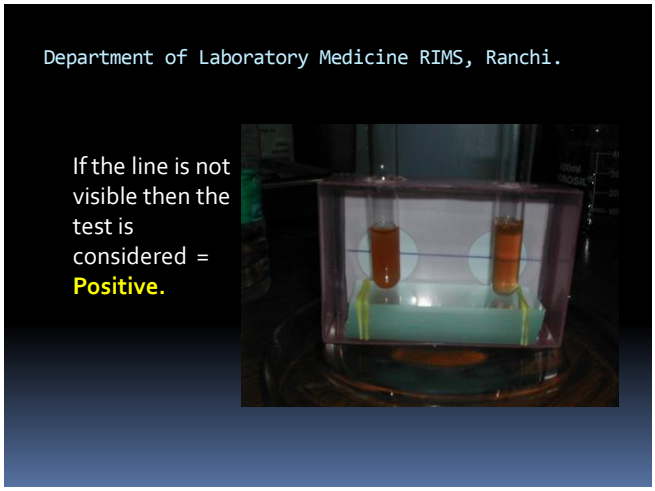
Procedure

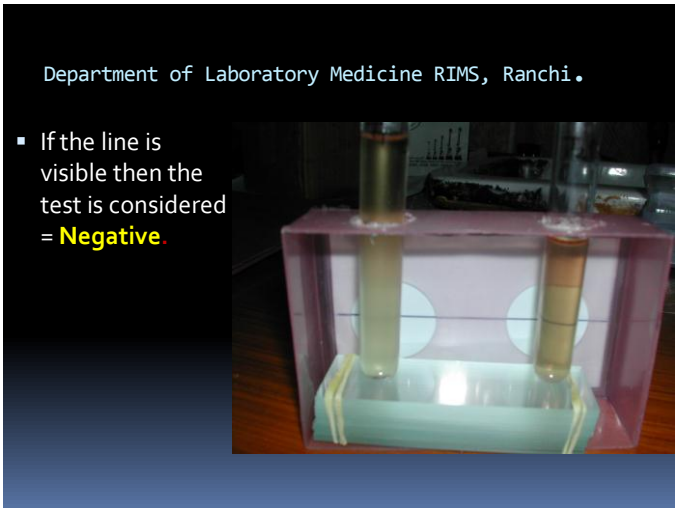
A volume of 2 ml of the working phosphate buffer is taken in a test tube and 20 ul of blood sample is added to it. The test tube is kept for 5 min before reading the test. A piece of white paper was taken and dark bold black lines are drawn on it for interpretation of the test as explained below.

The test is read as positive, if turbidity is present. To confirm turbidity the paper with bold black line is kept at a distance of 1 inch from the test tube against a bright light. If turbidity is present it impairs the visibility of dark bold lines. A negative test is indicated by a clear pinkish-violet solution. When dark lines are partially visible, it is marked as a doubtful result. All are also subjected to high-performance liquid chromatography (HPLC) simultaneously to differentiate sickle cell homozugous and hetrozygus.



ISSN: 0975-8585





Interpretation of Solubility test

The test tube on left is read as positive, as the bold dark lines of reader scale are not visible due to turbidity; the test tube on right is read as negative, as the bold lines of reader scale are clearly visible. were also subjected to HPLC. We also carried out sickling test on all samples having adequate quantity.

RESULTS

Observations

HPLC & Super Speed Sickle test in cases of different types Hemoglobinopathies

	1	2	3	4	5	6	7	Total
Findings of cases	Sickle Homo	Sickle cell Trait	Sickle Thal Trait	Beta Thal Trait	Delta Beta Thal Trait	Thal Major	HPFH	
by using HPLC	12	10	7	13	9	5	7	63
by using Super Sickle Test	8	7	4	1	0	1	0	21

