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Evaluation of HPLC patterns and red cell parameters in sickle cell anemia cases

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Abstract
Background: Sickle cell disease (SCD) and its variants are genetic disorders resulting from the presence of a mutated form of hemoglobin, hemoglobin S (HBS). In this study we want to profile various types of hemoglobins and their relative percentage in sickle cell cases. Also, we will analyze RBC indices such as Hb, HCT, MCV, MCH, MCHC and RDW-CV.
Methods: We analyzed blood from 150 patients suspected to have Sickle cell hemoglobinopathies and subjected it to Sickling screening test. All samples irrespective of the sickle cell screening test results will be subjected to HPLC to separate constituent hemoglobins and CBC analysis was done to check RBC indices.
Results: In sickle cell trait (SCT) patients, there is a significantly higher level of HbA2 and HbS and significantly lower level of HbA. 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 hematocrits, MCH and higher RDW CV.
Conclusions: While analyzing HPLC patterns, appearance of HbS, low levels of HbA and high levels of HbF and HbA2 should raise a suspicion for presence of Sickle cell hemoglobinopathy. There was a statistical difference in levels of Hb, HCT, MCH and RDW-CV between cases and controls. High index of suspicion should be maintained when these parameters are on the lower side, especially in populations who are prone to have sickle cell disorders and should be subjected to HPLC.

Keywords: HPLC, RBC indices, Sickle cell anemia

Introduction
Sickle cell anemia has an autosomal recessive inheritance pattern and leads to abnormal shaped red blood cells. This disease is associated with chronic hemolytic anemia and various complications secondary to vaso-occlusive phenomena. The molecular change in sickle cell disease (SCD) is base substitution of valine for glutamate in the sixth position of beta globulin gene leading to production of abnormal hemoglobin S.
In sickle cell anemia, once the hemoglobin S form is deoxygenated it has a tendency to polymerize that causes changes in red cell membrane structure and function leading to sickling of red cells and decrease in red cell deformity. The other mechanisms that play a role independent of polymerization are on vascular endothelial factors, environmental and psychosocial factors.
Sickling phenomena of the red blood cells are influenced by many psychosocial and environmental factors including dehydration, and hypoxemia.
Normal red blood cells have a life expectancy of 110-120 days. However, the red cells in SCD are destroyed at higher rates and have a life expectancy of 15-20 days.
The most common acute complication of SCD is acute vaso-occlusive crises (VOC) that cause pain crises and acute chest syndrome, which is considered the major cause of hospitalization and death among SCD patients. Chronic complications of SCD start to appear with age as organ failure due to the progressive ischemia leads to earlier death, cerebrovascular disease, pulmonary hypertension, retinopathy, and priapism. In addition, complications during pregnancy include preeclampsia and preterm delivery. Children with SCD who live in Sub-Saharan Africa have a high mortality rate estimated at 50-80% by five years old. The most common cause of death in children is infection, including invasive pneumococcal disease and malaria.

In developed countries, the life expectancy of SCD patients has been improved by early diagnosis, comprehensive treatment, and general medical care. Therefore, early detection supports the effective management of the disease. Detection of hemoglobin S and diagnosis of sickle cell disease depend mainly on the clinical laboratory, where a combination of biochemical and molecular tests is used in the detection and confirmation of the diagnosis\. The most popular methods for detecting these diseases are the full count of blood cells, Hb electrophoresis, and high-performance liquid chromatography (HPLC). These methods are considered the gold standard in the diagnosis of Sickle cell disease. HPLC has been shown to have a high degree of reproducibility and precision. HPLC has made hemoglobin abnormality detection much more accurate, faster, and automated.

Materials and Methods

This study was done in a tertiary care hospital in the city of Ahmedabad, Gujarat. Our population consists primarily of tribals from Central Gujarat. We received 2 ml venous blood in EDTA bulb from 150 patients suspected of having sickle cell hemoglobinopathies (i.e. patients suffering from anemia, positive family history, joint pains, weakness, abdominal pain etc.) which were subjected it to Sickling test and peripheral smears of the same were made. Later we collected blood from 30 normal healthy individuals which were taken as control. Cases and controls further underwent a CBC examination and HPLC to separate constituent hemoglobins.

