



Original Article

Variation in Hemoglobin A2 levels in Sickle cell disease, Sickle cell trait and sickle cell-beta thalassemia: A south Gujarat study

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ABSTRACT

Background: Sickle cell disorders are inherited hemoglobinopathies caused by presence of abnormal haemoglobin S (HbS). Hemoglobin A2(HbA2) is an important parameter in evaluation and differentiation and of various hemoglobinopathies, particularly in case of sickle cell disorders.

Aim & Objective: To evaluate and compare HbA2 levels in sickle cell disease, sickle cell trait and sickle cell-beta-thalassemia.

Material & Methods: An observational cross-sectional study was conducted over a period of 1 year among patients diagnosed with sickle cell disorder. EDTA-anticoagulated blood samples were analysed using HPLC (High performance liquid chromatography) on the BIORAD-10. HbA2 levels were evaluated and compared among different categories of sickle cell disorders.

Result: The study demonstrated variation in HbA2 levels among different sickle cell disorders. HbA2 levels were higher in patients with sickle cell-beta thalassemia compared with those with sickle cell disease and sickle cell trait. Elevated HbA2 levels were observed in 18.2% of patients with sickle cell disease and 16.1% of patients with sickle cell trait.

Conclusion: HbA2 estimation is an important component in interpretation of sickle cell disorders. Elevated HbA2 may occur in patients with sickle cell disease and sickle cell trait, therefore, HbA2 value should be interpreted in conjugation with other HPLC parameters, red cell indices and relevant clinical findings for accurate diagnosis.

Keywords: Sickle cell disorders, Hemoglobin A2, HPLC, Sickle cell disease, Sickle cell trait, sickle cell-beta thalassemia.

INTRODUCTION

Hemoglobin disorders are frequent genetic disease affecting 7% of world population. Hemoglobinopathies results from structural defect in globin chain whereas thalassemias are due to quantitative defect in globin chain production¹.

Sickle cell disease is among most prevalent monogenic disorder in India. It is characterised by presence of homozygous Hemoglobin S (HbS) or HbS in association with other haemoglobin variants. Sickle cell disease results from inheritance of beta-globin gene mutation that produces haemoglobin S. These genes are frequently observed in tribal region. Quantification of HbA₂ level using High performance liquid chromatography (HPLC) may help distinguish sickle cell disease, sickle cell trait from sickle cell-beta thalassemia².

Sickle cell-beta thalassemia occurs when patients inherit HbS gene and Beta globin thalassemia gene results in underproduction of beta globin chains in one gene, resulting in mild to moderate symptoms³.

- HbA₂ is minor adult hemoglobin comprising approximately 2.0-3.5% of total haemoglobin in healthy individuals, elevated HbA₂ level considering for beta thalassemia trait⁴.
- HbA₂ elevation is relatively frequent in SCD and SCT and should be interpreted cautiously in the presence of HbS¹.

MATERIAL AND METHOD-

Inclusion criteria- Include all age group diagnosed with sickle cell trait, sickle cell disease and sickle cell-beta thalassemia.

Exclusion criteria- Exclude babies with <6-month age, having blood transfusion history of < 3 months or post blood transfusion sample.

An observational cross section study was conducted in south Gujarat for period of 1 year after taking permission from ethical committee, where positive HPLC report of patients with sickle cell trait, sickle cell disease and sickle cell-beta thalassemia after satisfying inclusion and exclusion criteria were selected. HPLC was used to study various haemoglobin types using BIORAD D-10. BIORAD D-10 is fully automated analyser that perform a test from EDTA sample of whole blood followed by sample dilution. Hemoglobin fractions were separated according to their retention time in stationary phase. The level of each separated haemoglobin fraction was then measured photometrically at 415 nm. HPLC graphs were reviewed to extract level of HbA₂, HbF, HbA₀ and HbS. This study enrolled 96 patients of which 33 were homozygous for HbS, 56 were sickle cell trait and 07 were of sickle cell-beta thalassemia.

RESULT-

A total of 96 cases were included in study. Among these 56(58.3%) patients had sickle cell trait, 33(34.4%) had sickle cell disease and 07(7.3%) were diagnosed with sickle cell-beta thalassemia.

The overall mean age of study population was 23.02 years. The mean age was highest among sickle cell trait 27.38 years, followed by 18.55 years for sickle cell disease and 16.43 years for sickle cell-beta thalassemia.

Of the 96 patients, 62 were females and 34 were males indicating female predominance in study population.