ISSN: 0975-8585

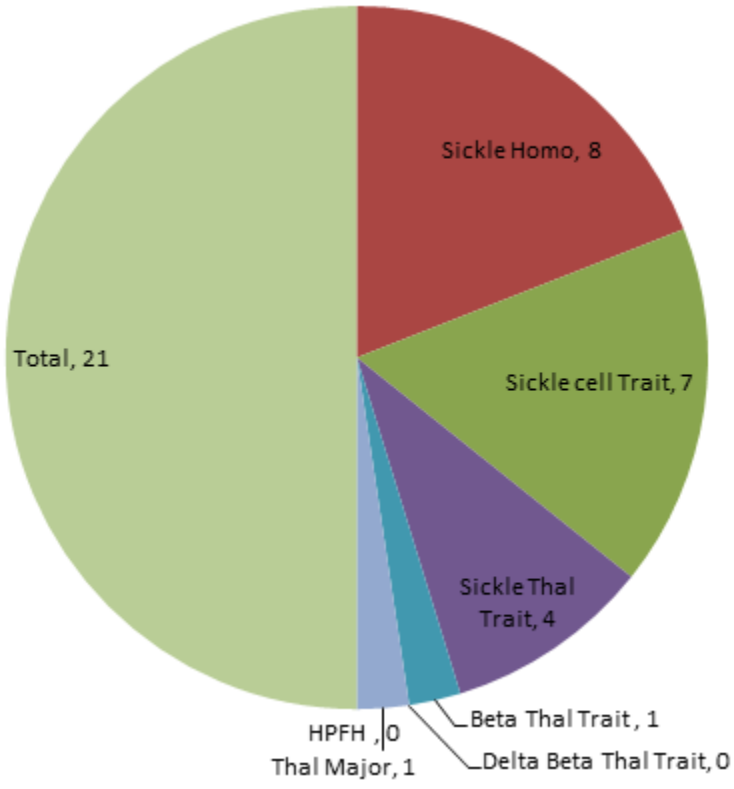
Super Speed Sickle test result in the following diseases

Disease	Positive	Doubtful	True Negative	Total
Sickle Homo	8	1	3	12
Sickle cell Trait	7	0	3	10
Sickle Thal Trait	4	1	2	7
Beta Thal Trait	1	7	5	13
Delta Beta Thal Trait	0	4	5	9
Thal Major	1	1	3	5
HPFH	0	4	3	7
Total	21	18	24	63

Note : Doubtful results were assumed as negative

Disease	Positive	**Negative** (doubtful+ true negative)	Total
Sickle Homo	8	4	12
Sickle cell Trait	7	3	10
Sickle Thal Trait	4	3	7
Beta Thal Trait	1	12	13
Delta Beta Thal Trait	0	9	9
Thal Major	1	4	5
HPFH	0	7	7
Total	21	42	63

Histogram showing prevelance of Sickle cell anemia & others



Among the 63 patients with different types of Hemoglobinopathies on HPLC of which Super speed Sickle Test was positive for 21, 'doubtful' for 18 , 'true negative' for 24 & negative for 42 cases.

ISSN: 0975-8585

Comparison between HPLC & Super Speed Sickle test in case of **Sickle Homozygous**

		HPLC		
		Positive	Negative	Total
Super Speed Sickle Test	Positive	12	9	21
	Negative	4	38	42
	Total	16	47	63

The number of True Positive (TP), True Negative (TN), False Positive (FP) and False Negative (FN) were determined.

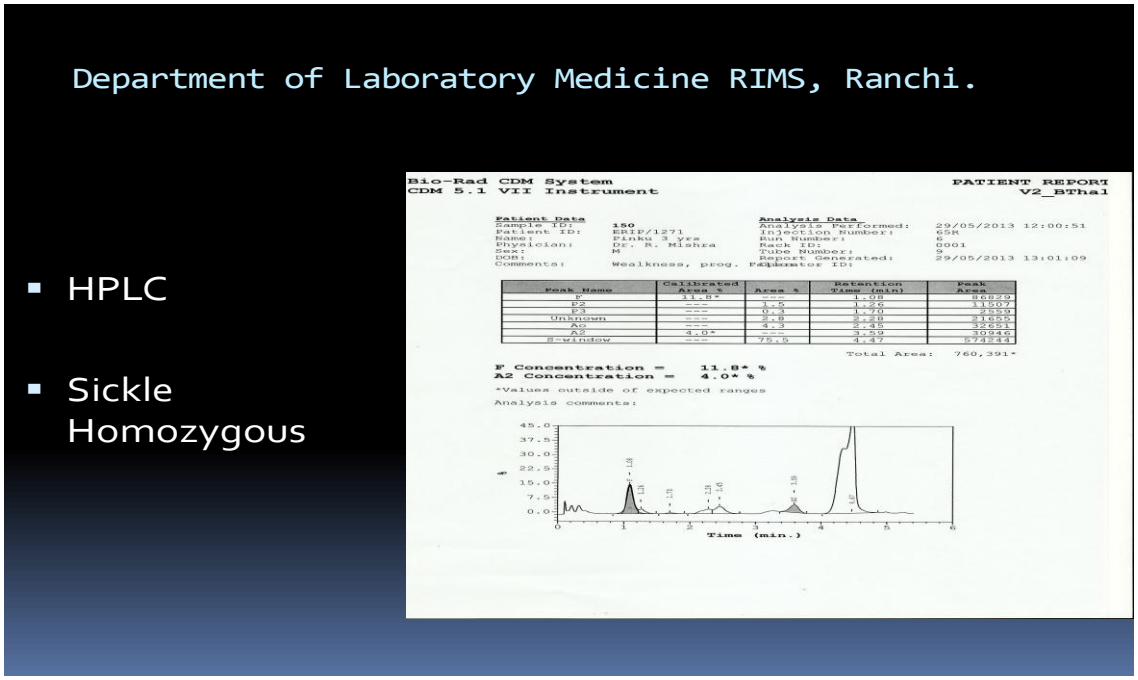
Sensitivity, specificity and predictive values were calculated as follows:

