Original Research Article

A clinico-haematological study of hereditary hemoglobinopathies: a tertiary care centre experience

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Received: 20 June 2021
Revised: 20 July 2021
Accepted: 21 July 2021

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ABSTRACT

Background: Hemoglobinopathies are the cause of concern in India for not only its effect on the quality of life in patients but also for their inheritance patterns. Tribal population of Visakhapatnam district has a high chance of inheriting hemoglobinopathies due to their culture of consanguineous marriage. Aim and objectives of current study were to know the distribution of various abnormal haemoglobins in cases with clinical suspicion of hemoglobinopathies.

Methods: This hospital-based observational study was conducted for a period of 10 months in the department of pathology, Andhra Medical College, Visakhapatnam. A total of 151 cases with suspected hemoglobinopathies, their parents, and siblings were screened for the presence of hemoglobinopathies. 3ml of venous blood was collected to perform complete blood count, peripheral smear, reticulocyte count, sickling test and High Performance liquid chromatography (HPLC).

Results: In the present study, out of 151 cases, 55 cases (36.42%) were adults, and 96 cases (63.57%) were children. 67 cases (44.37%) were asymptomatic and 84 cases (55.62%) were symptomatic. The most common symptom of subjects are fever (23 cases, 27.38%) and dyspnoea (22 cases, 26.19%). 85 cases (56.29%) had normal HPLC, and 66 cases (43.70%) had abnormal hemoglobin variants. The most common hemoglobinopathy detected by HPLC was sickle cell trait (36 cases, 23.84%) followed by homozygous sickle cell anemia 15 cases (9.93%). Other hemoglobinopathies detected were beta-thalassemia trait; 8 cases (5.29%) and compound heterozygous sickle beta-thalassemia 3 cases (1.98%).

Conclusions: Endemic areas for hemoglobinopathies has to be screened with HPLC along with complete hemogram in suspicious cases for the better diagnosis and management of the condition.

Keywords: Hereditary hemoglobinopathy, High performance liquid chromatography, Screening

INTRODUCTION

Hemoglobinopathies are the most common single-gene disorders in the world. World Health Organisation (WHO) estimates that 5% of the world's population are carriers of a potentially pathological haemoglobin gene.1 Consanguineous marriage being the choice of an estimated 10.4% of the global population, was found to be the major reason for the highest prevalence of Hemoglobinopathies.2 It has been estimated that in India, 0.37 per 1,000 foetuses have Hb disorder.3 Unawareness regarding hemoglobinopathies has been the primary reason for its highest prevalence in the country.4 Hemoglobinopathies are common in tribal population due to high practice of consanguineous marriage. The major tribal groups found in the district of Visakhapatnam are...
Bagata, Kotiya, Kondadora, Nookadora, Konda Kammara and Konda Kappu. Thalassemia and sickle cell anaemia are noteworthy for their significant impact on the day to day life of patients. Thalassemia is caused by the failure of the synthesis of alpha and beta chains of haemoglobin. The overall prevalence of beta-thalassemia in India is 3-4%. An estimate of around 10,000-12,000 children are born every year with beta-thalassemia major. The carrier rate for beta-thalassemia gene ranges from 1% to 3% in Southern and 3% to 15% in Northern parts of India. Lehman and Cutbush in 1952 first described the sickle hemoglobin in the tribal populations in the Nilgiri hills in India. The prevalence of sickle cell carriers among different tribal groups in India is 1-40%. Sick cell disease is caused by mutation in 6th position of beta chain of hemoglobin which causes replacement of glutamate by valine. The lifelong morbidity in homozygotes of sickle cell anaemia such as acute painful crises, infections and risk of recurrent stroke mandates effective screening and prevention programmes for sickle cell disease. Screening is pivotal in both individuals with clinical symptoms for hemoglobinopathies and his/her family members by complete hemogram of an individual and confirmation by estimating various hemoglobin fractions using high performance liquid chromatography (HPLC). Thus the present study aimed to know the distribution of various abnormal hemoglobins and to evaluate the role of high-performance liquid chromatography (HPLC) in cases with clinical suspicion of hemoglobinopathies in asymptomatic and symptomatic cases.

METHODS

This is a hospital based observational study carried out for a period of 10 months (August 2020 to June 2021) in the department of pathology, Andhra medical college, Visakhapatnam. A total of 151 cases were included in this study.

Sampling technique

Sampling in current study was done through convenience sampling technique.

