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Study of sickle cell disease by hematological parameter and HPLC at tertiary care center

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Abstract
Background: Sickle Cell Disease is an inherited disease characterized by the presence of an abnormal hemoglobin called hemoglobin S (HbS) which is mutated form of hemoglobin. In this study we want to profile various types of hemoglobin's and their relative percentage in sickle cell cases. Also, we will analyse RBC indices such as Hb, MCV, MCH, MCHC.
Ams and Objectives: To study various hematological parameters and peripheral blood smear findings of sickle cell disease, to determine incidence of sickle cell disease according to age and sex group and classify sickle cell disease based on HPLC findings.
Methods: We analyzed blood from 145 patients suspected to have Sickle cell hemoglobinopathies and subjected it to Sickling screening test. All positive cases will be subjected to HPLC to separate hemoglobin and CBC analysis was done to check RBC indices.
Results: 51% patients were having sickle cell trait (AS), 9% showed sickle cell disease (SS) and 40% were sickle beta thalassemia (Sβ). In sickle cell disease patients, there were significantly higher levels of HbA2, HbF and HbS and significantly lower levels of HbA. Both sickle cell trait and sickle cell disease patients had significantly lower levels of MCH.
Conclusion: Peripheral blood smear examination, Sickling test and HPLC findings are very important investigations to diagnose Sickle Cell Disease. Sickle cell disease is very common in our populations mainly in rural area. High index of suspicion should be maintained when these parameters are on lower side, especially in population who is prone to have sickle cell disorders.

Keywords: HPLC, hematological parameter, Sickle cell disease

Introduction

- Sickle Cell Disease is an inherited disease characterized by the presence of an abnormal hemoglobin called hemoglobin S (HbS) which is mutated form of hemoglobin [1].
- It is due to the substitution of glutamic acid by valine at the 6th amino acid position in the β globin polypeptide chain [2].
- Although SCD is primarily a disorder of the red cells, it has been found that the white blood cells (WBC) and the platelets are also affected by the inherited mutation [3].
- Clinical presentation of sickle cell disease is variable, with some patients having a normal life; however, some patients show increased morbidity and mortality due to Vaso-occlusive, severe thrombotic, aplastic and sequestration crisis [4, 5].
- Initial step of diagnosis is carrying out complete blood count and sickling test followed by Hemoglobin Electrophoresis and High-Performance Liquid Chromatography (HPLC) is used to confirm the diagnosis.

Aims and Objectives

- To study various hematological parameters and peripheral blood smear findings of sickle cell disease.
- To determine incidence of sickle cell disease according to age and sex group.
- To classify sickle cell disease based on HPLC findings.

Methodology

- The Retrospective study of 145 cases was carried out at the Department of Pathology, B.J. Medical College and Civil Hospital, Ahmedabad (tertiary care center) for a period

of 8 months (May 2023 to December 2024).

- Blood sample is collected in EDTA vacuette tube for hematological study and for HPLC analysis.
- Various hematological parameters received in computerized software Laboratory Information System such as hemoglobin, MCV, MCH, MCHC, anisocytosis, poikilocytosis were evaluated.
- Study of sickle cell disease according to patient's details like age, sex and locality were also included in this study
- The ultimate diagnostic tool was to classify Sickle cell disease according to HPLC findings.

Inclusion criteria

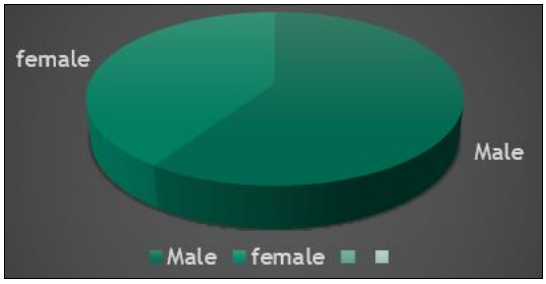
- Findings of sickle shaped RBCs on peripheral blood smear.
- Positive sickling test.
- Siblings and family members in case of abnormal Hb variant.

Exclusion criteria

Rejected samples in pre-analytical phase-e.g. samples not adequate, clotted samples etc.

Results

- In the present study, total 145 cases were studied during period of 8 months from May 2023 to December 2023.
- In this retrospective study, sickle shaped RBCs observed on peripheral blood smear examination and sickling test positivity was studied.
- Classification of Sickle cell disease done according to HPLC findings.