- Sensitivity = TP x 100/(TP+FN) = 75.00% %
- Specificity = TN x 100/(TN+FP) = 80.85 %
- Predictive value of a positive test= TP x 100/ (TP+FP) = 57.14 %
- Predictive value of a Negative test = TP x 100/ (TN+FN)= 90.47 %
- Accuracy = (TP+TN)/Total = 79.37 %

Positive Super Sickle blood samples are subsequently confirmed for Sickle Homozygous by HPLC = 16

Table 1: Results of Super Sickle specially in Sickle Homozygous

SUPER SICKLE	Total Subject n= 63	B-Thalassemia trait n = 16
Positive	21	12
Negative	42	14



ISSN: 0975-8585

Comparison between HPLC & Super Speed Sickle test in case of Sickle Cell Trait

Super Speed Sickle Test	HPLC		
	Positive	Negative	Total
Positive	10	11	21
Negative	3	39	42
Total	13	40	63

The number of True Positive (TP), True Negative (TN), False Positive (FP) and False Negative (FN) were determined.

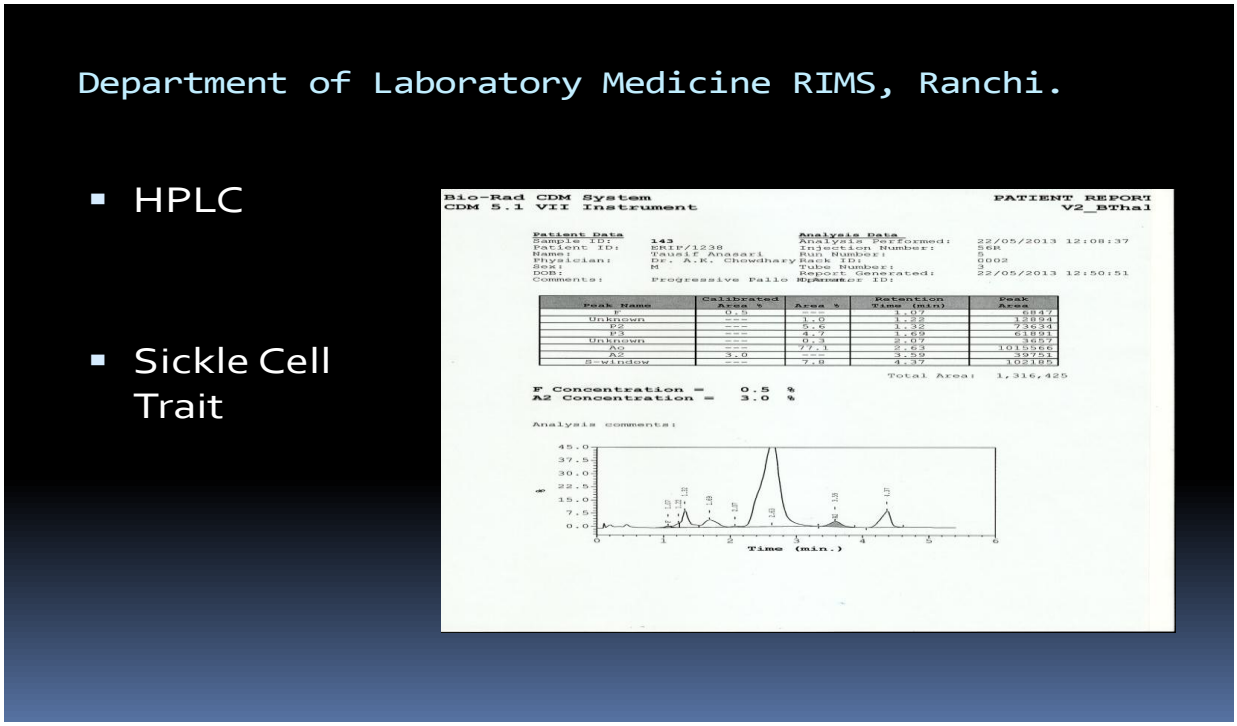
Sensitivity, specificity and predictive values were calculated as follows:

- Sensitivity = TP x 100/TP+FN = 76.92 %
- Specificity = TN x 100/TN+FP = 97.5 %
- Predictive value of a positive test TP x 100/ TP+FP 4) = 57.14 %
- Predictive value of a Negative test = TP x 100/ TN+FN= 92.8 %
- Accuracy = 77.77 %

Positive Super Sickle blood samples are subsequently confirmed for Sickle Cell Trait by HPLC = 13

Table 1: Results of Super Siclkle specially in Sickle Cell Trait

SUPER SICKLE	Total Subject n= 63	Sickle Cell trait n = 13
Positive	21	10
Negative	42	3



Comparison between HPLC & Super Speed Sickle test in case of Sickle Thal Trait

ISSN: 0975-8585

Super Speed Sickle Test	HPLC			
		Positive	Negative	Total
	Positive	7	14	21
	Negative	3	39	42
	Total	10	53	63

The number of True Positive (TP), True Negative (TN), False Positive (FP) and False Negative (FN) were determined.

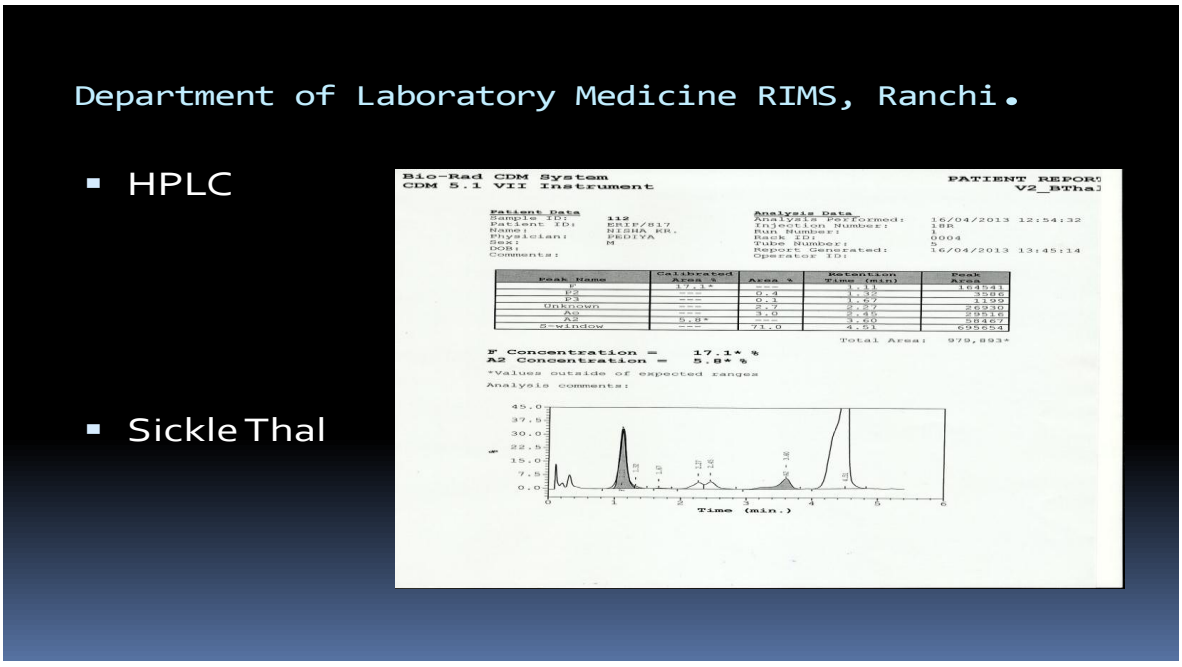
Sensitivity, specificity and predictive values were calculated as follows:

- Sensitivity = TP x 100/TP+FN = 70.0 %
- Specificity = TN x 100/TN+FP = 73.58 %
- Predictive value of a positive test TP x 100/ TP+FP 4) = 33.33 %
- Predictive value of a Negative test = TP x 100/ TN+FN= 92.85 %
- Accuracy = 73.0 %

Positive Super Sickle blood samples are subsequently confirmed for Sickle Thal Trait by HPLC = 10

Table 1: Results of Super Siclkle specially in Sickle Thal Trait

SUPER SICKLE	Total Subject n= 63	Sickle Thal trait n = 10
Positive	21	7
Negative	42	3



DISCUSSION

More than 50% of the total global sickle cell anaemia (SCA) cases are found in India [27].

