# Study of Hemoglobinopathies at a Referral Laboratory in a Western District of West Bengal among the Antenatal Women and Premarital Men and Women: A 2 Years Study

**Priyaranjan Chattopadhyay[1], Soumya Kundu[2], Tarak Nath Saha[3], Kausik Chatterjee[4]**

**ABSTRACT**

**Introduction:** Thalassemia and related hemoglobinopathies are a major preventable hematological disorder, affecting most parts of the Indian subcontinent. Prevention of these disorders lies in screening of at-risk population. This study was undertaken to assess the disease burden amongst pre-defined at-risk population in western districts of West Bengal.

**Material and methods:** A total of 903 cases of antenatal mothers and premarital men and women in the age range of 18-40 years referred for Hemoglobin High Performance Liquid Chromatography were studied during a two year period. Blood samples were collected and tested in an automated hematology analyzer for hematological assessment and HPLC for hemoglobin pattern. Suitable statistical methods were used for analysis of the data.

**Results:** Among the study population, 86.32% were normal and 13.69% showed abnormal hemoglobin fractions. β Thalassemia trait was the most common abnormal hemoglobin comprising 8.34% of the cases. Heterozygous for sickle cell disease (2.22%) was the second most common abnormality, followed by HbE Heterozygous (1.56%).

**Conclusion:** The present cross-sectional study closely follows the thalassemic gene burden in similar studies done in this area previously. With increasing awareness and with this simple screening procedure, it may be possible to eradicate or at least lessen the present community burden of thalassemia in foreseeable future.

**Keywords:** At-risk Population, Hemoglobinopathies, HPLC, Thalassemia

## INTRODUCTION

Thalassemia and other hemoglobinopathies are one of the most common preventable hematological disorders of the South East Asian population, of which India carries a major burden. These disorders are caused by genetic mutation in the globin gene sequence, resulting in a spectrum of clinical manifestations ranging from asymptomatic career state to major disease, requiring repeated blood transfusions and associated increased morbidity and mortality. The prevention of these disorders lies in effective screening of at-risk population, namely premarital men and women and anti-natal women. Various studies have been done in the past which have showed different prevalence patterns of these diseases in different parts of the country.[1,2]

The present study was undertaken to assess the disease burden amongst the antenatal women and premarital men and women, so that a cost-effective screening program can be taken up to prevent the birth of every single child with thalassemia disease.

## MATERIAL AND METHODS

Antenatal mothers and premarital men and women in the age range of 18-40 years referred for Hemoglobin High Performance Liquid Chromatography (Hb HPLC) testing in a referral laboratory in Medinipur town between from April 2017 to March 2019, were included in this cross sectional, descriptive study. A total of 903 cases were studied from West Medinipur and Jhargram Districts of West Bengal which includes predominantly rural areas with a large tribal population. Written consent was collected from each patient.

Subjects with a history of blood transfusion within last one month and age group outside the defined age range were excluded. Medical records were reviewed and 4 cases were excluded due to history of recent blood transfusion.

2.5 ml of venous blood were collected in the EDTA vacutainer from each subject.

The blood samples were tested by automated hematology analyzer (ABX Horiba Pentra 60 C) for Hemoglobin and RBC indices.

Hemoglobin HPLC with proper dual mode calibration and control was done in Biorad D10 (Bio-Rad Laboratories, Hercules, CA, USA). HbA2 value of 3.5% was taken as upper limit of normal. A range of 4 -7% Hb A2 was taken for diagnosis of β-thalassemia trait.[3] HPLC results were interpreted on the basis of retention time of Hb fractions, percentage of Hb and peak characteristics. All data were entered in MS Excel 2010 and analyzed using Origin PRO 8 SRO software.

[1]Assistant Professor, Department of Pathology,Midnapore Medical College, Medinipur, West Bengal, [2]Assistant Professor, Department of Pathology, Midnapore Medical College, Medinipur, West Bengal, [3]Associate Professor, Department of Pathology, Midnapore Medical College, Medinipur, West Bengal, [4]Consultant Epidemiologist, DPMU, Zilla Swasthya Bhawan, Jhargram, West Bengal, India

**Corresponding author:** Dr Soumya Kundu. Flat No B8/3, Samadrita Housing Society, EKTP Phase 3, Kolkata 700107, West Bengal, India

**How to cite this article:** Priyaranjan Chattopadhyay, Soumya Kundu, Tarak Nath Saha, Kausik Chatterjee. Study of hemoglobinopathies at a referral laboratory in a western district of West Bengal among the antenatal women and premarital men and women: a 2 years study. International Journal of Contemporary Medical Research 2019;6(6):F24-F27.