Inclusion criteria

Inclusion criteria for current study were; patients with suspicion of haemoglobinopathy by clinical history and haematological findings and patients with a clinical history of pallor, fatigue, breathlessness, and jaundice. The family members of hemoglobinopathy patients were also included in the study.

Exclusion criteria

Patients who underwent blood transfusion in the past three months were excluded from the study.

A written informed consent from adults and consent from a parent in paediatric subjects was obtained from all participants before enrolling into the study. Consent was obtained from a parent in paediatric subjects. 3ml of venous whole blood in EDTA (ethylene diamine tetra acetic acid) vacutainer and direct peripheral smear from each subject was collected. Clinical history and family history were also collected in all cases. Informant was a parent in paediatric cases. A complete blood count (CBC) in five part automated haematology analyser (sysmex), peripheral smear with leishman stain, reticulocyte count by new methylene blue stained smears, sickling test with freshly prepared 2% sodium metabisulphite were performed immediately in all cases. Samples were stored at 2-8˚Celsius to run in batches for HPLC (TOSHO G8 analyzer). The data was analysed in Microsoft office excel 2007 for percentage distribution.

RESULTS

In the present study, out of 151 cases, 85 cases (56.29%) had normal HPLC, and 66 cases (43.70%) had abnormal haemoglobin variants in HPLC. The most common hemoglobinopathy detected by HPLC was sickle cell trait (36 cases, 23.84%) followed by homozygous sickle cell anemia (15 cases, 9.93%). Other hemoglobinopathies detected were beta-thalassemia trait 8 cases (5.29%) and compound heterozygous sickle beta-thalassemia (3 cases, 1.98%), high Hb F (4 cases, 2.64%). Elevated Hba1c was observed in two cases who also found to have inclined blood sugar levels on follow-up. One case was a 3-month-old infant with clinical features of Wolfram syndrome such as bilateral optic nerve atrophy and diabetes mellitus. Detailed history also revealed diabetes mellitus in mother. Another case was an elderly female patient with diabetes mellitus and pallor. Distribution of various hemoglobinopathies detected was mentioned in (Table 1). Normal chromatogram of an adult male patient presented with severe anemia is shown in (Figure 1). While screening the siblings of hemoglobinopathy patients, an interesting case of 8 year old boy with clinical and radiological features of tuberous sclerosis was diagnosed with homozygous sickle cell anaemia by HPLC. His chromatogram showed a peak in F window of retention time 1.13min corresponding to Hb F (25.3%) and S window of retention time 4.35 min corresponding to Hb S 65.9% in HPLC (Figure 2a-f). A 3 year old male asymptomatic sibling of sickle cell anemia child was diagnosed to have sickle cell trait with Hba0-56.1%, HbS-27.8%, and Hba2-1.4% by chromatogram. His sickling test was positive (Figure 3). HPLC of a 4 year old female child having microcytic hypochromic anemia with plenty of target cells showed beta-thalassemia trait with HbA2 -6.5% (Figure 4a-b).

An adolescent female patient with microcytic hypochromic anemia of mild degree had sickle beta-thalassemia trait with HbS- 23.6% and HbA2-4.3% on HPLC (Figure 5). Of 66 cases with abnormal haemoglobins, 29 cases (43.93%) belong to the adult age group.
group, 14 cases (21.21%) belong to the adolescent age group, 15 cases (22.72%) belong to 1-10 years of age and 8 cases (12.12%) belong to infancy. The age distribution of cases with hemoglobinopathies was mentioned in (Table 2).

Table 1: Distribution of cases with various abnormal haemoglobins in the present study (n=151).

| HPLC findings                        | N (%) |
|--------------------------------------|-------|
| Normal                               | 85 (56.29) |
| Sickle cell trait                    | 36 (23.84) |
| Sickle cell anaemia                  | 15 (9.93) |
| Beta thalassemia trait               | 8 (5.29) |
| Sickle beta thalassemia double       | 3 (1.98) |
| heterozygous trait                   |       |
| High Hb F (infants)                  | 4 (2.64) |
| High HbA1c                           | 2 (1.3) |

