

Hemoglobin Patterns in Sickle Cell Hemoglobinopathies- A Large Prospective Study in North Maharashtra

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Abstract

Introduction: Sickle cell haemoglobinopathy is an inherited hemoglobinopathy resulting from a mutation occurring in beta-globin gene, on chromosome 11. The gene is prevalent in some tribes of North Maharashtra. The main aim of the study is to determine haemoglobin patterns in cases with sickle cell hemoglobinopathies in North Maharashtra using HPLC testing system. **Material and Methods:** This is a prospective study done over a period of 6 years. 10081 patients having positive solubility test or negative solubility test but having clinical suspicion of Sickle cell hemoglobinopathies were studied in detail and all samples were subjected for HPLC testing. **Results:** Prevalence of sickle cell hemoglobinopathy in this study was 70.36%. Most common pattern of haemoglobin observed was SA (89.72%). A slight female preponderance (54%) was noted. Predominant age group was paediatric (39.96%), followed by 12-20yrs (33.97%). Oldest case for HbSS was 55yrs male. Predominant category affected was ST (82.05%). **Conclusion:** A very high prevalence of Sickle cell hemoglobinopathy was noted in this study. This is because the study was done in areas where Pawara and Bhil community resides who have a high frequency of HbS gene. Solubility test was found to be cost effective and easy screening test (Sensitivity being 70.36%). HPLC found to be Rapid and accurate test for diagnosis of hemoglobinopathy and had helped in diagnosis of some rare heterozygous disorders like SA-HBQ India, SA-Hereditary persistence of foetal haemoglobin, HBD-SA. This is one of the largest and first of its kind prospective study which will help in prevention and cost effective management in targeted population.

Keywords: Hemoglobinopathies, HPLC, Solubility Test, Sickle Cell Anemia

1. Introduction

Hemoglobinopathies are common genetic disorders resulting from formation of one of the abnormal globin chain of the haemoglobin molecule. Sickle cell disease was first described in early 20th century¹. It is an inherited haemoglobinopathy resulting from a mutation occurring in beta-globin gene, on chromosome 11¹. Approximately 5% of the world's population carries a gene for sickle-cell anaemia or thalassaemia, the percentage of carriers can reach 25% in some regions². It includes disorders affecting the structure, function or production of haemoglobin³. There is a substitution of glutamate with valine in position 6 of the beta globin resulting in the formation of haemoglobin S⁴.

Sickle cell Hemoglobin is biochemically unstable and results in the polymerization of hemoglobin forming large tactoids which deform the red cells into the typical pointed, slightly curved "sickle" cells when in the deoxygenated state. The sickle cell hemoglobinopathy can manifest in two forms. The heterozygous carrier state or Sickle Cell Trait (SCT) results in the production of both haemoglobin A and S. Hemoglobin A being more than haemoglobin S and Hemoglobin S being 30-40 %. The homozygous state with near 100% HbS results in Sickle Cell Disease (SCD). It is said that HbSS (sickle cell homozygous) is the most common pathological haemoglobin variant worldwide and majority of children born with Sickle cell disease die before reaching five years of age⁵.

The pathophysiological consequences of sickling are

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two-fold: Small vessel obstruction by sickle cells (vaso-occlusive events which can be extremely painful) and haemolytic anaemia due to the greatly reduced half-life of SS cells when compared with normal red blood cells (12 vs. 120 days)⁶. Sickle cell trait has predominantly benign clinical picture, as the cells only sickle at very low oxygen tension. Sickle cell trait provides some protection against the consequences of *Plasmodium falciparum* malaria⁷.

Sickle cell disease is a chronic debilitating disease characterized by chronic haemolytic anemia, recurrent intermittent vaso-occlusion and severe pain, progressive organ damage and early death.

Diagnosis: Hemoglobin disorders are responsible for an extremely complex series of clinical phenotypes. Sickle cell anaemia can cause chronic ill health and life threatening situations, so it is very important to have reliable detection and identification methods for haemoglobin variants and because this can lead to the prevention of more severe disorders such as sickle cell disease in infants.

Peripheral Blood Smear examination (PBS): Examination of the peripheral blood film may reveal sickle forms or an elevated reticulocyte count relative to the haemoglobin level, but these may not be present in sickle cell trait where the red cell morphology may be essentially normal⁸.

Solubility test: It is only screening test. False positive results can be seen.

Serum electrophoresis: It is definitive test but it does not give percentage of abnormal hemoglobins

High Performance Liquid Chromatography (HPLC): HPLC is rapid and accurate tool for diagnosis of sickle cell hemoglobinopathies.

2. Materials and Methods

2.1 Study Design

It is a hospital based prospective study carried out in GMC Dhule, a tertiary care centre over a period of 6 years. Here patients come from Dhule, Nandurbar, Nashik and Jalgaon district. All these districts have high prevalence rate of sickle cell anaemia.