As per this chart most of the patients were male

Fig 1: Sex wise distribution of 145 cases of sickle cell disease

Table 1: Age wise distribution of 145 cases of sickle cell disease

Age(years)	Total no.	No. Of male	No. Of female
<12 Year (pediatric age group)	29(20%)	18(12.4)	11(7.5%)
13-20	39(27%)	24(16.5%)	15(10%)
21-30	47(32.4%)	27(18.6%)	20(13.7%)
31-40	18(12.4%)	10(9%)	08(5.5%)
41-50	09(6.2%)	06(4%)	03(2%)
>=50	03(2%)	01(0.6%)	02(1.3%)
Total	145(100%)	86(60%)	59(40%)

The predominant age group detected was 13-30 years of age

Table 2: Distribution of cases according to clinical history

HISTORY	NO. OF MALE	NO. OF FEMALE
Breathlessness and palpitation	23	35
Generalized weakness and fatigue	48	43
Pain over joints, hands and feet	59	38
Hematuria	08	14
Vaso-occlusive crisis	36	16
Hand foot syndrome	07	06
Abdomen-chest pain	27	18

As per clinical history, most common reason for hospitalization was vaso-occlusive crisis in male and joint pain in females

Table 3: Distribution of cases according to Hemoglobin (Hb), MCV, MCH and MCHC level

Parameter	level	No. of cases
HB	<=6	15(10.3%)
	6.1-10	87(60%)
	>=10	43(29.6%)
MCV	<60fl	07(4.8%)
	60.1-80 fl	33(22.7%)
	80.1-100	86(59%)
	>101	19(13%)
MCH	<27	76(52%)
	27.1-30	33(22.7%)
	>30.1	36(24.8%)
MCHC	<33	115(79.3%)
	33.1 - 37	29(20%)
	>37.1	01(0.6%)

As per this table most of the patients had Hb level within 6 to 10 mg/dl range, MCV within normal range, MCH value and MCHC value was significantly decrease in majority of the patients

Table 4: Distribution of cases according to findings on Peripheral Smear

Findings of poikilocytosis	No. Of patients
Target cell	96
Tear drop cell	32
Elliptocyte	127
Polychromasia	68
Basophilic stippling	05
Laptocyte	21
Howell-jolly bodies	19
Sickle shaped RBCs	138

On peripheral blood smear examination, most of the cases had dimorphic and microcytic pictures. In poikilocytosis, sickle shaped RBCs were most common

followed by elliptocytes, target cells, and polychromasia

Table 5: Classification of Sickle Cell Anemia based on HPLC findings

Hplc level	No. Of patients	Status of patient
>50% of HbS, <2% of HbF	13(9%)	Homozygous state: Sickle Cell Anemia (HbSS)
<50% of HbS, HbA>50% (HbA>HbS)	74(51%)	Heterozygous state: Sickle cell trait (HbAS)
>50% of HbS, HbF>2%, HbA2>3.5%	58(40%)	Sickle cell beta thalassemia (HbS/beta 0/+)

As per this table 74 patients were having sickle cell trait (AS) (51%), 13 showed sickle cell disease (9%) (SS) and 58 were sickle beta thalassemia (Sβ) (40%).

Discussion

- The study of total 145 cases was carried out in which positive sickling test and sickle shaped RBCs were found on peripheral blood smear examination.
- According to the study most of the patients were male. The predominant age group was 13-20 years in male and 21-30 years in female.
- SCD was found to be more common male in the present study similar in the study done by Thakkar CC *et al.* [10]
- In the present study, the most common age group affected in SCD was 13 to 30 years age group, which is agree with the studies done by Akinbami A *et al*., Thakkar CC *et al.* and Emmanuelchide O *et al.* [9, 10, 11].
- Reduced Hb among SCD patients was also seen in the studies conducted by Jawarkar A *et al.* and Akinbami A *et al.* [8, 9].
- In the studies conducted by Akinbami A *et al.*, Rao SS *et al.* and Antwi Boasiako C *et al.*, MCV and MCHC was found to be signicantly decreased in SCD patients. Which is also seen in present study [12, 13].
- According to clinical history, most common reason for hospitalization is vaso-occlusive crisis in male and joint pain in female. Majority of patients had moderate to severe anemia with generalized weakness. Most of the patients had Hb, MCH and MCHC values were significantly decreased.
- On peripheral blood smear examination, most of the RBCs had dimorphic picture with poikilocytosis, sickle shaped RBCs followed by elliptocytes, target cells and polychomasia were noted.
- Analysis of HPLC patterns revealed the following findings: 74 patients were having sickle cell trait (AS) (51%), 13 showed sickle cell disease (9%) (SS) and 58 were sickle beta thalassemia (Sβ) (40%).

Conclusion

- Sickle cell disease is very common in our populations mainly in rural area.
- Most common clinical presentation is vaso-occlusive crisis followed by joint pain.
- Peripheral blood smear examination, Sickling test and HPLC findings are very important investigations to diagnose Sickle Cell Disease.
- Hb-S and Hb F in HPLC, when compared to heterozygous sickle cell disease patients in our study. Thus, these parameters can serve as a useful preliminary tool in assessing patients with SCD.
- There is no widely acceptable and readily available cure for patients with sickle cell anemia 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. Instead of it, supportive management, symptomatic treatment, preventive management and curable approaches are used.

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Author's Contribution
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Conflict of Interest
Not available.

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