Abnormal hemoglobins were common in females (40 cases, 60.60%) than males (26 cases, 39.39%). In the present study, 37.31% of asymptomatic cases had hemoglobinopathy which was predominantly sickle cell trait followed by beta-thalassemia trait. Interestingly, these asymptomatic cases were siblings and parents of haemoglobinopathy patients. 61.22% of cases with symptoms such as fever, shortness of breath, and pallor had haemoglobinopathy which was predominant symptom of sickle cell anemia. Among 21 cases with hepatosplenomegaly, 14 cases (66.7%) had haemoglobinopathy which was beta-thalassemia trait and sickle cell trait. HPLC findings in cases with clinical suspicion of hemoglobinopathy in asymptomatic and symptomatic cases were tabulated in (Table 3). 38.88% non-anaemic patients had heterozygous hemoglobinopathies such as sickle cell trait and beta-thalassemia trait. 10 cases with severe anemia (33.33%) had sickle cell anemia. Out of 3 cases of double heterozygous sickle beta-thalassemia trait, 2 cases had moderate degree anemia with Hb-10g/dl and 1 case had mild degree anemia with Hb-10g/dl. All 3 cases had microcytic hypochromic RBCs in peripheral smear. HPLC findings in cases with anaemia and non-anaemic cases were mentioned in (Table 4).

Figure 2: a-b) A 8-year-old boy with clinical features of tuberous sclerosis such as facial angiofibroma, and ash leaf macules, c) MRI brain showing numerous cortical tubers, d) sickling test was positive, showing sickle cells, e) peripheral smear showing sickle cells and microcytic hypochromic anemia, f) chromatogram showing a peak in F window of retention time 1.13 minutes (yellow peak) corresponding to Hb F (25.3%) and S window of retention time 4.35 minutes corresponding to HbS 65.9% (black arrow) in HPLC.

Figure 3: Chromatogram of sickle cell trait with HbA0-56.1%, Hb S-27.8% (yellow arrow) and HbA2-1.4%.

All the cases of sickle cell anemia and beta-thalassemia trait (100%) had low MCV, low MCH values in the present study. High reticulocyte response was observed in 5 cases of beta-thalassemia trait and 11 cases of sickle cell anemia. RBC indices findings in various hemoglobinopathies were mentioned in (Table 5).
Table 2: Age distribution of cases with hemoglobinopathies (n=66).

| Age group               | Infants | Toddler (1-3 years) | Pre school (3-5 years) | School (5-10 years) | Adolescent (11-18 years) | Adults (>18 years) |
|-------------------------|---------|---------------------|------------------------|---------------------|--------------------------|-------------------|
| Sickle cell trait       | 04      | 2                   | 5                      | 2                   | 6                        | 17                |
| Sickle cell anemia      | 0       | 1                   | 0                      | 2                   | 5                        | 7                 |
| Beta thalassemia trait | 0       | 0                   | 1                      | 2                   | 1                        | 4                 |
| Sickle-beta thalassemia trait | 0   | 0                   | 0                      | 0                   | 2                        | 1                 |
| High HbF                | 04      | -                   | -                      | -                   | -                        | -                 |
| Total; N (%)            | 8 (12.12) | 3 (4.54)             | 6 (9.1)                | 6 (9.1)             | 14 (21.21)               | 29 (43.93)        |

Figure 4: a) peripheral smear showing microcytic hypochromic anaemia with plenty of target cells, b) Chromatogram showing beta-thalassemia trait with Hb A2 -6.5% (yellow arrow).

Figure 5: Chromatogram of sickle beta-thalassemia trait showing HbS-23.6% (yellow arrow) and Hb A2-4.3% (black arrow).

DISCUSSION

Haemoglobinopathies are broadly divided into the presence of structurally abnormal hemoglobin variants and defective synthesis of normal haemoglobin chains called thalassemia. In the present study, 66 cases (43.70%) had hemoglobinopathy which is significant and higher in comparison with the study of Sachdev et al (12.57%), Haryana. Unlike the study by Buch in western Maharashtra with a sample of 3465 cases (2016), beta-thalassemia were the predominant hemoglobinopathy detected, the current study shows predominance in sickle cell trait and sickle cell disease. Female preponderance in hemoglobinopathies 40 cases, (60.60%) was seen in the present study which was similar to the study of Santhosh et al (40.81%). The asymptomatic and nonanemic parents and siblings of hemoglobinopathy cases were screened for hemoglobinopathy by HPLC. The present study had 37.31% of haemoglobinopathy among 67 asymptomatic cases which call attention to the screening among the general population and also to cascade screening of family members. In a study done by Gorakshakar and Colah, it was concluded that cascade screening can detect more number of carriers beta thalassemia trait within the family than screening the general population. Red cell indices such as MCV (mean corpuscular volume), MCH (Mean Corpuscular Haemoglobin) and MCHC (mean corpuscular hemoglobin concentration) have a significant role in suspicion of beta-thalassemia. Low MCV, MCH and MCHC values were observed in cases of beta-thalassemia trait in the present study which correlated with Sorathiya et al. In a case of microcytic hypochromic anaemia, RDW is less than 14.5% in iron deficiency and 14.5% in beta thalassemia trait. The RDW value of less than 14.5% was observed in 7 cases of beta thalassemia trait in the present study.
In the present study, cut-off of Hb A2 was taken as 3.5 % for the diagnosis of thalassemia. Iron deficiency anaemia lowers the value of HbA2 but studies suggest that HPLC can diagnose elevated HbA2 levels in thalassemia even in presence of iron deficiency anaemia.13 In the present study, cases with normal serum iron profile, serum vitamin B12, serum folate were considered for HPLC evaluation to avoid dilemmas with falsely low and falsely high hemoglobin fractions.