2.2 Aim of Study

- To determine haemoglobin patterns in cases with sickle cell hemoglobinopathies in North Maharashtra using HPLC testing system.
- To find the categories at high risk of sickle cell hemoglobinopathies in North Maharashtra so as to

target this population for cost effective prevention and management of hemoglobinopathies.

- To see demographic characteristics of these patients
- To see the sensitivity and efficacy of solubility test in screening of sickle cell hemoglobinopathies.

2.3 Period of Study

Six years from Year 2008 to 2013.

Study subject: A total 10081 patients having positive solubility test or negative solubility test but having clinical suspicion of sickle cell hemoglobinopathies were included in the study. These patients included, patients coming to OPD for investigations of anaemia, IPD patient in wards, ANC patients and patients from various camps taken in primary and secondary units.

Ethical consideration: Informed consent was taken before enrolling the patients in the study.

2.4 Data Analysis

Screening tests: Solubility test was done as screening test in all cases. All patients having positive screening test or having negative screening test but having clinical suspicion of hemoglobinopathies were subjected for HPLC studies. Diagnosis of hemoglobinopathies was made based on laboratory tests and clinical findings as mentioned in literature⁹.

2.5 Demographic Study

Age, sex and category wise distribution of patients and patterns of hemoglobin were observed.

2.6 Procedure

- 2-3ml of blood samples were collected after obtaining informed consent using Ethylene Diamine Tetra acetic acid as an anticoagulant from each patient ensuring that they were free of blood transfusion for at least one month.
- First samples were run on cell counter to note Hb% and red blood cell indices.
- Samples were tested within a week of collection and stored at 2-8°C.
- The samples were run on BIO-RAD Variant Hemoglobin Testing System which utilizes the principle of HPLC.
- An HbA₂F calibrator and two levels of controls (BIO-RAD) were analyzed at the beginning of each run. The total area acceptable was between one to three million¹⁰.

3. Results

From this Table 1 it is seen that prevalence of Sickle cell hemoglobinopathies is 70.36%. Sensivity of solubility test is also 70.36%

Table 1. HPLC report

Result	Normal	Sickle cell hemoglobinopathies	Other hemoglobinopathies	Total
No cases	2716 (26.94%)	7093(70.36)	272(2.69)	10081

From this Table 1 it is seen that prevalence of Sickle cell hemoglobinopathies is 70.36%. Sensivity of solubility test is also 70.36%

Table 2. Sex distribution of cases

Year	Male	Female	Total
2008	289(47%)	325(53%)	614(100%)
2009	612(48%)	662(52%)	1274(100%)
2010	528(47%)	595(53%)	1123(100%)
2011	522(43%)	693(57%)	1215(100%)
2012	856(48%)	928(52%)	1784(100%)
2013	433(40%)	650(60%)	1083(100%)
Total	3240(46%)	3853(54%)	7093(100%)

From the given Table 2 it is seen that male to female ratio is 46/54.

Table 3. Patterns of sickle cell hemoglobinopathies

Year	SA	SS	SA-BTT	SA-HBQ-India	SA-HPFHB	Total
2008	524	89	1	-	-	614
2009	1106	164	3	1	-	1274
2010	1050	68	5	-	-	1123
2011	1103	105	5	1	1	1215
2012	1649	110	22	1	2	1784
2013	932	131	20	-	-	1083
Total	6364(89.72%)	667(9.40%)	56(0.78%)	3(0.04%)	3(0.04)	7093(100%)

SA-HPFHB- Hereditary persistence of foetal haemoglobin

Table 4. Age wise distribution of cases

Year	Ped	12-20	21-30	31-40	41-50	>50	Total
2008	212	268	85	28	16	5	614
2009	458	470	232	59	42	13	1274
2010	438	415	180	45	33	12	1123
2011	502	402	198	60	39	14	1215
2012	655	548	433	66	62	20	1784
2013	570	307	147	30	22	7	1083
Total	2835 (39.96%)	2410(33.97%)	1275 (17.97%)	288 (4.06%)	214 (3.01%)	71 (1.00%)	7093 (100%)

From the given Table 3 it is seen that the most common hemoglobinopathy in this study is Sickle cell trait (89.72%).

From the Table 4 it is seen that predominant age group affected was pediatric (39.96%), followed by 12-20 yrs (33.97%). Oldest case of Sickle cell disease was a 55 yrs male.

From the above Table 5 it is seen that sickle cell hemoglobinopathy was most prevalent in ST category.