Among 56 cases of sickle cell trait 42(75%) had HbA₂ value <3.5, 5 (8.9%) had HbA₂= 3.5 and 9 (16.1%) had HbA₂>3.5. while 33 cases of sickle cell disease 26 (78.8%) had HbA₂<3.5, 1 (3.0%) had HbA₂=3.5 and 6(18.2%) had HbA₂> 3.5. In case of sickle cell-beta thalassemia all 7 (100%) had HbA₂ >3.5.

All these data show 9(16.1%) cases of sickle cell trait and 6(18.2%) cases of sickle cell disease show more heterozygosity of HbA₂.

Table 1- Demographic characteristics

Variables	SCT	SCD	Sickle cell-B thal	Total
Number of patients	56	33	07	96
Age(Mean± SD)	27.38±16.09	18.55±9.78	16.43±11.83	23.02±14.65
Sex(Male/Female)	Male-16, Female-40	Male-14, Female-19	Male-04, Female-03	Male-34, Female-62

Table 2- Age group wise distribution

Age group	SCT		SCD		Sickle cell-B thal		Total
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	
0-10	06	10.7%	07	21.2%	02	28.6%	15
11-20	13	23.2%	12	36.4%	03	42.9%	28
21-30	22	39.3%	12	36.4%	01	14.3%	35
31-40	06	10.7%	01	3.0%	01	14.3%	08
41-50	03	5.4%	01	3.0%	00	00%	04
≥51	06	10.7%	00	0.0%	00	0.0%	06

Table 3-Sex wise distribution

Sex	SCT		SCD		Sickle cell-B thal		Total
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	
Male	16	28.6%	14	42.4%	04	57.1%	34
Female	40	71.4%	19	57.6%	03	42.9%	62

Table 4- Range of HPLC parameters

Parameters	SCT	SCD	Sickle cell-B thal
HbA ₀	8.70-72.00	2.30-23.10	3.30-61.70
HbA ₂	2.20-4.30	1.00-6.40	4.10-5.70
HbF	0.80-4.80	2.90-29.30	0.90-29.80
HbS window	3.90-38.60	47.10-80.50	23.50-85.90

Table 5- Mean of HPLC Parameters

Parameters	SCT	SCD	Sickle cell- B thal
HbA ₀	56.96 ± 8.35	4.56 ± 4.53	13.60 ± 21.24
HbA ₂	3.20 ± 0.42	2.88 ± 1.35	4.87 ± 0.75
HbF	1.18 ± 0.80	16.30 ± 6.82	12.90 ± 10.15
HbS window	28.25 ± 6.31	68.08 ± 7.20	60.81 ± 19.29

Table 6- Median of HPLC parameters

Parameters	SCT	SCD	Sickle cell-B thal
HbA ₀	58.50	3.30	5.80
HbA ₂	3.20	2.80	5.00
HbF	0.80	16.70	11.65
HbS window	27.95	67.40	63.30

Table 7- HbA₂ distribution

HbA ₂ (%)	SCT	SCD	Sickle cell- B thal
<3.5	42	26	00
3.5-5	14	03	04
>5	00	04	03

Table 8- Frequency of HPLC parameters

Parameters	Frequency	Percentage
SCT	56	58.3%
SCD	33	34.4%
Sickle cell-B thal	07	7.3%

Table 9-Comparison of HbA₂ value

Disorder	Total Number	HbA ₂ <3.5	%	HbA ₂ =3.5	%	HbA ₂ >3.5	%
SCT	56	42	75.0%	5	8.9%	9	16.1%
SCD	33	26	78.8%	1	3.0%	6	18.2%
Sickle cell-B thal	7	0	0.0%	0	0.0%	7	100.0%

DISCUSSION-

India is distinguished by its extensive geographical scope and considerable ethnic diversity, resulting in a diverse spectrum of haemoglobin variant across various region and demographic groups. There is significant prevalence of SCD in certain region of country, simultaneously with beta-thalassemia and iron deficiency anemia¹.

