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Original Research Article

A study of HPLC patterns in patients of sickle cell anemia with analysis of red cell parameters

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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 haemoglobins and their relative percentage in sickle cell cases. Also, we will analyse RBC indices such as Hb, HCT, MCV, MCH, MCHC and RDW-CV.
Methods: We analysed blood from 200 patients suspected to have Sickle cell hemoglobinopathies and subjected it to Sickling screening test. All positive cases will be subjected to HPLC to separate constituent haemoglobins 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 haematocrit, MCH and higher RDW CV.
Conclusions: While analysing 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 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 lower side, especially in population who is prone to have sickle cell disorders.

Keywords: HPLC, RBC indices, Sickle cell anemia

INTRODUCTION

Sickle cell disease (SCD) and its variants are genetic disorders resulting from the presence of a mutated form of hemoglobin, hemoglobin S (HbS).[1] It has an autosomal recessive inheritance.[2]

In 1957, Vernon Ingram discovered that sickle haemoglobin resulted from a single amino acid substitution in the haemoglobin molecule.[3,4] The disease results from a single base A>T mutation in the triplet encoding the sixth residue of the β-globin chain, leading to a substitution of valine for glutamic acid and the abnormal haemoglobin S (HbS).

The primary pathophysiology is based on the polymerization of deoxy HbS with formation of long fibres within the Red Blood Cells (RBCs) causing a distorted sickle shape which eventually leads to increased haemolysis and vaso-occlusion of sickle red cells. However, the clinical presentation of SCD patients is extremely variable and there are several events that may

trigger vaso-occlusion. Recent work has shown the importance of red cell dehydration, abnormal adhesion of RBCs to the vascular endothelium, inflammatory events, and activation of all the cells in the vessel and abnormalities of nitric oxide metabolism in the pathophysiology of this multi-organ disease.[5]

In India, sickle cell anemia is more prevalent in tribal population. The first description of sickle haemoglobin in India was by Lehman and Cutbush in 1952 in the tribal populations in the Nilgiri hills in south India.[6] In the same year, Dunlop and Mazumder also reported the presence of sickle haemoglobin in the tea garden workers of Upper Assam who were migrant labourers from tribal groups in Bihar and Odisha.[7]

Since then, many population groups have been screened and the sickle cell gene has been shown to be prevalent among three socio-economically disadvantaged ethnic groups, the scheduled tribes, scheduled castes and other backward classes in India.

In Gujarat, the Dhodia, Dubla, Gamit, and Naika tribes have a high prevalence of HbS (13-31%).[8] More recently very extensive population surveys have been done by the Indian Red Cross Society, Gujarat State Branch where 1,68,498 tribals from 22 districts were screened and the overall prevalence of sickle cell carriers was 11.37 per cent. Some tribal groups in south Gujarat like Chaudry, Gamit, Rohit, Vasava and Kukana have shown both a high prevalence of HbS (6.3 to 22.7%) as well as β-thalassaemia trait (6.3 to 13.6 %).[9]

In this study we want to profile various types of haemoglobins and their relative percentage in sickle cell cases versus controls in our study population. Also, we will compare RBC indices such as Hb, HCT, MCV, MCH, MCHC and RDW-CV in sickle cell cases versus controls.

METHODS

This study was done in a tertiary care hospital in Western Indian city of Vadodara, Gujarat. Our population consists primarily of tribals from Central Gujarat and southern Madhya Pradesh. We collected 2ml venous blood in EDTA bulb under aseptic conditions from 200 patients suspected of having sickle cell hemoglobinopathies (i.e. patients suffering from anemia, joint pains, weakness, abdominal pain etc.) and subjected it to Sickling test. 151 patients showed sickling test positive and were selected as cases.

Also, we collected blood from 40 normal healthy individuals and subjected it to the same tests as above. They were sickling test negative and were selected as controls. Cases and controls further underwent a CBC examination and HPLC to separate constituent haemoglobins. Thea following criterial were used to identify hemoglobinopathies on HPLC patterns (10) (Table 1).

Table 1: Criteria used for differentiating HPLC patterns.