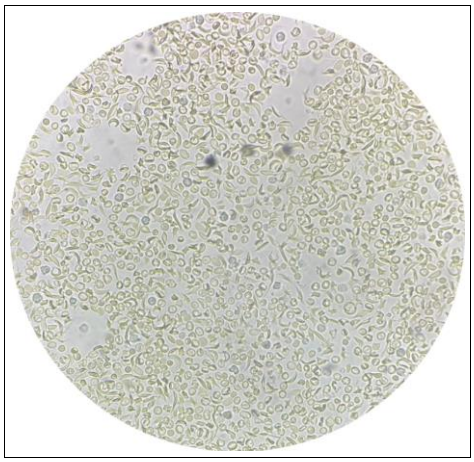

Fig 1: Presence of sickle shaped RBC on sickling test

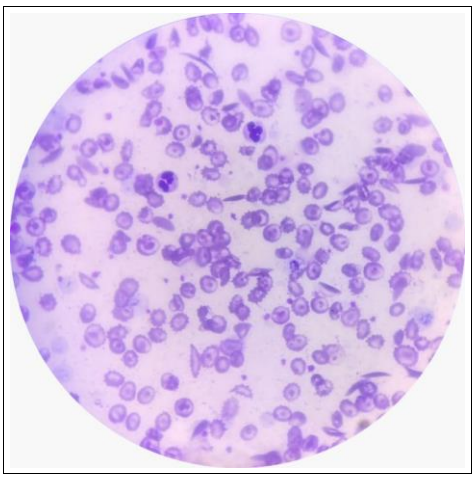

Fig 2: Sickle shaped RBC on peripheral smear.

The following criteria were used to identify hemoglobinopathy on HPLC patterns.

Table 1: Criteria used for differentiating HPLC patterns

Condition	HbA	HbF	HbA2	HbS
Normal Adult	96% to 98%	<2%	2 to 4%	0%
Sickle cell trait	56 to 60%	<1%	<4%	30-40%
Sickle cell anaemia	<10%	>5%	<5%	>50%
Sickle cell Beta thalassemia	56 to 60%	>5%	>5%	>50%

Statistical analysis

Age and sex was expressed in actual numbers and percentages. Continuous variables were presented as mean ± 2SD. Continuous variables were compared between cases and controls by performing unpaired t test. Categorical variables were compared by performing chi square statistics. $p<0.005$ was statistically significant. Microsoft Excel and GraphPad calculator was used for data analysis.

Results

Out of 150 patients suspected of having sickle cell anemia, 120 tested positive by sickling test. Analysis of HPLC patterns revealed the following findings. 100 patients were having sickle cell trait (AS) (66.6%), 18 showed sickle cell disease (12%) (SS) and 2 were sickle beta thalassemia (Sβ) (1.3%). We are not including the latter two categories in our study.

Table 2 shows age distribution of our patients diagnosed with sickle cell trait against controls.97% patients of sickle cell trait were below the age of 31 and 3% patients were above the age of 30. This was very similar to the control group where 6.66% of the population was above 30 years of age.

Table 2: Age distribution of patients with sickle cell trait

Age	SCT No	%	Control	%
0-10	36	36	9	30
11-20	26	26	14	46.66
21-30	35	35	5	16.66
31-40	3	3	2	6.66
Total	100	100	30	100

Table 3: Age distribution of sickle cell disease patients versus controls

Age	SCD No	%	Control	%
0-10	5	27.77	9	30
11-20	8	44.44	14	46.66
21-30	4	22.22	5	16.66
31-40	1	5.55	2	6.66
Total	18	100	30	100

Table 3 shows the age distribution of our patients diagnosed with sickle cell disease against controls. 94.44% patients of sickle cell disease were below the age of 31 and only 2.4% patients were above the age of 30. In the control group, 6.66% of patients were above 30 years old.

Table 4: Sex distribution of sickle cell trait patients versus controls

	SCT No	SCT %	Control	Control %
Females	73	73	13	43.33
Males	27	27	17	56.66
Total	100	100	30	100

27% patients of sickle cell trait were males and 73% were

females. 56.66% control were males, and 43.33% were females. Sex distribution of our sickle cell trait patients compared with controls is given in Table 4.

Table 5: Sex distribution of sickle cell disease patients versus controls

	SCD No	SCD %	Control	Control%
Females	7	38.8	13	43.33
Males	11	61.1	17	56.66
Total	18	100	30	100

50% patients of sickle cell disease were males and 49.4% were females. 56.66% control were males, and 43.33% were females. Sex distribution of our sickle cell disease patients compared with controls is given in Table 5. Sickle cell trait patients demonstrated a significantly higher level of HbA2 and HbS and significantly lower level of HbA as compared to the control group. The difference in HbF levels were not statistically significant in our study. The mean level of HbA, HbA2, HbF and HbS in sickle cell trait patients as compared with the control group is given in Table 6.

