

Study of prevalence of haemoglobin disorders in sindhi community with reference to HPLC

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Abstract

Introduction: Inherited haemoglobin disorders are major health problems in certain communities in different geographical areas in India. These disorders pose a huge burden on affected families physically, financially & psychologically. This study is carried out to find the Prevalence of these disorders in Sindhi community in Aurangabad city in state of Maharashtra, India.

Materials and Methods: The present cross-sectional study was undertaken among 338, apparently healthy Sindhi individuals from community in Aurangabad, Maharashtra from July 2014 to March 2017. All the samples were tested in automated hematology cell counter and then for HPLC on Bio-Rad variant classic. Various CBC parameters and Hemoglobin patterns were derived on all samples.

Result: The overall prevalence of Hemoglobinopathy was 60/338 (17.75%), comprised of 42 (12.42%) cases of BTT, 3 (0.88%) cases of BTM, 3 (0.88%) cases of SCT, 11 (3.25%) cases of Hb Q-India & One (0.29%) case of Hb Q-India+BTT.

Conclusion: Out of 338 samples, BTT and Hb Q-India were found to be more common Hb disorders in Sindhi community. Screening of community and counselling regarding the nature of these disorders are very important measures to reduce the burden of disease.

Keywords: Hemoglobin disorders, Prevalence, HPLC, β -thalassaemia trait, Hb Q-India, Maharashtra, India.

Introduction

Inherited haemoglobin disorders were originally characteristic of the tropics and subtropics but are now common worldwide due to migration.¹ These disorders comes mainly under two groups – structural haemoglobin variants and thalassaemias. Both these groups inherit recessively. The structural variants generally result from single amino acid substitutions in the α or β chains. Most of known structural variants are harmless but in some cases they may alter the stability or functional properties of the haemoglobin and lead to a clinical manifestation.² Thalassaemias are the most common monogenic gene disorders in the world. Thalassaemia is a group of disorders where complete absence or reduced synthesis of globin chains occurs in haem protein of haemoglobin.³

It has been estimated that 7% of the World populations are carriers of such disorders and that 300000-400000 babies with severe forms of these diseases are born each year.⁴ Around 1.1% of couples worldwide are at risk for having children with a haemoglobin disorder and 2.7 per 1000 conceptions are affected. Over 9 million carriers become pregnant annually. The risk that their partner is also a carrier ranges from 0.1–40% (global average 14%).¹

The general incidence of β -thalassaemia trait and sickle cell disorders in India varies between 0-17 per cent and 0-44 per cent, respectively. In India, as a consequence of high consanguinity, caste and area endogamy, some communities exhibits higher incidences of the diseases, what determines a major public health problem.^{5,6}

In central India the incidence of β -thalassaemia has been mainly attributed to its high prevalence in the migrant population of Sindhi origin. Sindhi's migrated to India at the time of partition from Sindh province of present day Pakistan & they show high prevalence of 5-15% of thalassaemia carriers.^{7,8}

Most children with thalassaemia are born in low-income countries. Worldwide, transfusion is available for a small fraction of those who need it, and most transfused patients will die from iron overload unless an available and potentially inexpensive oral iron chelator is licensed more widely. The patients' predicament underlines the need for combined treatment and prevention programmes. Wherever combined programmes exist survival is steadily improving, affected births are falling, and numbers of patients are stabilizing. The policy is spreading because of its demonstrable cost-effectiveness, and thalassaemia is gradually becoming contained.¹

Screening of healthy population is required to determine the carrier rates and gene frequencies & it is also most effective approach to minimize the problem of hemoglobinopathies in India. Here, we discuss the laboratory aspects of various hemoglobin disorders and diagnostic difficulties in cases like borderline HbA2 values & few rare variants which at times require correlation with genetic study.

Prevention of these disorders decreases birth of babies with severe forms of these disorders & also cost effective, as the ratio of treatment to prevention is 4:1. By using the prevention programme incidence of

thalassaemia major in Sardinia & Cyprus has decreased substantially.⁹

The main objective of the Present study is to evaluate the role of high performance liquid chromatography along with adjunctive tests in studying prevalence of hemoglobin disorders in Sindhi community, and to educate those who are carriers regarding the nature of these disorders & also about the partner selection.