In cases with borderline HbA2 level (3-3.4%), genetic testing was advised to rule out alpha thalassemia or delta beta thalassemia. In the present study, a child with clinical features of tuberous sclerosis had homozygous sickle cell anemia by HPLC. He had facial angiofibromas, ash-leaf macules on trunk, therapy refractory recurrent seizures, rhabdomyoma of heart since birth, multiple cortical tubers and multiple subependymal nodules in brain. Genetic analysis for mutation in tuberous sclerosis gene was not done due to lack of facility. Coexistence of these double genetic diseases in a child is very rare.

The structurally abnormal haemoglobin variants vary in prevalence based on the geographical distribution and ethnicity of the population. In the study of Pant et al in Delhi with a sample size of 4800 cases, HbE disease and Hb D trait, Hb J Meerut and Hb Hope were diagnosed by HPLC.14 In the present study, abnormal variant haemoglobins such as Hb D Punjab, Hb E, and Hb C were not encountered which can be probably due to the small sample size and hospital-based sample collection for the study during COVID-19 pandemic. In a study done by Mondal et al 96.6% of cases with significant HbA1c levels had high blood sugar values whereas, in the present study, 100% of cases with elevated HbA1c had diabetes mellitus.2

**Limitations**

Small sample size and hospital-based study design were the major limitations of the study and hence results cannot be generalized to the community burden of hemoglobinopathies. The genetic analysis for confirmation of hemoglobinopathies was not done due to the non-availability of a facility in the hospital. Infants with high HbF were lost to follow up and diagnosis of hereditary persistence of fetal globin could not be confirmed.

**CONCLUSION**

The present study conducted using HPLC reflects the magnitude of hemoglobinopathies in a hospital based setting which infact is the tip of an iceberg. Heterozygous state of hemoglobinopathies needs special attention for diagnosis due to the high frequency of asymptomatic carriers and normal hemogram picture. Hence, HPLC has a definite role as a screening and diagnostic tool for various hemoglobinopathies in high incidence areas.

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**Table 4: HPLC findings in cases with anaemia and non anaemic cases (n=151).**

| Degree of anaemia | HPLC findings N (%) |
|------------------|---------------------|
| Non-anaemic (36) | Normal 20 (55.55)   |
|                  | Haemoglobinopathy 14 (38.88) |
|                  | High HbA1c 2 (5.55) |
| Mild degree anaemia (40) | Normal 27 (67.5) |
|                    | Haemoglobinopathy 13 (32.5) |
| Moderate degree anaemia (45) | Normal 27 (60) |
|                    | Haemoglobinopathy 18 (40) |
| Severe degree anaemia (30) | Normal 20 (66.66) |
|                   | Haemoglobinopathy 10 (33.33) |

**Table 5: RBC Indices in various hemoglobinopathies (n=66).**

| RBC Indices | Sickle cell trait | Sickle cell anaemia | Beta thalasemia | Sickle beta Thalasemia | High HbF | Total |
|-------------|------------------|--------------------|-----------------|------------------------|---------|-------|
| MCV (fl)    | Low              | 24                 | 15              | 8                      | 3       | 2     | 52 |
|             | Normal           | 12                 | 0               | 0                      | 0       | 2     | 14 |
| MCH (pg)    | Low              | 23                 | 15              | 8                      | 3       | 2     | 51 |
|             | Normal           | 13                 | 0               | 0                      | 0       | 2     | 15 |
| MCHC (%)    | Low              | 22                 | 12              | 6                      | 2       | 2     | 44 |
|             | Normal           | 14                 | 3               | 2                      | 1       | 2     | 22 |
| RDW (%)     | Normal           | 9                  | 2               | 7                      | 0       | 1     | 19 |
|             | High             | 27                 | 13              | 1                      | 3       | 3     | 47 |
| RC (%)      | Normal           | 15                 | 4               | 3                      | 0       | 3     | 25 |
|             | High             | 21                 | 11              | 5                      | 3       | 1     | 41 |