Table 6: The mean level of HbA, HbA2, HbF and HbS in sickle cell trait patients as compared with the control group

Hb Type (%)		N	Mean	SD	P-Value	Significance
HbA	Control	30	85.7	2.12	< 0.0001	Yes
	Trait	100	60.4	3.61		
HbA2	Control	30	2.5	0.38	< 0.0001	Yes
	Trait	100	3.2	0.51		
HbF	Control	30	0.5	0.56	0.003	Not significance
	Trait	100	0.8	0.50		
HbS	Control	30	0	0	< 0.0001	Yes
	Trait	100	28.4	41		

Sickle cell disease patients show significantly higher levels of HbA2, HbF and HbS and significantly lower levels of HbA as compared to the control group. The mean level of HbA, HbA2, HbF and HbS in sickle cell disease patients as compared with control group Table 7.

Table 7: The mean level of HbA, HbA2, HbF and HbS in sickle cell disease patients as compared with the control group

Hb Type (%)		N	Mean	SD	P-Value	Significance
HbA	Control	30	85.7	2.12	< 0.0001	Yes
	Sad	18	3.55	3.7		
HbA2	Control	30	2.5	0.38	< 0.0001	Yes
	Sad	18	3.53	1.13		
HbF	Control	30	0.5	0.56	< 0.0001	Yes
	SCD	18	15.6	7.7		
HbS	Control	30	0	0	< 0.0001	Yes
	Sad	18	75.2	8.4		

Analysis of hematological indices was done and the following results were obtained. Sickle cell disease patients had significantly lower levels of Hb as compared with controls but sickle cell trait patients it was not significantly lower. Analysis of Hb in cases versus controls is shown in Table 8.

Table 8: P value of Hb in sickle cell anemia patients versus controls

	N	Mean	SD	P-Value	Significance
Control	30	12	1.26		
SCT	100	11.03	1.83	0.007	No
SCD	18	8.3	1.56	< 0.0001	Yes

Table 9: P value of MCV in sickle cell anemia patients versus control

	N	Mean	SD	P-Value	Significance
Control	30	80.6	5.78		
SCT	100	70.4	8.04	< 0.0001	Yes
SCD	18	69	9.4	< 0.0001	Yes

The difference in MCV values between controls and sickle cell trait patients was statistically significant and also there was significant statistical difference between controls and sickle cell disease patients. Analysis of MCV values in cases versus controls is shown in Table 9.

Table 10: P-Value of MCH in sickle cell anemia patients versus controls

	N	Mean	SD	P-Value	Significance
Control	30	26.7	2.26		
SCT	100	22.02	3.24	< 0.0001	Yes
SCD	18	21.5	4.92	< 0.0001	Yes

The values of MCH were significantly lower than the control group in both sickle cell trait and sickle cell disease patients. Analysis of MCH in cases versus controls is shown in Table 10.

Table 11: P-Value of MCHC in sickle cell anemia patients versus controls

	N	MEAN	SD	P-Value	Significance
Control	30	32.1	1.22		
SCT	100	31.3	1.51	0.001	No
SCD	18	29.4	3.59	0.0003	No

The value of MCHC was not significantly lower than the control group in sickle cell trait patients and sickle cell disease patients.
Analysis of MCHC in cases versus controls is shown in Table 11.

Table 12: P value of HCT in sickle cell anemia patients versus controls

	N	MEAN	SD	P-Value	Significance
Control	30	37.1	3.87		
SCT	100	35	5.44	0.090	No
SAD	18	27.3	5.24	<0.0001	Yes

Sickle cell disease patients had significantly lower levels of hematocrits as compared with controls. While compared to sickle cell trait there was no significant difference as compared to controls. Analysis of HCT in cases versus controls is shown in Table 12.

Discussion

Few patients didn't give sickling test positive but due to low Hb, red cell parameters and family history were subjected to HPLC Test and those individuals turned out to be Sickle cell Trait. Also, all the suspected cases didn't show positive findings on peripheral smear but still turned out to have sickle cell hemoglobinopathies.
In this study, HPLC results obtained from a normal healthy control group revealed that means of HbA, HbA2 and HbF were consistent with other studies.
Our study indicated a higher percentage of Sickle cell trait prevalence versus sickle cell disease in our study population compared to study done by Jawarkar A, Bhatia V.

Looking at the age distribution we could conclude that sickle cell trait patients had a higher life expectancy as compared to sickle cell disease patients (38% of sickle cell trait patients were in the age group of 21-40 against only 27.77% sickle cell disease patients). This was consistent with study done in Boston in 1994.

Our study reflected that in sickle cell trait patients, there is a significantly higher level of HbA2 and HbS and significantly lower level of HbA as compared to the control group. The difference in HbF levels were not statistically significant in our study. This is in accordance with studies done by Shirley L *et al* and Eman A *et al*. In sickle cell disease patients, there were significantly higher levels of HbA2, HbF and HbS and significantly lower levels of HbA as compared to the control group. This is consistent with various studies done by Eman A *et al* and Jawarkar A, Bhatia V.