Materials and Methods

Present study was carried out for detecting prevalence of haemoglobin disorders in Sindhi Community of Aurangabad. Study was carried between July 2014 to march 2017. 338 blood samples were randomly collected for screening of haemoglobin disorders. Almost all of these individuals are migrants from Sindh state in present day Pakistan to Aurangabad and are living here since over 65 years.

Orientation camps have been arranged for educating the people regarding the genetic nature of haemoglobin disorders, camps for sample collection has

been arranged in the locality of Sindhi community, detailed history was taken and clinical examination was done and informed consent was taken from the every individual participated in the camp, in case of minors the consent of their parents was taken, 2-3 ml blood was collected in EDTA tube & all the investigations were done in the Department of Pathology of a Tertiary care hospital.

Laboratory investigations were carried out following the standard procedures after cross checking for quality control from time to time. Haematological profile of cases was done, which included CBC, PS, Reticulocyte count, sickling.

Hematological parameters were studied by using an automated Blood Cell Counter (cellenium, trivitron 3 part analyser).¹⁰

The sickling test was performed on red cells by using freshly prepared 2% sodium metabisulphite solution as reducing agent.¹¹

Reticulocyte count was done by mixing 2-3 ml of blood with equal amount of Azure B dye.¹²

Table 1: Analysate identification windows:¹³

Retention time (minutes)	Band (minutes)	Window (minutes)	Range
F	1.15	0.15	1.00-1.30
P2	1.45	0.15	1.30-1.60
P3	1.75	0.15	1.60-1.90
A0	2.60	0.40	2.20-3.30
A2	3.83	0.15	3.68-3.98
D-window	4.05	0.15	3.98-4.12
S-window	4.27	0.15	4.12-4.42
C-window	5.03	0.15	4.88-5.18

Normal haemoglobin and variants were quantified by HPLC, their proportion of the total haemoglobin, retention time and peak characteristics were all analyzed and final diagnosis based on HPLC reading was given. Prevalence of haemoglobin disorders overall and prevalence of individual haemoglobin disorders was calculated. Our machine the 'Biorad Variant', β -thalassaemia short programme, manufactured by BIORAD laboratories USA, uses the principle of High Performance Liquid Chromatography (HPLC). Calibrators were run before each examination.

DNA was isolated from peripheral blood leucocytes using the standard phenol-chloroform method.¹⁴ Amplification Refractory Mutation System (ARMS)-PCR method was used for the diagnosis of common Asian β -thalassaemia mutations IVS1-1G \rightarrow A, IVS1-5G \rightarrow C, cd41/42, cd8/9, 619bps deletion¹⁵ and for screening and analysis of HbQ (alpha) 64 Asp_ His mutation.¹⁶ All primers were procured from invitrogen, USA and used along with other PCR reagents (Life Technology, USA) using an ARMS PCR program in a

dual block Thermal cycler (Bio-Rad, USA). A standard positive (known) and negative (DI water) controls were included in each ARMS-PCR assay. Analyses of the wild and mutant alleles were performed in 1% agarose gel electrophoresis using the quantity one software, Bio-Rad gel documentation system (USA). Double heterozygotes for the both mutation were cross verified by parent study.

Family studies were carried out to confirm the diagnosis, wherever it was felt necessary.

Inclusion Criteria: 1. Individuals belonging to sindhi community.

Exclusion Criteria: 1. Individuals other than the sindhi community; 2. Patients below 1 year of age as significance of HbF (Fetal Hemoglobin) levels are hard to interpret; 3. Patients having recent blood transfusions as HPLC will not be able to distinguish between patients own cells and transfused cells.

Results

In the present study, total 338 individuals from Sindhi community were screened for haemoglobin disorders by HPLC. Out of 338 subjects 170 were males and 168 are females. Out of 338 subjects 278 shows normal pattern by HPLC and 60 cases were diagnosed as haemoglobin disorders based upon HPLC pattern.