Hemoglobin	Disease
A>S	Sickle cell trait, sickle alpha-thalassemia
S, F and no A	Sickle cell anemia, Sickle-beta thalassemia
S> A and F	Sickle-beta thalassemia
A>C	HbC trait
C, F and no A	HbC disease, HbC-beta thalassemia
C>A	HbC-beta thalassemia

Statistical analysis

Age and sex was expressed in actual number 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 200 patients suspected of having sickle cell anemia, 151 tested positive by sickling test. Analysis of HPLC patterns revealed the following findings. 58 patients were having sickle cell trait (AS) (38.9%), 83 showed sickle cell disease (55.7%) (SS), 2 were diagnosed to have HbS due to recent transfusion and 8 were sickle beta thalassemia (Sβ) (5.3%). We are not including the latter two categories in our study.

Table 2: Age distribution of patients with sickle cell trait.

Age	SCT no.	%	Controls no.	%
0-10	14	24.1	10	25
11-20	13	22.4	13	32.5
21-30	14	24.1	9	22.5
31-40	6	10.3	3	7.5
41-50	6	10.3	3	7.5
51-60	4	6.8	1	2.5
61-70	1	1.7	1	2.5
Total	58	100	40	100

Table 2 shows age distribution of our patients diagnosed with sickle cell trait against controls. 81.2% patients of sickle cell trait were below the age of 40 and 18.8% patients were above the age of 40. This was very similar to the control group where 12.5% population was above 40 years of age.

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

Age	SCD No.	%	Controls No.	%
0-10	31	37.3	10	25
11-20	34	41.0	13	32.5
21-30	14	16.9	9	22.5
31-40	2	2.4	3	7.5
41-50	2	2.4	3	7.5
51-60	0	0.0	1	2.5
61-70	0	0.0	1	2.5
Total	83	100	40	100

Table 3 shows age distribution of our patients diagnosed with sickle cell disease against controls. 97.6% patients of sickle cell disease were below the age of 40 and only 2.4% patients were above the age of 40. In the control group, 12.5% patients were above 40 years old.

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

	SCT (No.)	SCT (%)	Controls No.	Controls (%)
Males	33	56.8	17	42.5
Females	25	43.1	23	57.5
Total	58	100	40	100

56.8% patients of sickle cell trait were males and 43.1% were females. 42.5% control were males, and 57.5% 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 %	Controls No.	Controls (%)
Males	42	50.6	17	42.5
Females	41	49.4	23	57.5
Total	83	100.0	40	100

50.6 % patients of sickle cell disease were males and 49.4% were females. 42.5% control were males, and 57.5% were females. Sexfig distribution of our sickle cell disease patients compared with controls is given in Table 5.

Next, we analysed the HPLC patterns in patients and controls. Sickle cell trait patients demonstrated a significantly higher level of HbA2 and HbS and significantly lower level of HbA as compared to 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 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 control group.

Hb type (%)		N	Mean	SD	P value	Significance
HbA	Control	40	95.4	3.11	<0.0001	Yes
	Trait	58	66.05	5.85		
HbA2	Control	40	2.42	0.38	<0.0001	Yes
	Trait	58	3.17	0.65		
HbF	Control	40	1.11	3.25	0.968	Not significant
	Trait	58	1.13	1.62		
HbS	Control	40	0	0	<0.0001	Yes
	Trait	58	28.92	5.4		

Sickle cell disease patients show significantly higher levels of HbA2, HbF and HbS and significantly lower levels of HbA as compared to 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 control group.

Hb type (%)		N	Mean	SD	P value	Significance
HbA	Control	40	95.4	3.11	<0.0001	Yes
	scd	83	5	10.74		
HbA2	Control	40	2.42	0.38	<0.0001	Yes
	scd	83	3.4	1.2		
HbF	Control	40	1.11	3.25	<0.0001	Yes
	scd	83	17.3	8.4		
HbS	Control	40	0	0	<0.0001	Yes
	scd	83	74	10.8		

Analysis of hematological indices was done and the following results were obtained. Both sickle cell trait and sickle cell disease patients had significantly lower levels of Hb as compared with controls. 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	40.0	13.3	1.4		
SCT	58.0	11.0	4.8	0.0049	Yes
SCD	83.0	8.0	1.8	<0.0001	Yes

The difference in RBC counts was not statistically significant between controls and both sickle cell trait and sickle cell disease patients. Analysis of RBC count in cases versus controls is shown in Table 9. Both sickle cell trait and sickle cell disease patients had significantly lower levels of haematocrit as compared with controls.

Analysis of HCT in cases versus controls is shown in Table 10.

Table 9: P value of RBC count in sickle cell anemia patients versus controls.