Sickle Cell Gene In Tribal Area Of Rajnandgaon District Of Chhattisgarh revealed that 9.75% of population is carrying sickle cell gene [27].

In our observations prevalence sickle cell gene is widely spread in district of Ranchi & in surroundings area was 30.0 % . The predictive value of positive and negative solubility test in case of Sickle

Cell Homozygous was 57.14 % and 80. 85 % respectively. The sensitivity of the solubility test was 75.0 %, whereas the specificity was 80.85 %. Some of the solubility tests that were doubtful were labelled as negative. On HPLC all of these turned to be negative, which was responsible for decreasing the specificity percentage in our study.

Much later, Neel [16] and Beet [17] clarified the genetic basis of Sickle cell anemia by demonstrating that hetrozygosity for the sickle cell trait without significant clinical symptoms, while homozygosity resulted in sickle disease.The high prevalence of the gene for sickle cell haemoglobin in the areas of the world where malaria has been common suggests that persons with sickle cell trait have a selective advantage over normal individuals when they are exposed to this disease [18].

It is reported that the steady –state haemoglobin level of patients with sickle cell disease is usually between 5 and 11 gm/dl. The range of red cell densities is increased in sickle cell anemia , but the average cellular MCHC is normal [19,20].

Correct diagnosis depends upon documentation of the presence of sickle haemoglobin, preferably by HPLC . There are rapid methods that are less reliable for the detection of sickle hemoglobin, including the observation of sickling of red cells containing sickle haemoglobin microscopically under a cover slip by suspending the cells in a droplet of 2% solution of sodium metabisulfite[22] and sickle Hb by solubility tests. The solubility test depends on the low solubility of reduced sickle haemoglobin, which results in the development of turbidity under appropriate conditions [21-23].

However, such tests do not detect haemoglobin C or Beta-Thalassemia and do not reliably distinguish between sickle cell trait and sickle cell homozygous and therefore of limited value.

Misunderstanding concerning the significance carrier states as led to unwarranted harm to individuals who are detected as carriers in the screening programmes [24].

Sickle cell gene is widely recognized specially in the central parts of India11. The highest frequency of sickle cell gene in India is reported in Orissa followed by Assam, M.P., U.P., Tamilnadu and Gujarat [26].

Numbers of screening programmes have been carried out in these areas, but very few screening programmes have been carried out in Chhattisgarh [27].

So this screening programme was carried out for detection of the prevalence rate of sickle cell gene in Rachi District of Jharkhand and also to distinguish between Homozygous & Heterozygous so as to prevent unwarranted harms to the individuals who are detected as Heterozygous sothat advised may be given to prevent the birth of homozygous by genetic counseling.

After taking history these subjects were screened by the method of Nalbundian [23] rapid solubility test for detection of HbS. Blood sample of subjects of positive solubility test were subjected to HPLC [28].

Rajnadgaon District is bordered by Vidarbha (Nagpur) Region of Maharashtra state, where high prevalence of sickle cell gene has been found. R.S. Balgir et al. [26] reported a frequency of 20% of sickle cell gene in old Madhya Pradesh that imparts the threatening about this disease in this area. In Rajnadgaon District of Chhattisgarh state [27] prevalence of sickle cell gene was found to be 9.75. %. [27]. The sickle cell gene in this area is not only prevalent in the tribal or schedule caste but it has also penetrated in to the general caste.

The present study is a pilot study on this very genuine health problem; therefore there is an urgent need to do the well-designed and large-scale study involving the most area of Chhattisgarh state. The present study is a pilot study on this very genuine health problem; therefore there is an urgent need to do the well-designed and large-scale study involving the most area of Jharkhand state.

We also refer to the paper by Okwi *et al*. [29] Cost benefit analysis of screening for sickle cell disease (SCD) using different methods cannot be done in isolation.