So by clinical details, complete haematological workup, HPLC study and family study (where-ever possible) cases are classified as:

Group A Beta Thalassaemia Trait (BTT) – 42 cases

Group B: Beta Thalassaemia Major (BTM) – 3 cases

Group C: Sickle Cell Trait (SCT) – 3 cases

Group D: Hb Q-India (Hb Q) – 11 cases

Group E: Hb Q-India-Beta Thalassaemia Trait (Hb Q-BTT) – 1 case

The overall prevalence of hemoglobinopathy was 60/338 (17.75%), comprised of 42 (12.42%) cases of BTT, 3 (0.88%) cases of BTM, 3 (0.88%) cases of SCT, 11 (3.25%) cases of Hb Q-India & One (0.29%) case of Hb Q-India+BTT. 278 members were normal. Most common hemoglobin disorder was beta thalassaemia trait with 12.42% prevalence & second most common disorder was Hb Q-India with 3.25% prevalence.

Table 2: Age wise distribution of cases among all the groups (n=60)

Age (Yrs)	Gr-A BTT (n=42)	Gr-B BTM (n=3)	Gr-C SCT (n=3)	Gr-D Hb Q (n=11)	Gr-E Hb Q-BTT (n=1)
0 to 10	8 (19.04%)	3 (100%)	0 (0%)	3 (27.27%)	1 (100%)
11 to 20	7 (16.66%)	0 (0%)	0 (0%)	2 (18.18%)	0 (0%)
21 to 30	10 (23.80%)	0 (0%)	2 (66.67%)	1 (9.09%)	0 (0%)
31 to 40	12 (28.57%)	0 (0%)	0 (0%)	3 (27.27%)	0 (0%)
41 to 50	2 (4.76%)	0 (0%)	1 (33.33%)	0 (0%)	0 (0%)
51 to 60	2 (4.76%)	0 (0%)	0 (0%)	2 (18.18%)	0 (0%)
61 to 70	1 (2.38%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

In Group-A i.e. Beta Thal Trait: Maximum cases i.e 12 were in 31 to 40 years of age group and minimum cases are from age group 61 to 70yrs i.e 01.

In Group-B i.e. Beta Thal Major: All three cases 3 were in 0 to 10 years age group.

In Group-C i.e. Sickle Cell Trait: Maximum cases i.e 2 were in 21 to 30 years age group and minimum cases were 01 in 41 to 50 years age group.

In Group-D i.e. Hb Q-India: Maximum cases i.e 3 were in 0 to 10 and 31 to 40 years age group each and minimum cases were 1 in 21 to 30 years age group.

In Group-E i.e. Hb Q-India-Beta thal Trait: One case is in age group 0 to 10 years.

Table 3: clinical presentation of cases among all groups: (n=60)

Clinical Feature	Group-A (BTT)	Group-B (BTM)	Group-C (SCT)	Group-D (Hb Q)	Group- E (Hb Q-BTT)	Total
Pallor	14	3	2	0	1	20
Jaundice	0	2	0	0	0	02
Hepatomegaly	0	1	0	0	0	1
Splenomegaly	0	1	0	0	0	1

- Majority of the cases presented clinically with Pallor i.e 20 cases out of which 14 cases were seen in Group A i.e. BTT.
- Next common presentation was jaundice which was seen in Group B i.e. BTM.
- Hepatomegaly and splenomegaly was seen in one patient of Group B i.e. BTM.

Table 4: Mean Hematological parameters in all age groups: (n=60)

Parameters	Group-A (BTT)	Group-B (BTM)	Group-C (SCT)	Group-D (Hb Q)	Group-E (Hb Q-BTT)
Hb (gm%)	11.13±1.52	4.33±0.95	9.56±0.70	13.29±1.46	10.3
RBC count (million/mm ³)	5.36±0.52	2.75±0.78	4.29±0.48	5.06±0.59	5.39
MCV (fl)	70.04±8.36	59.66±10.50	78±6.58	84.21±8.27	62.2
MCH (pg)	21.20±2.94	16.20±3.89	24.1±5.58	26.75±2.70	19.1
RDW (%)	16.52±2.15	26.76±1.55	15±2.94	14.32±1.02	18.3

(All Values in mean ± S.D.)