	N	Mean	SD	P value	Significance
Control	40	3.67	0.54		
SCT	58	4.19	1.29	0.0181	No
SCD	83	3.29	0.98	0.0239	No

Table 10: P value of HCT in sickle cell anemia patients versus controls.

	N	Mean	SD	P value	Significance
Control	40	39.8	4.63		
SCT	58	31.63	1.94	<0.0001	yes
SCD	83	34.41	5.87	<0.0001	yes

Table 11: P value of MCV in sickle cell anemia patients versus controls.

	N	Mean	SD	P value	Significance
Control	40	83.4	5.46		
SCT	58	77.14	13.82	0.0078	No
SCD	83	75.8	11.1	<0.0001	Yes

The difference in MCV values between controls and sickle cell trait patients was not statistically significant. However, there was significant statistical difference between values of MCV between controls and sickle cell disease patients. Analysis of MCV values in cases versus controls is shown in Table11.

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

	N	Mean	SD	P value	Significance
Control	40	29.96	2.49		
SCT	58	25.4	5	<0.0001	Yes
SCD	83	25.1	4.8	<0.0001	Yes

The values of MCH were significantly lower than control group in both sickle cell trait and sickle cell disease patients. Analysis of MCH in cases versus controls is shown in Table 12.

The value of MCHC was significantly lower than control group in sickle cell trait patients. However, the difference with sickle cell disease patients was not significant. Analysis of MCHC in cases versus controls is shown in Table 13.

The value of RDW CV was significantly higher in both sickle cell trait and sickle cell disease patients versus controls. Analysis of RDW cv in cases versus controls is shown in Table 14.

Table 13: P value of MCHC in sickle cell anemia patients versus controls.

	N	Mean	SD	P value	Significance
Control	40	34.76	1.74		
SCT	58	32.9	2.6	<0.0001	Yes
SCD	83	33.07	4.21	0.0163	No

Table 14: P value of RDW-CV in sickle cell anemia patients versus controls.

	N	Mean	SD	P value	Significance
Control	40	14.24	1.09		
SCT	58	17.7	5.4	<0.0001	Yes
SCD	83	22.7	5.25	<0.0001	Yes

DISCUSSION

In this study, HPLC results obtained from normal healthy control group revealed that means of HbA, HbA2 and HbF were consistent with other studies.[11]

Our study indicated a higher percentage of Sickle cell disease prevalence versus sickle cell trait in our study population which contrasts with the studies done so far, where prevalence of sickle cell trait is more than sickle cell disease.[12-15]

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 (29.2% of sickle cell trait patients were in age group of 31-70 against only 4.8% sickle cell disease patients). This was consistent with study done in Boston in 1994.[16]

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 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.[17,18]

In sickle cell disease patients, there were significantly higher levels of HbA2, HbF and HbS and significantly lower levels of HbA as compared to control group. This is consistent with various studies done by Eman A et al and Cotton F et al.[17,19]

Both sickle cell trait and sickle cell disease patients had significantly lower levels of Hb as compared with controls. This corelates with various studies done by Walke et al and Chikhlikar et al.[20,21]

The difference in RBC counts was not statistically significant in our study between controls and both sickle cell trait and sickle cell disease patients. This is in contrast with studies by Chikhlikar et al, Pathak et al, and Yasmin et al.[21-23]

Both sickle cell trait and sickle cell disease patients had significantly lower levels of haematocrit as compared with controls. This is in accordance with studies by Chikhlikar et al, and Pathak et al.[21,22]

The difference in MCV values between controls and sickle cell trait patients was not statistically significant. However, there was significant statistical difference between values of MCV between controls and sickle cell disease patients. This contrasts with studies done by Chikhlikar et al and Brittenham et al.[21,24]

The values of MCH were significantly lower than control group in both sickle cell trait and sickle cell disease patients which is in accordance with various studies.[21-23]

The value of MCHC was significantly lower than control group in sickle cell trait patients. However, the difference with sickle cell disease patients was not significant. This contrasted with various studies.[21-23]

The value of RDW CV was significantly higher in both sickle cell trait and sickle cell disease patients versus controls. This was similar in study done by Chikhlikar et al.[21]

CONCLUSION

We conclude that sickle cell hemoglobinopathies are very common in our population which consists of a large proportion of tribals. Sickle cell disease is more common than trait in our geographic area which has to do with consanguineous and same caste marriages and lack of prenatal counselling and detection. 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 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 lower side, especially in population who is prone to have sickle cell disorders such as tribals.

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