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Thalassemia is diagnosed by elevated HbA2 level in HPLC. The cut-off of Hb A2 level as 4% for the diagnosis of Beta-thalassemia was taken by Sorathiya et al study.9 In the present study, cut-off of HbA2 was taken as 3.5 % for the diagnosis of thalassemia. Iron deficiency anaemia lowers the value of HbA2 but studies suggest that HPLC can diagnose elevated HbA2 levels in thalassemia even in presence of iron deficiency anaemia.13
ACKNOWLEDGEMENTS

We thank the multidisciplinary research unit (MRU), department of health and research, New Delhi and principal, Andhra medical college for the infrastructure provided to conduct the study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Kumar BM, Saikia M, Baruah A. Pattern of hemoglobinopathies and thalassemias in upper Assam region of North Eastern India: High performance liquid chromatography studies in 9000 patients. Indian J Pathol Microbiol. 2014;57:236-43.
2. Bittles AH, Black ML. Consanguineous marriage and human evolution. Annu Rev Anthropol. 2010;39:193-207.
3. Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: A 10-year high-performance liquid chromatography study of 119,336 cases. Asian J Transfus Sci. 2016;10(1):105-10.
4. Babu STV, Manjula S. An incidence of beta-thalassemia in south india-a review. Int J Res Pharm Biosci. 2016;3(5):1-6.
5. Aggarwal R, Prakash A, Aggarwal M. Thalassemia: An overview. J Sci Soc. 2014;41:3-6.
6. Joseph N, Pai S, Sengupta S, Bharadwaj S, Dhawan S, Khare K. A clinic-epidemiological study of thalassemia cases in India. J Nat ScBiol Med. 2018;9:236-41.
7. Bhatia HM, Rao VR. Genetic atlas of Indian Tribes. Available at: https://catalogue.nla.gov.au/Record/1171067. Accessed on 20 April 2021.
8. Sachdev R, Dam AR, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: Report of 2600 cases. Indian J Pathol Microbiol. 2010;53:57-62.
9. Buch A, Iqbal B, Bordawekar R, Jain A, Jariwala P, Rathod H. Patterns of hemoglobinopathies diagnosed by high-performance liquid chromatography in and around Pune (Western Maharashtra, India): A pilot study. J Med Soc. 2016;30:111-5.
10. Bhokare SB, Phulgirkar PP, Joshi AR, Bindu RS. Spectrum of hemoglobinopathies by high performance liquid chromatography with special reference to role of HbA2 levels at tertiary care centre. Int J Res Med Sci. 2016;4:5269-76.
11. Gorakshakar AC, Colah RB. Cascade screening for b-thalassemia: a practical approach for identifying and counselling carriers in India. Indian J Community Med. 2009;34:354-6.
12. Sorathiya VP, Vachhani NA, Nandani SL, Vekariya DJ, Kashtiyani HN, Colah RB. Experience with NESTROFT for screening for thalassemia trait/minor: evaluation against CBC and HPLC in a high prevalence region in Saurashtra, Gujarat, India. Int J Res Med Sci. 2020;8:1108-13.
13. Khera R, Singh T, Khuna N, Gupta N, Dubey AP. HPLC in characterization of hemoglobin profile in thalassemia syndromes and hemoglobinopathies: a clinicohematological correlation. Indian J Hematol Blood Transfus. 2015;31(1):110-5.
14. Pant L, Kalita D, Singh S, Kadesia M, Mediratta S, Mittal M et al. Detection of Abnormal Hemoglobin Variants by HPLC Method: Common Problems with Suggested Solutions. Int Scho Res Notices. 2015;5:1-9.

Cite this article as: Atla B, Botta VSK, Balakrishnan P, Lalam N, Argi A, Natukula K. A clinico-haematological study of hereditary hemoglobinopathies: a tertiary care centre experience. Int J Res Med Sci 2021;9:2309-14.