1. Lowest Hb was found in Group B i.e. Beta thal major and highest level of Hb was found in Group D i.e. Hb Q-India.
2. Lowest RBC count was found in Group B i.e. Beta thal major and highest level of RBC count was found in Group A i.e. Beta thal trait.
3. Lowest MCV was found in Group B i.e. Beta thal major and highest level of MCV was found in Group D i.e. Hb Q-India.

4. Lowest MCH was found in Group B i.e. Beta thal major and highest level of MCH was found in Group D i.e. Hb Q-India.
5. Lowest RDW was found in Group D i.e. Hb Q-India and highest RDW was found in Group B i.e. Beta thal major.

Table 5: Mean of different haemoglobin types in all age groups: (n=60)

Hb Type	Group-A (BTT)	Group-B (BTM)	Group-C (SCT)	Group-D (Hb Q)	Group-E (Hb Q-BTT)
HbF%	0.96±0.88	92.06±0.85	0.6±0.55	0.17±0.18	1.4
HbA0%	83.20±1.70	3.96±1.09	51.53±1.30	72.08±1.68	75.9
HbA2%	5.61±0.80	3.76±1.0	3.9±0.36	1.52±0.15	6
HbS%	0	0	38.5±1.70	0	0
Other Hb%	0	0	0	16.33±0.60	7.7

(All Values in mean ± S.D.)

1. In the present study in beta thal trait group average HbA2 level was found to be 5.61±0.80%. The cut off value for HbA2 > 3.9% was used to diagnose beta thalassaemia trait.¹³

2. In beta thal major group average HbF levels were high as compared to other groups.
3. In sickle cell trait group average HbS level was 38.5%.

Representative graph of each group:

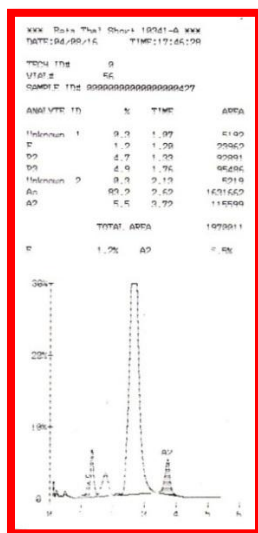


Fig. 1: BTT

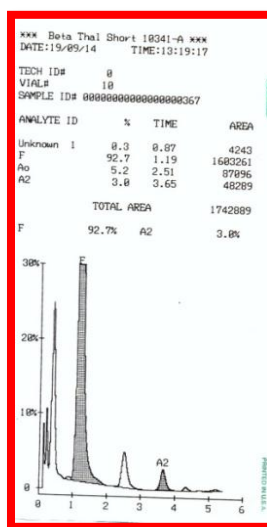


Fig. 2: BTM

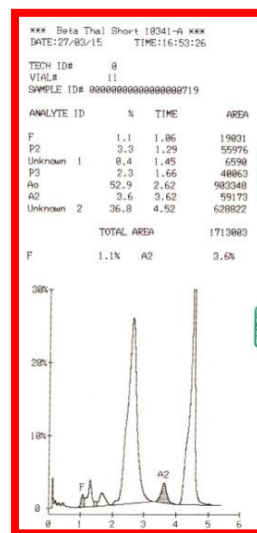


Fig. 3: SCT

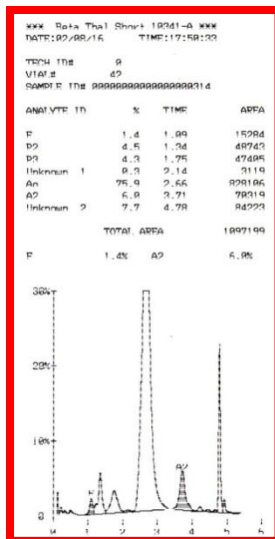


Fig. 4: Hb Q

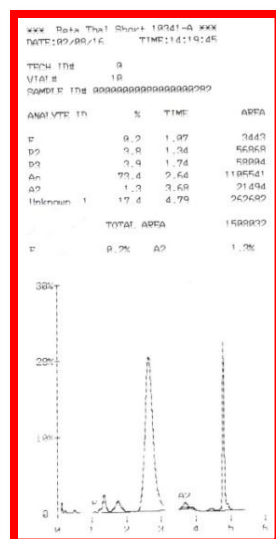


Fig. 5: Hb Q-BTT

DNA Analysis:



Fig. 6: Hb Q- India

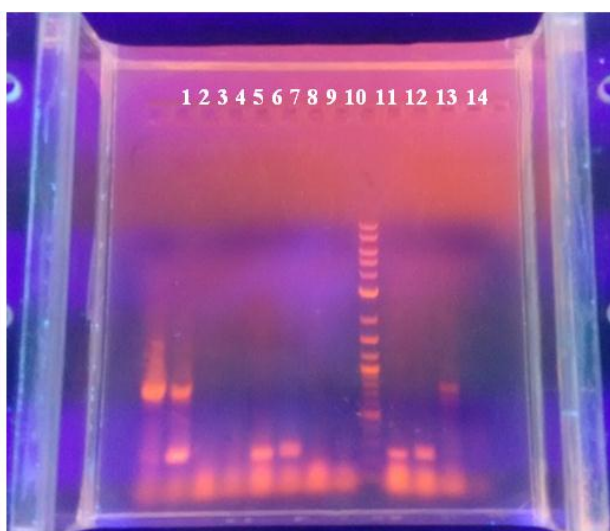


Fig. 7: BTT

Lane: 1-2Kb Ladder, Lane-2 to 6-Q samples, Lane: 5-HbQ+BTT
 Lane 1, 3, 5, 7, 10, 12- Normal for IVS1-5 (G→C) mutation and Lane 2, 4, 6, 8, 11 & 13-Mutant for IVS1-5(G→C) mutation, Lane-9: 2kb ladder

Table 6:

	HbF (%)	HbA0 (%)	HbA2 (%)	Hb Q (%)	Interpretation
Index case	1.4	75.9	6.0	7.7	Hb Q-India-BTT.
Father	0.3	74.2	1.5	16.6	Hb Q-India
Mother	1.0	83.4	6.8	0	Beta thal trait
Brother	0.4	73.5	1.7	17	Hb Q-India

We found one case of Hb Q-India with beta thalassaemia trait, male child 10 years of age, his father is heterozygous for HbQ-India & his mother is Beta thalassaemia trait, his brother is Hb Q-India trait, we

confirmed this case with family studies & also with molecular studies.

Discussion

Present study shows that there is high prevalence of beta thalassaemia trait in sindhi community which is in accordance with the other studies. Present cross sectional prospective study was conducted in such a way so that there will be no bias in age selection. There is no restriction on age of the individual participating in the study and we have not divided them into sub castes which can create bias. We studied for all the haemoglobin disorders and not for beta Thalassaemia trait only which is actually more common in Sindhi community.

WHO recommended that Screening for haemoglobin disorders should be an intrinsic part of health care in most Countries. A policy of detecting carriers and informing them of their risk, and possibilities for reducing it, usually leads to a fall in births and deaths of affected children. Requirements are the same for Thalassaemias and Sickle-cell disorders. In most countries, the approach develops in three stages- First, retrospectively informing parents with affected children of their 25% recurrence risk allows them to limit family size and, where average family sizes are typically large, this approach can significantly reduce

affected birth prevalence. Second, introduction of prenatal diagnosis for couples with affected children enables them to have a family, but has little further effect on affected birth prevalence. Third, information and prospective carrier screening is provided for the whole population. Choice of strategy varies with social attitudes, costs and opportunities within the health system.¹

According to we have done counseling of unmarried thalassaemia carriers and sickle cell carriers and their parents regarding the inheritance pattern of these disorders and advised them to not to marry another carrier so that birth of babies of severe forms of these disorders can be reduced. Also counseling of those couples who are both carriers of thalassaemia was done and information regarding the prenatal diagnosis in next pregnancy was given to them.

