Study of sickle cell cases by High Performance Liquid Chromatography (HPLC) Patterns in tertiary care hospital

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Abstract

Background

Genes for haemoglobin S are found in high frequencies in Gujarat. The clinical presentation of HbS- β thalassemia is enormously variable, ranging from an asymptomatic state to a severe disorder similar to homozygous sickle cell disease.

Materials and Methods

Haemoglobin A_2 and HbF were determined in sickle cell anaemia patients attending Dhiraj general hospital, wagodia, Vadodara by high performance liquid chromatography (HPLC). Hematological parameters were estimated using Sysmex KX-21 and peripheral blood smear examination was assessed using Romanosky staining technique.

Results

A total of 596 cases were studied, out of these 380 (63.7%) cases were HbS positive on HPLC. From all 380 cases of HbS positive, 49(12.9 %) were HbSS , 184 (48.4 %) were Hb AS and 147 (38.7%) were double heterozygous for HbS-β thalassemia.

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.

Keywords

HPLC, thalassemia, Sickle cell disease

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]. It comprises sickle cell disease and other compound heterozygous state such as haemoglobin SC disease, Sβ-thalassaemia, and SD-Punjab. 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. [3]

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. SCD has different genotypes with substantial variations in presentation and clinical course [4,5] The combination of the sickle cell mutation and beta-thalassemia (β-Thal) mutation gives rise to a compound heterozygous condition known as Hb S/β thalassemia (Hb S/β-Thal), which was first described in 1944 by Silvestroni and Bianco. [6]

Sickle beta-thalassaemia (S/beta-thalassaemia) is a condition, which results from coinheritance of a sickle cell gene and a beta-thalassaemia gene. The clinical phenotype depends on the type of beta-thalassaemia gene (beta (+) or beta (o)) inherited.[7]

Current methods used by laboratories in the evaluation of haemoglobin disorders include sickle solubility test, alkaline and acid electrophoresis, isoelectric focusing (IEF), high performance liquid chromatography (HPLC), capillary electrophoresis, globin chain electrophoresis and DNA analysis/protein analysis . However, it is important to stress that in conjunction with any of these methods, evaluation of the peripheral blood smear, as well as correlation with the results of a full blood count(FBC) are very important as many of the clinically significant haemoglobin disorders show characteristic peripheral blood findings, and are often co-inherited.[8].

The present study evaluated the high performance liquid chromatographic patterns of sickle cell patients to determine frequency of sickle cell disease, trait and the co-inheritance of HbS and Beta thalassemia in our institute.

Material and method

This is a cross sectional study in which a total of 380 sickle cell patients were recruited for the study. These patients comprised of 198 males and 182 females attending Dhiraj general hospital,

Piparia, Vadodara, Gujarat, were recruited for period of 7(seven) months from July 2019 to January 2020. 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 [9].The sample size of 380 subjects recruited in this study was obtained using Thumb's rule statistical formula [10].

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. The chromatographic patterns were evaluated for the identification and quantification of different Hb variants. Each haemoglobin variant has its characteristic retention time. Retention time is the elapsed time from the sample injection to the apex of a haemoglobin peak. The printed chromatogram shows all the haemoglobin variants eluted, the retention times, the areas of the peaks and the values (%) of different haemoglobin components. If a peak eluted at a retention time that is not pre-defined, it is labelled as an unknown.

Patients with HbS and elevated A2>= 4.0 % were considered as Sβ thalassemia trait.

Result

A total of 596 cases were studied, out of these 380 (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 380 cases of HbS positive, 49(12.9 %) were HbSS , 184 (48.4 %) were Hb AS and 147 (38.7%) were double heterozygous for HbS-β thalassemia.

Common pattern seen in our study were sickle cell trait ie. 184 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 %.

Table 1: Total Hb S positive cases per month

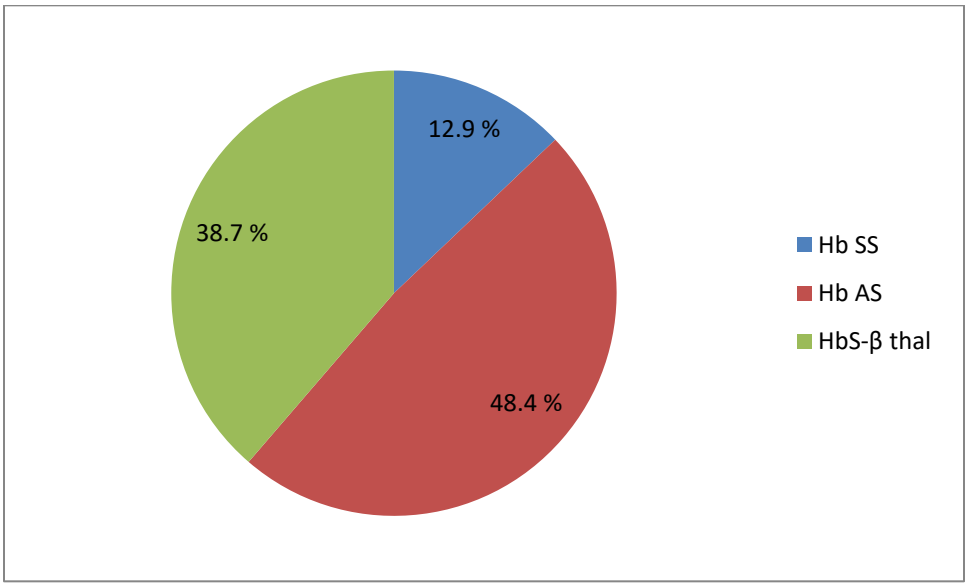
Months (2019-20)	Total cases	HbS positive
July	85	58
August	66	40
September	94	57

October	88	56
November	91	45
December	89	58
January	93	66
Total	596	380 (63.7%)

Table 2: Variants of Hb S diagnosed on HPLC per month

Months (2019-20)	HbS positive	Hb SS	Hb AS	HbS-β thal
July	58	11	33	14
August	40	7	14	19
September	57	4	26	27
October	56	6	36	14
November	45	5	22	18
December	58	6	25	27
January	66	10	28	28
Total	380	49(12.9 %)	184 (48.4%)	147 (38.7%)

Figure 1: Percentage of different HbS in seven months



Discussion

Sickle cell Anemia is prevalent in east and west areas of Gujarat.

It is a monogenic disease that characterizes the homozygous state of hemoglobin S (Hb S) [11]. The complex pathophysiology includes anaemia, vaso occlusive crises, aplastic crises, sequestration crises and hemolytic crises all of which could be of acute or chronic course and may eventually lead to death [12].

The use of HPLC is increasing for separation and quantity measurement of variousabnormal 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.

Molecular methods can be used for diagnosing exact type of mutation in double heterozygous cases of Hb S-β thalassemia.

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.

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