CONCLUSION

Sickling test (metabisulfide) is cumbersome and time consuming and, thus, it is inconvenient for large sample screening. Also improper sealing by coverslip may lead to drying of sample especially in hot season. Solubility test is better for mass screening, because it is rapid (takes just about 5 min), reliable with minimal observer variation, does not need any microscope and requires a very small blood sample. It is also a cost-effective test.

A faster diagnostic tool for SCD is also needed in developed countries where financial constraints may pose less of a barrier for the conventional diagnostic methodologies to ensure that a patient presenting with symptoms of SCD actually has SCD before beginning treatment.

Reasons for screening: (i) early detection of the disease for timely intervention to minimise morbidity and mortality; (ii) patient and family education on SCD; (iii) genetic counselling as part of a long-term strategy to prevent live homozygous SCD (SS) births; and (iv) short-and long-term cost saving by means of (i), (ii) and (iii) above.

Limitations

This test cannot differentiate between SCA and Sickle cell disease[30]; hence, confirmation is needed by HPLC.

ACKNOWLEDGEMENT

We are thankful to our technician Mr. Jawed Akhtar, Senior Lab. Technician, Department of Laboratory Medicine, RIMS, Ranchi.

REFERENCES

[1] Mousa SA. and MH Qari. Methods Mol Biol 2010;663: 291-307.
[2] Mousa SA and MH Qari. Methods Mol Biol 2010;663: 291-307.
[3] Niscola P, F Sorrentino, L Scaramucci, P de Fabritiis, and P Cianciulli. Pain Med 2009;10(3): 470-80.
[4] Tsaras G, A Owusu-Ansah, FO. Boateng, and Y Amoateng-Adjepong. Am J Med 2009;122(6): 507-12.
[5] Lille RD, Solankhi BR, Bhagwat RB, Ingle VN, Shah PM. J Assoc Physicians India 1962; 10: 263.
[6] Matusik JE, JB Powell and DM Gregory. Clin Chem 1971;17(11): 1081-2.
[7] Pagana KD and TJ Pagana, Mosby's Manual of Diagnostic and Laboratory Tests. 3rd ed. 2006, St. Louis, MO: Mosby Elsevier.
[8] Wethers DL. Am Fam Physician 2000;62(5):1013-20, 1027-8.
[9] Frenette PS and GF Atweh. J Clin Invest 2007;117(4): 850-8.
[10] Wilson C, P Hopkins, B Cabello-Inchausti, S Melnick, and R MJ. Lab Med 2000;31(8): 445-7.
[11] RG Huntsman, GPT Barclay , DM Canning, And GI Yawson. Practical Haematology, Sir John Dacie, Ninth edition, 2001.
[12] Clinical Diagnosis and Management, J.B Henry , 20th Edition, 2001.
[13] Hi Media , Mumbai, India.
[14] Taliajerro WH, Huck JG. Genetics 1923;8: 594.
[15] Neel JV. Science 1949;110: 64.
[16] Beet EA. Ann Evgen (London) 1949;14:279.
[17] Luzzatto L. Blood 1979;54: 961.
[18] Glader BE, Proper RD, Buchanan GR. AM J Clin Pathol 1979;72:63.
[19] Mohandas N, Johnson A, Wyatt J, et al. Blood 1989;74: 442.
[20] Reid CD, Charache S, Lubin B, Johnson C, Ohene frem Pong K. Management and Therapy of sickle cell Diseases. Bethesda, MD, National Institute of Health, Heart, Lung and Blood Institute, PP 96 – 2117, 1995.
[21] Daland GA, Castle WB. J Lab Clin Med 1948;33:1082.
[22] Hanry RL, Nalbandian RM, Nichols BM, et al. Clin Biochem 1971;4:196.
[23] Beutler E, Boggs DR, Heller P, et al. N Engl J Med 1971;285:1485.
[24] Negi RS. Bull Anthropol Survey India 1972;17:439-49.
[25] Balgir RS. JAPI 1996;44:25-8.

[26] Paunipagar PV, Patil SKB, Singh CM, Arya RC. Indian J Prev Soc Med 2006;37(3& 4).
[27] Nalbundian RM, Nichols BM, Camp FR, Lusher JM. Clin Chem 1971;17:1028.
[28] Okwi AL, Ocaido M, Byarugaba W, Ndugwa CM, Parkes A. S Afr Med J 2009;99(12):887-891.
[29] Hunstman RG, Barclay GP, Canning DM, Yawsen GT. J Clin Pathol 1970; 23: 781–3.