In present study 2 out of 35 couples showed history of consanguineous marriage, consanguineous marriage increases the incidence of hemoglobin disorders in high risk communities like sindhi community, this is because hemoglobin disorders are transmitted only through heredity.

Prevalence of Haemoglobin Disorders:

Table 7: Studies on specific groups (Sindhi Community)

Studies	Prevalence of haemoglobin disorders	Prevalence of Beta thal. trait	Prevalence of Beta thal. major	Prevalence of Sickle cell trait
Jawahirani et al. ¹⁸ (2006) (n=1563)	-	16.4%	-	-
Mulchandani D. V. et al. ⁸ (2008) (n=446)	-	16.81%	-	-
Saraswathy K. N. et al. ¹⁹ (2009) (n=210)	-	8.6%	-	-
Balgir RS ²⁰ (2009) (n=508)	22.9%	20.5%	0.19%	No case found
Zia H. Khan et al. ⁹ (2013) (n=1888)	-	11.01%	-	-
Rakholia R, Chaturvedi P ²¹ (2013) (n=550)	-	17.2%	-	-
Kaur M. et al. ³ (2013) (n=500)	24%	15.4%	2.8%	1.8%
Das K et al. ²² (2013) (n=1498)	-	14.49%	-	-
Bhanvadia V. M. et al. ²³ (2015) (n=109)	19.26%	15.59%	-	3.66%
Present study (2017) (n=338)	17.75%	12.42%	0.88%	0.88%

1. Study for prevalence of haemoglobin disorders in sindhi community was done by Balgir RS,²⁰ Kaur M. et al.³, Bhanvadia V. M. et al.²³ Present study has overall prevalence of 18.95% which is comparable with results of these studies.
2. Other studies like Zia H. Khan et al.⁹, Mulchandani D. V. et al.⁸, Rakholia R, Chaturvedi P²¹ Jawahirani et al.¹⁸, Saraswathy K. N. et al.¹⁹ studied the prevalence of only beta thalassaemia trait in sindhi community.

- Jawahirani et al.¹⁸ showed variable prevalence of beta thal. Trait in sindhi community based on prevalence in different sub castes of sindhi population which can cause bias in selection for sampling.
- Mulchandani D. V. et al.⁸ studied the prevalence of beta thalassaemia trait in sindhi community but they have taken 10 year and above individuals for screening which cannot be considered as representative sample because of bias in age selection.
- Similarly Rakholia R, Chaturvedi P²¹ had taken individuals from age 3 year to 28 years from sindhi community which again cannot be considered as representative of whole community.
- Das K et al.²² showed variable frequency of beta thal. Trait in sub castes of sindhi community of Nagpur similar to Jawahirani et al. Frequency of beta thal. Trait was 14.49% which is close to present study.

Prevalence of Haemoglobin Disorders:

Table 8: Studies on prevalence of haemoglobin disorders in different communities with sindhi community included

Studies	Prevalence of BTT (Overall)	Prevalence of SCT (Overall)	Prevalence of BTT in sindhi com.	Prevalence of SCT in sindhi com.
Patel A. P. at al. ²⁴ (2008) (n=317539)	1.95%	6.54%	8.1%	-
Urade B. P ⁷ (2013) (n=5172)	2.3%	6.1%	10.4%	0.2%
Present study (2017) (n=338)			12.42%	0.88%

Urade B.P⁷ and Patel A. P. at al.²⁴ studied the prevalence of HBS and BTT in different communities with sindhi community included in that. There results clearly showed the higher frequency of beta thal. Trait in sindhi community over the other communities.

Conclusion

- Standard treatment and care for thalassaemic child puts emotional and financial stress on the family as it is associated with frequent hospital visits, expensive treatments and procedures.
- Only curative treatment is bone marrow transplant which is out of reach of majority of people. So focus has to be shifted from treatment to prevention birth of such child's in future.
- Mass screening puts huge burden on resources and manpower, so high risk screening of vulnerable community is better as it targets population most likely to be benefited from screening.
- So present study emphasizes the need of community based targeted study so that health care resources can be planned accordingly to reduce the disease burden.

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