ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 01 | January - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

LABORATORY PARAMETERS IN STUDY OF SICKLE CELL DISEASES AT TERTIARY CARE CENTRE (A STUDY OF 200 CASES).

Pathology

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ABSTRACT

INTRODUCTION: Sickle cell anaemia is an inherited disease characterized by the presence of an abnormal haemoglobin called haemoglobin S (HbS). During deoxygenation, the red blood cell (RBC) shape changes from the biconcave shape to the sickle shape due to the abnormal haemoglobin. The shape of the RBC changes back to the biconcave shape after reoxygenation. However, the frequent sickling and unsickling leads to haemolysis and anaemia. This distortion prevents the cell from passing through small blood vessels; leading to occlusion of vascular beds, followed by tissue ischemia and infarction.

AIMS & OBJECTIVES

- To evaluate HbS level by HPLC with clinical and haematological parameters .
- To classify the Sickle cell diseases based on genetic abnormality, which is evaluated by HPLC findings.
- To determine incidence of age and sex groups associated with Sickle cell diseases.

MATERIAL AND METHODS: The study design is Hospital Based Analytical Observational Study conducted at a tertiary centre in B. J. medical college, civil hospital, Ahmedabad. The present study was carried out during the period of 2 years from august 2017 to August 2019. During this period total 200 cases of positive Sickle cell were found and their Clinical details and laboratory parameters were studied. The data was retrieved from computerised software laboratory information system (LIS). **RESULTS:** Sickle cell diseases classified as Sickle cell anaemia (HbSS), Sickle cell trait (HbAS), Sickle cell (HbS/β-Thalassemia) based on HPLC findings. In the present study 92 % cases were sickle cell anaemia (Homozygous state), 6% were sickle cell trait (Heterozygous state) and 2 % were Sickle cell β Thalassemia (Double heterozygous state). In the present study the cases are mainly from the rural area (74 %). Peak age of presentation of Sickle cell Diseases was 13 to 20 years age in male (20%), and 20 to 30 years in female (20%) noted in present study. **CONCLUSIONS:** Apart from appearance of HbS on HPLC, low levels of HbA and high levels of HbA2 should raise a suspicion for presence of sickle cell haemoglobinopathy. HPLC is reliable method for screening and diagnostic purpose. Sickling test is also an acceptable method for purpose of screening.

KEYWORDS

Sickle cell anaemia, Sickle cell trait, HPLC.

INTRODUCTION:

Sickle cell anaemia is an inherited disease characterized by the presence of an abnormal haemoglobin called haemoglobin S(HbS). During deoxygenation, the red blood cell (RBC) shape changes from the biconcave shape to the sickle shape due to the abnormal haemoglobin. The shape of the RBC changes back to the biconcave shape after reoxygenation. However, the frequent sickling and unsickling leads to hemolysis and anaemia [1].

There are three types of normal haemoglobin: haemoglobin A(HbA), haemoglobin F(HbF), and haemoglobin A2(HbA2).Sickle cell anaemia (SCA) is due to the substitution of adenine with thymine in the glutamic DNA codon (GAG→GTG), which results in turn, in substitution of β6 valine for glutamic acid. Patients with sickle cell anaemia (homozygous to HbS gene) have HbS instead of HbA associated with formation of HbF and HbA2. Some patients with sickle cell disease (double heterozygous) have got HbS together with other types of abnormal haemoglobin or even they are sickle-thalassemia [2].

DIAGNOSIS: Initial or first step of diagnosis for this disease is carrying out blood test and it is known as simple Sickling test and goes for further testing if required. On the basis of test we can indentify whether the patient is belongs to homozygous or heterozygous .Sickle gene is inherited from parent to child. Parent suffering from Sickle Cell abnormality, the child may be inherited Sickle Cell from parent and become as Sickle trait or can be normal. Person having Sickle trait known as heterozygous[3]. High-performance liquid chromatography (HPLC) is used to confirm the diagnosis. Genetic study is not frequently done as electrophoresis and HPLC are accurate in detecting HbS and HbC [4].

LABORATORY FEATURES: The haemoglobin level is usually between 5 and 11 g/dL. Anaemia is normochromic and normocytic, but considerable variation in red cell size and shape is noted. Sickled cells and target cells are seen on the blood. Reticulocytosis is almost always present. Leukocytosis and thrombocytosis are common, even in patients without acute problems; these may be caused by a reactive marrow along with demargination of peripheral leukocytes, and possibly contributed by functional asplenia [5].

Furthermore, there is no widely acceptable and readily available cure for patients with SCA at present. Curable methods such as gene therapy and bone marrow transplantation, which may be associated with several complications, are not readily available in developing nations. Many patients with SCA are in reasonably good health most of the time and achieving a steady state level of fitness [6,7,8].