Sickle cell disease (SCD) remains one of the important inherited hemoglobin disorders in India, with a particularly high prevalence in tribal populations of Gujarat, Maharashtra, Chhattisgarh, Odisha, and Madhya Pradesh.^{5,6,7} Early and accurate diagnosis is essential not only for patient management but also for genetic counselling and prevention programmes. High-performance liquid chromatography (HPLC) has become an important laboratory method for screening and characterisation of hemoglobinopathies because it permits simultaneous assessment of HbA, HbA₂, HbF and HbS with good reproducibility.^{8,9,10} However, interpretation of HbA₂ values in sickle cell disorders remains challenging because biological and analytical factors may influence HbA₂ estimation.^{9,11}

The present study included 96 patients with sickle hemoglobinopathies, of whom 58.3% had sickle cell trait (SCT), 34.4% had sickle cell disease (SCD), and 7.3% had sickle cell-beta-thalassemia. The predominance of SCT observed in the present study is consistent with the higher frequency of the carrier state compared with clinically manifest SCD in population-based screening programmes. Studies from India have documented substantial variation in the distribution of HbS across tribal populations, with particularly high frequencies in western and central India.^{9,10,11}

The overall mean age of the study population was 23.02 years, with patients having sickle cell-beta-thalassemia and SCD presenting at a younger age than those with SCT. This may reflect the earlier clinical recognition of symptomatic homozygous and compound heterozygous disorders, whereas individuals with SCT are frequently identified incidentally during screening, family studies, antenatal evaluation, or genetic counselling.^{5,6} The female predominance observed in the present study may be related to the hospital-based nature of the study and greater healthcare utilization or screening among women, particularly during antenatal care; it should not be interpreted as evidence of a biological sex difference because SCD is an autosomal recessive disorder.¹²

The principal objective of the present study was to evaluate HbA₂ values among different sickle cell disorders. HbA₂ estimation is widely used as an important laboratory parameter for detection of beta-thalassemia trait, with HbA₂ values above approximately 3.5% commonly considered suggestive of beta-thalassemia carrier status.^{13,14} However, borderline HbA₂ values require careful interpretation, and different laboratories may use somewhat different diagnostic thresholds.^{14,15} Interpretation becomes particularly difficult in the presence of HbS because chromatographic and biological factors can influence the measured HbA₂ fraction.^{9,11}

In the present study, the mean HbA₂ concentration was 3.20% in SCT, 2.88% in SCD, and 4.87% in sickle cell-beta-thalassemia. As expected, the highest mean HbA₂ concentration was observed in patients with sickle cell-beta-thalassemia. Half of these patients demonstrated HbA₂ values greater than 5%. These findings support the usefulness of HbA₂ estimation as one component of the laboratory assessment for identifying possible coexisting beta-thalassemia.

Our observations are also relevant to the recent Indian study by Pendharkar and Nirmal, which evaluated HbA₂ values in a large cohort of Indian patients with SCD. In that study, 17% of patients had HbA₂ values $\geq 4\%$, and the authors emphasised that elevated HbA₂ in SCD may reflect heterozygosity for beta-thalassemia but may also be influenced by analytical co-elution and other factors. They recommended molecular testing when confirmation is required.¹ The presence of elevated HbA₂ in a subset of patients with SCD in this study therefore supports the need for cautious interpretation as 18.2% SCD and 16.1% SCT cases had HbA₂ >3.5 .

This observation emphasises that isolated HbA₂ estimation should not be considered sufficient for diagnosing sickle cell-beta-thalassemia. Correlation with clinical findings, family history, complete blood count, red-cell indices, HbA/HbS pattern, and, when necessary, molecular genetic studies remain important.^{1,9,11}

Similarly, HbS percentages followed the expected pattern, with the highest values observed in SCD followed by sickle cell-beta-thalassemia and SCT. HbA levels were markedly reduced in SCD and sickle cell-beta-thalassemia, reflecting absent or reduced production of normal β -globin chains in these disorders. These findings are consistent with established HPLC patterns of sickle hemoglobinopathies and support the usefulness of automated HPLC as an initial diagnostic modality.^{8,9,10}

The major strength of our study is that it provides regional data from South Gujarat, an important endemic area for sickle hemoglobinopathies in western India. Simultaneous evaluation of SCT, SCD, and sickle cell-beta-thalassemia enabled comparison of HbA₂ and other haemoglobin fractions within the same study population using a standardized HPLC-based approach.

Limitations of our study are limited sample size and unavailability of molecular testing.

CONCLUSION-

This study analysed various sickle cell disorders shows that 18.2 % SCD and 16.1% SCT cases show elevated HbA₂ value than usual. HbA₂ values should always be interpreted with other HPLC parameters, various blood indices and clinical findings. Because HbA₂ may be influenced by iron deficiency, megaloblastic anemia, HbS-related analytical factors, alpha-thalassemia and by coinheritance of beta-thalassemia or other genetic factors, molecular confirmation should be considered in diagnostically challenging cases. Accurate interpretation of HPLC findings can improve diagnostic precision, facilitate appropriate genetic counselling, and strengthen screening programmes in regions with a high prevalence of hemoglobinopathies.

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