STUDY OF SICKLE CELL CASES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) PATTERNS IN TERTIARY CARE HOSPITAL

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Introduction

- Haemoglobinopathies are inherited disorders characterised by either an abnormality in the structure of haemoglobin such as in sickle cell anaemia or reduced production of one or more globin chains in thalassaemia[1].
- Sickle cell anemia (SCA) is the most common, accounting for about 70% of the world's major haemoglobinopathies[2]. About 5% of the world's populations are carriers of genes responsible for haemoglobinopathies and about 300,000 children are born annually with haemoglobin disorders. Sickle cell disease (SCD) results from a single amino acid substitution in the gene encoding the β-globin subunit (β6Glu > Val) that produces the abnormal hemoglobin (Hb) named Hb S.

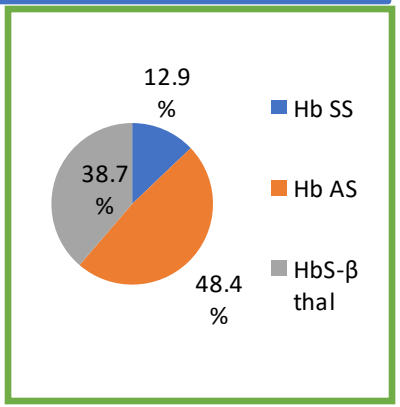
Material and method

- This is a cross sectional study in which a total of 187 sickle cell patients were recruited for the study. These patients comprised of 98 males and 89 females attending Dhiraj general hospital, Piparia, Vadodara, Gujarat, were recruited for period of 3(three) months and 10 days from September 2023 to 10th December 2023. Pregnant women were excluded from the study in order to prevent variations in HbF or HbA2 concentration which may be induced by physiological stress of pregnancy.
- 2.5ml of venous blood was collected in an ethylenediamine tetra acetic acid (EDTA) anticoagulated bottle after obtaining written informed consent from parents/guardians.
- High Performance Liquid Chromatograph of samples was carried out using Bio-Rad Laboratories, Hercules, CA. Retention time (RT) is the elapsed time from the sample injection to the apex of a haemoglobin peak & each haemoglobin variant has its unique RT. The printed chromatogram shows all the haemoglobin variants eluted, the retention times, the areas of the peaks and the values (%) of different haemoglobin components. Patients with HbS and elevated A2>= 4.0 % were considered as Sβ thalassemia trait.

Result

- A total of 293 cases were studied, out of these 187(63.7%) cases were HbS positive on HPLC. Our institute is situated near the sickle belt of Gujarat, so that's the reason we get good number of HbS positive patients.
- From all 187 cases of HbS positive, 24(12.9%) were HbSS, 91(48.4%) were Hb AS and 72(38.7%) were double heterozygous for HbS-β thalassemia.
- Common pattern seen in our study were sickle cell trait i.e. 91 cases (48.4%). It showed S window between 20-30%. In Hb SS, Hb F was around 5- 25% and Hb S was 60-90%. In double heterozygous state, Hb A2 was between 4-9%.

Month (2023)	Total cases	HbS positive
Sept.	94	67
Oct.	88	56
Nov.	91	55
Dec.	20	9
Total	293	187 (63.7%)



Months (2023)	HbS positive	Hb SS	Hb AS	HbS-β thal
Sept.	67	12	30	25
Oct.	56	10	26	20
Nov.	55	1	31	23
Dec.	9	1	4	4
Total	187	24(12.9 %)	91(48.4%)	72(38.7%)

Discussion

- Sickle cell Anemia is prevalent in east and west areas of Gujarat. The use of HPLC is increasing for separation and quantity measurement of various abnormal hemoglobin. It is a reliable tool for early and accurate measurement so it is used in prevention and management of various hemoglobinopathies. In our study, we diagnosed cases on HPLC showing S window along with considering percentage of Hb F, Hb A2 and Hb A.
- Most common pattern was Sickle cell trait because our institute is situated near the sickle belt of Gujarat.
- Double heterozygous states of Hb S- β thalassemia were also found in significant percentage (38.7 %). Therefore, its important to consider β thalassemia in sickle cell anemia patients.

Conclusion

- These findings confirm that the frequency of beta thalassaemia in sickle cell patients in Gujarat is higher. It is therefore important to consider the possibility of this variant in patients with sickle cell anaemia since their course may differ from that of patients with homozygous sickle cell anaemia.
- Sickle cell anemia is relatively more among males (52.1%) than females (47.8 %) out of all patients.

References

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2. Angastiniotis, Modell B, Englezos P, Boulyjenkov V. Prevention and control of haemoglobinopathies. World Health Organisation. Sickle cell disease in the African Region: WHO Africa Regional Report. AFR/RC/56/17.