Both sickle cell trait and sickle cell disease patients had significantly lower levels of Hb as compared with controls. This correlates with various studies done by Walke *et al* and Chikhlikar *et al*. Sickle cell disease patients had significantly lower levels of hematocrits as compared with controls while that of sickle cell trait patients there was no significant difference. This is not in accordance with studies by Chikhlikar *et al*, and Pathak *et al*. The difference in MCV values between controls and sickle cell trait patients was statistically significant and same was with controls and sickle cell disease patients. This is in accordance with studies done by Chikhlikar *et al* and Brittenham *et al*. The values of MCH were significantly lower than the control group in both sickle cell trait and sickle cell disease patients which is in accordance with various studies.

The values of MCHC were not significantly lower than the control group in sickle cell trait patients and sickle cell disease patients were not significant. This contrasted the study done by Jawarkar A, Bhatia V.

Conclusion

In Our study we found more female dominance which is 73% and male with 27%. 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 hemoglobinopathy. There was a statistical difference in levels of HB, HCT, MCV and MCH between cases and controls. High index of suspicion should be maintained when these parameters are on the lower side, and should undergo HPLC to rule out sickle cell hemoglobinopathy.

References

1. Jawarkar A, Bhatia V. A study of HPLC patterns in patients of sickle cell anemia with analysis of red cell parameters. Int J Res Med Sci 2018;6:2390-5.
2. Jain R, Saxena S. The efficacy and reliability of Solubility test followed by High-Performance Liquid Chromatography (HPLC) for sickle cell disorders in Gujarat: An original research article. Tropical Journal of Pathology and Microbiology. 2020;6(2):199-204. https://doi.org/10.17511/jopm.2020.i02.13
3. Walke VA, Walde MS. Hematological study in sickle cell homozygous and heterozygous children in the age group 0-6 years. Ind J Pathol Microbiol. 2007;50(4):901-4.
4. Chikhlikar K, Wilkinson A. A study of red cell parameters in patients of Sickle Cell Trait. J Dental Med Sci. 2014;13(3):46-50.
5. Pathak K, Kishore S, Anshu, Shivkumar VB, Gangane N, Sharma S. Study of haemoglobin S percentage and Haematological parameters in sickle cell trait. Ind J Pathol Microbiol. 2003;46(3):420-24.
6. Khan Y, Thakur AS, Mehta R, Kundu RK, Agnihotram G. Hematological profile of sickle cell disease: A Hospital based study at CIMS, Bilaspur, Chattisgarh. International J App Biol Pharm Technol. 2010;1(2):717-21.
7. Brittenhem G, Lozoft B, Harris JW, Mayson SM, Miller A, Huissman THJ. Sickle cell anemia and trait in southern India. Further studies. Am J Hematol. 1979;6:107-23.
8. Wilson M, Forsyth P, Whiteside J: Hemoglobinopathy and sickle cell disease continuing education in anaesthesia. Critical Care and Pain. 2009.
9. Tarer V, Julan EM, Diara JP, Belloy MS, Mukaza MM, Elion J, *et al*. Sickle cell anemia in Guadeloupean children: Pattern and prevalence of acute clinical events. Eur J Haematol. 2006;76(3):193-9.
10. Al-Allawi NA, Al-Dousky AA. Frequency of hemoglobinopathy at premarital health screening in Dohuk, Iraq: Implications for a regional prevention programme. East Mediter Health J. 2010;16(4):381-5.
11. Bayoumi RA, Abu Zeid YA, Abdul Sadig A, Awad Elkarim O. Sickle cell disease in Sudan. Trans R Soc Trop Med Hyg. 1988;82(1):164-8.
12. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, *et al*. Mortality in sickle cell disease, Life expectancy and risk factors for early death: N Engl J Med. 1994;330(23):1639-44.
13. Ajjack EA, Awooda HA, Abdalla SE. Hemoglobin patterns in patients with sickle cell hemoglobinopathies. Inter J Hematol Disorders. 2014;1(1):8-11.
14. Shirley L. Hemoglobinopathy and Thalassaemias. In: The ABCs of Lab Evaluation. 2009;35.
15. Cotton F, Lin C, Fontaine B, Gulbis B, Janssens J, Vertongen F. Evaluation of a capillary electrophoresis method for routine determination of hemoglobins A2 and F. Clin Chem. 1999;45(2):237-43.
16. Walke VA, Walde MS. Hematological study in sickle cell homozygous and heterozygous children in the age group 0-6 years. Ind J Pathol Microbiol. 2007;50(4):901-4.

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