4. Discussion

- In India, sickle cell gene is widely spread in the district of Eastern Maharashtra, North Maharashtra¹¹ and some parts of Marathwada region¹². The incidence of sickle cell hemoglobinopathies in India ranges from 1-44%⁹. The highest frequency of sickle cell gene in India is reported in Orissa (9%), followed by Assam (8.3%), Madhya Pradesh (7.4%), Uttar Pradesh (7.1%), Tamil Nadu (7.1%) and Gujarat(6.4%)¹³⁻¹⁵. But in light of population migration it is becoming a worldwide phenomenon.

70.36% cases in this study showed Sickle cell hemoglobinopathies. A study by Balgir et al.,¹⁶ showed 39.3% cases showing Sickle cell hemoglobinopathies. This is because the present study was done in high prevalence zone for sickle cell hemoglobinopathies.

Table 5. Category wise distribution of cases

Year	Open	OBC	VJ-NT	SC	ST	Muslim	Total
2008	37	50	31	110	337	49	614
2009	7	32	13	89	1107	26	1274
2010	34	39	28	90	921	11	1123
2011	6	30	6	73	1094	6	1215
2012	36	98	36	89	1516	9	1784
2013	22	108	33	70	845	5	1083
Total	142	357	147	521	5820	106	7093
	(2.00%)	(5.03%)	(2.07%)	(7.34%)	(82.05%)	(1.49%)	(100%)

Table 6. HPLC report comparison with other study

Result	Normal	Sickle cell hemoglobinopathies	Other hemoglobinopathies	Total
Our study	2716(26.94%)	7093(70.36)	272(2.69)	10081
Balgir etal ¹⁶	348(34.3%)	398(39.3%)	269(26.5%)	1015

Table 7. Sex distribution of cases-comparison with other study

Study	Male	Female
Our study	45.69%	54.34%
Ajjack etal ¹⁷	48.6%	51.4%

From this Table 7 it is seen that our reports are comparable with other study.

- From the Table 8 it is seen that predominant pattern in our study was SA (89.7%). Balgir et al.,¹⁶ showed SA pattern in 75.9% cases. Sickle cell homozygous patients showed HBS values more than 70% along with raised HBF levels. Our findings are comparable with study by Kar et al¹⁸.
- HPLC has helped in identification of compound heterozygous disorders like SA-BTT, SA-HBQ India, SA- Hereditary persistence of foetal Hb, HBD-SA. These were diagnosed based on quantification of levels of HBE, HBS and HBA.

When compared with other study our study shows predominant category affected was ST category. It is established that sickle cell gene harbour amongst different caste groups but very high prevalence amongst SC, ST and OBC category^{19,20}. The castes residing here like Bhill, Pawara, Konkana etc come under ST Category which carries high prevalence of sickle cell hemoglobinopathies.

- It is also observed that solubility has a sensitivity of 70.36%. It is a good screening test as it is cost effective and easy test and can rule out sickle cell hemoglobinopathy in clinically nonsuspicious cases. This will avoid burden on HPLC testing as it is a costly test.

5. Conclusion

- Prevalence of sickle cell hemoglobinopathy in this study was 70.36%.
- Our study showed a slight female preponderance (54%) over male (46%).

Table 8. Patterns of sickle cell hemoglobinopathies - comparison with other studies

Study	SA	SS	SA-BTT	SA-HBQ India	SA-HPFHB	SA/C	SC	SD
Our study	6364 (89.7%)	667 (9.4%)	56 (0.8%)	3 (0.04%)	3 (0.04%)	-	-	-
Ajjack etal ¹⁷	52.9%	28.6%	11.4	-	-	1.4%	1.4%	4.3%
Balgir etal ¹⁶	75.9%	19.3%	4.3%	-	-	-	-	0.5%

Table 9. Category wise distribution of cases-comparison with other study

Study	General	SC	ST
Our study	752(10.60%)	521(7.34%)	5820(82.05%)
Balgir etal ¹⁶	64.6%	27.4%	8%

- Predominant sickle cell hemoglobinopathy in our study was sickle cell trait (89.72%).
- The most common age group was paediatric (0-12yrs) i.e., 39.96%, followed by 12-20 yrs(33.97%) and least common was age more than 50 yrs (1%).
- Sickle cell Hemoglobinopathy was most prevalent in ST category (82.05%) as Pawara and Bhill community in this region belongs to this category.
- We encountered some rare compound heterozygous conditions like SA-HBQ India, SA-Hereditary persistence of foetal haemoglobin, HBD-SA.
- HPLC provides rapid and accurate tool for diagnosis of hemoglobinopathies as identification of abnormal hemoglobin based on electrophoretic mobility is only presumptive and have to be confirmed by another technique applying different principle.
- To the best of our knowledge, it is one of the largest and the first of its kind study reported from North Maharashtra.
- This study helps us to focus on communities and categories having high prevalence of sickle cell gene, so it may help us in prevention by screening programme, genetic counselling and public education as well as cost effective management of patients.

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7. Competing Interests

None declared.

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