MATERIAL AND METHODS:

This study will be a Analytical observational study of all positive Cases received submitted to the pathology laboratory of BJ Medical college, Ahmedabad from August 2017 to August 2019 in which Sickle shape RBCs were observed on peripheral blood smear examination and positive Sickling test. For this purpose, Peripheral blood will be drawn from all study subjects under aseptic precautions in EDTA vacutainer. Peripheral smear examination will be done with slides stained in Giemsa stain. Haemogram will be obtained for each case using automated methodology analyser Horriba Penta XLR. Haemoglobin variants HbS detected by performing test on an instrument manufactured by Bio-Rad utilizes the principle of "High Performance Liquid Chromatography". Sickling test was done by using sickling solution made by sodium Bimetathiosulphate powder with using DPX Mountant for sealing of slide with coverslip. Reticulocyte count with using supravital stain Methylene blue .

ETHICAL CONSIDERATIONS:

All procedures performed were in accordance with the ethical standards of the institution.

OBSERVATION AND RESULTS:

Sickle cell diseases classified as Sickle cell anaemia (HbSS), Sickle cell trait (HbAS), Sickle cell (HbS/β-Thalassemia) based on HPLC findings. In the present study the 148 (74%) cases are mainly from the rural area. Peak age of presentation of Sickle cell Diseases was 13 to 20 years age in male (20%), and 20 to 30 years in female (20%) noted in present study. In looking at age distribution, we concluded that Sickle cell trait and Sickle cell Anaemia mainly occur in Adult age group in 21 to 30 years of age and shows male preponderance (54.5%) in the

present study. RDW is also high in Sickle cell anaemia as it is due to changes in shape of RBCs. In the present study 115(57.5%) cases have ≥16.1 RDW level. Among 200 cases, 132(66%) cases show elevated S. billirubin level range between 1.1 to 4 mg/dl. In majority of cases are admitted in hospital on sickle cell crisis basis and have haemolytic picture on smear examination. 193(96.5%) cases have low HCT level ≤35%. A various haematological parameters such as Haemoglobin, RBCs indices, RBCs morphology on peripheral smear examination and Reticulocyte count were studied and results are given on table No. 1 to 6.

TABLE 1: DISTRIBUTION ACCORDING TO HB LEVEL

Hb level	No. of Male	No. of Female	Total No. & percentage
≤ 6	15 (7.5%)	15 (7.5%)	30 (15%)
6.1 to 10	81 (40.5%)	64 (32%)	144 (72%)
≥ 10.1	13 (6.5%)	12 (6%)	26 (13%)
			Total =200

TABLE 2: DISTRIBUTION ACCORDING TO MCV RANGE

MCV(fl)	No. of Male	No. of Female	Total no. of patient & percentage
≤60	0	2 (1%)	2 (1%)
60.1 to 80	36 (18 %)	23 (11.5 %)	59 (29.5%)
80.1 to 100	61 (30.5%)	52(26%)	113 (56.5%)
≥101	12 (6%)	14 (7%)	26 (13%)
			Total =200

TABLE 3: DISTRIBUTION ACCORDING TO MCH RANGE

MCH (pg)	No. of Male	No. of Female	Total no. of patient & percentage
≤ 27	56 (28%)	45 (22.5%)	101 (50.5%)
27.1 to 30	28 (14 %)	21 (10.5%)	49 (24.5%)
≥ 30.1	25 (12.5%)	25 (12.5%)	50 (25%)
			Total =200

TABLE 4: DISTRIBUTION ACCORDING TO MCHC RANGE

MCHC (gm/dl)	No. of Male	No. of Female	Total no. of patient & percentage
≤ 33	83 (41.5%)	74 (37.5%)	157 (78.5%)
33.1 to 37	43 (21.5%)	26 (13 %)	43 (21.5%)
≥ 37.1	0	0	0
			Total =200

TABLE 5: DISTRIBUTION ACCORDING TO RBCs MORPHOLOGY ON PERIPHERAL SMEARS

FINDINGS	NO. OF CASES & PERCENTAGE
ANISOCYTOSIS	
Microcytic RBCs	40 (20%)
Macrocytic RBCs	6 (3%)
Normocytic RBCs	33 (16.5%)
Dimorphic population	121 (60.5%)
POIKILOCYTES	
Target cell	138 (69%)
Tear drop cell	77 (38.5%)
Elliptocytes	184 (92%)
Polychromasia	97 (48.5%)
Basophillic stippling	4 (2%)
Leptocytes	25 (12.5%)
Howell jolly bodies	25 (12.5%)
Sickle shape RBCs	193 (96.5%)

TABLE 6: DISTRIBUTION ACCORDING TO RETICULOCYTE COUNT

Reticulocyte (%)	No. of Male	No. of Female	No. of patient & percentage (%)
≤2.5	51(25.5 %)	38 (19 %)	89 (44.5%)
2.6 to 10	52 (26%)	48 (24%)	100 (50%)
≥10.1	5 (2.5%)	5 (2.5 %)	10 (5%)

TABLE 7: CLASSIFICATION OF SICKLE CELL ANAEMIA BASED ON HPLC FINDINGS

HPLC PATTERN	No. of patients	Status of patient
>50 % of HbS, HbF <2%	184 (92 %)	Homozygous state - Sickle cell Anaemia (HbSS)
<50 % HbS, HbA >50 % (HbA > HbS)	12 (6%)	Heterozygous state - Sickle cell trait (HbAS)
>50% HbS, HbF >2%, HbA2 > 3.5%	4 (2%)	Sickle cell β Thalassemia (Hb S/β0/+)

As per Table no.7, 92 % cases were sickle cell anaemia (Homozygous state), 6% were sickle cell trait (Heterozygous state) and 2 % were Sickle cell β Thalassemia (Double heterozygous state).

TABLE 8: COMPARISON OF MEAN VALUE HPLC FINDINGS WITH OTHER STUDY

	Mean value Hb A2	Jawarkar A et al9 Mean value Hb A2	Mean Hb F	Jawarkar A et al9 Mean value Hb F	Mean HB S	Jawarkar A et al9 Mean value HB S
Sickle cell anaemia (HbSS)	2.59	3.4	8.13	17.3	80.6	74
Sickle cell trait (HbAS)	2.93	3.17	0.75	1.13	25.86	28.92
Sickle cell Thalassemia (HbS/β0/+)	21.07	-	15.9	-	66.8	-

As per table no. 8 it is reflected that in Sickle cell trait patient had higher level of HbS and relative normal level of HbA2 and lower level of HbA. HbF level is not significant statistically in this study. In Sickle cell anaemia patients, there were significant higher level of HbS and HbF. In sickle cell β thalassemia patient, there were significant higher level of HbF, HbA2, HbS in the present study.

TABLE 9: RARE INCIDENCE OF MALARIA IN 200 CASES OF SICKLE CELL DISEASES.

NO. of patient	Type of malaria	Age	Sex
1 (0.5%)	Trophozoite form of p. vivax	32	Male
1 (0.5%)	Gametocyte form of p. falciparum	20	Male
Total=2 (1%)			

As per Table no.9, malaria infection is very rare in sickle cell disorder. In the present study we found 2(1%) cases of malarial infection during peripheral smear examination. It is shown that 0.5% of incidence of p. vivax and p.falciparum malaria infection in present study. Both cases have diagnosed as homozygous Sickle cell Anaemia (HbSS) on HPLC finding.

SUMMARY &CONCLUSION:

We conclude that sickle cell haemoglobinopathies are very common in our population which consists of a large proportion of tribals area. Sickle cell disease is more common than trait in our geographic area which has to do with consanguineous and same caste marriages and lack of prenatal counselling and detection. Apart from appearance of HbS on HPLC, low levels of HbA and high levels of HbA2, HbF should raise a suspicion for presence of Sickle cell haemoglobinopathy.

- HPLC is reliable method for screening and diagnostic purpose.
- Sickling test is also an acceptable method for purpose of screening test.
- As per HPLC reports, Out of 200 cases 92% patients showed Sickle cell Anaemia, 6% patients showed Sickle cell trait, and 2% patients showed Sickle cell-β thalassemia.
- Out of 200 cases 96.5% cases show Sickle shape RBCs on peripheral Smear examination & all cases having a Sickling test positivity.
- Maximum number of cases (72%) have Haemoglobin range between 6.1 to 10 gm/dl.
- 56.5% cases show MCV range between 80.1 to100 fl, 50.5% cases show MCH range <27 pg, 78.55% cases show MCHC range < 33gm/dl.
- Peripheral Smear examinations show mainly Haemolytic picture and Moderate to Severe anaemia with mainly Sickle shaped RBCs (96.5%), Elliptocytes (92%) and Target cell (69%).
- In the present study, 50 % cases have high Reticulocyte count.
- Rare 1% incidence of malarial infection in Sickle cell anaemia found in present study.

For marriages, instead of matching HOROSCOPE of brides and groom, certificate of Sickle cell anaemia test should be matched. It will

be a great help to mankind by Sickle cell diseases and giving birth to healthy baby and healthy society.

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