**DOI:** http://dx.doi.org/10.21276/ijcmr.2019.6.6.31

## RESULTS

Amongst study group of 899 cases, 208 were male and 691 were female. (Table 1)

Females outnumbered males in our study on account of routine antenatal check-up. Mean age of the study population was 29.1± 5.04 years for male and 24.25± 4.85 years for female. (Mean ± 1 Standard Deviation)

Analyzing the HPLC data, 776 cases (86.32%) were normal and 123 cases (13.69%) showed abnormal hemoglobin fractions. β Thalassemia trait was the most common abnormal hemoglobin in the present study, comprising 8.34% of the cases. (Table 1) Heterozygous for sickle cell disease (2.22%) was the second most common abnormality, followed by HbE

| Patterns of hemoglobin | Number of cases | | Total | |
|---|---|---|---|---|
| | Male | Female | Number | Percentage |
| Normal | 177 | 599 | 776 | 86.32 |
| β-Trait | 19 | 56 | 75 | 8.34 |
| HBS Heterozygous | 5 | 15 | 20 | 2.22 |
| HBS Homozygous | 1 | 2 | 3 | 0.33 |
| HBE Heterozygous | 3 | 11 | 14 | 1.56 |
| HBE Homozygous | 1 | 2 | 3 | 0.33 |
| HBD Heterozygous | 1 | 0 | 1 | 0.11 |
| Compound Heterozygous for HBE β-Thalassaemia | 0 | 3 | 3 | 0.33 |
| Compound Heterozygous for HBS β-Thalassaemia | 0 | 1 | 1 | 0.11 |
| HPFH Heterozygous | 1 | 2 | 3 | 0.33 |
| Total | 208 | 691 | 899 | 100 |

**Table-1:** Distribution of hemoglobin patterns in the study population

| Patterns of Hemoglobin | Number of cases (N=899) | | HbF(%) | HbA0(%) | HbA2(%) | Abnormal Peak Value |
|---|---|---|---|---|---|---|
| | M | F | M+F | M+F | M+F | M+F |
| Normal | 177 | 599 | 1.97±0.23 | 84.68±4.42 | 2.97±0.41 | |
| β-Trait | 19 | 56 | 2.01±1 | 82.47±1.0 | 5.23±0.0 | |
| HbS Heterozygous | 5 | 15 | 1.25±0.5 | 59.96±3.38 | 5.82±1.0 | 27.31±3.0 |
| HbS Homozygous | 1 | 2 | 10.23±0.45 | 8.10±2.13 | 3.37±1.28 | 75.27±4.0 |
| HbE Heterozygous | 3 | 11 | 1.28±0.34 | 64.84±2.37 | 26.76±2.91 | |
| HbE Homozygous | 1 | 2 | 2.3±0.57 | 9.85±0.49 | 95.55±2.19 | |
| HbD Heterozygous | 1 | 0 | 0.8 | 58.2 | 1.9 | 27.2 |
| Compound Heterozygous for HbE β-Thalassemia | 0 | 3 | 27.1±9.32 | 12.0±1.81 | 60.8±15.27 | 66.2 |
| Compound Heterozygous for HbS β-Thalassemia | 0 | 1 | 7.7 | 25.1 | 5.9 | 56.5 |
| HPFH Heterozygous | 1 | 2 | 32.3±12.03 | 54.87±10.64 | 2.50±0.17 | |
| Total | 208 | 691 | | | | |

M=Male, F= Female, Hb= Hemoglobin, β= Beta, Values mentined are Area Count(%) mean± Standard Deviation

**Table-2:** Distribution of major Hemoglobin fractions in the study group

| Patterns of hemoglobin | Numberof cases (N=899) | | Hb(gm/dl) | MCV(fl) | MCH(pg) | RDW-SD(%) |
|---|---|---|---|---|---|---|
| | Male | Female | Male + Female | Male + Female | Male + Female | Male + Female |
| Normal | 177 | 599 | 11.90±2.37 | 80.93±9.28 | 26.83±11.63 | 13.20±3.88 |
| β-Trait | 19 | 56 | 10.68±1.59 | 65.48±5.28 | 20.96±4.94 | 15.19±2.14 |
| HBS Heterozygous | 5 | 15 | 12.07±2.22 | 74.65±8.45 | 24.15±3.50 | 13.44±2.19 |
| HBS Homozygous | 1 | 2 | 7.03±2.22 | 87.33±12.90 | 27.33±4.64 | 14.87±1.20 |
| HBE Heterozygous | 3 | 11 | 11.91±2.57 | 75.31±5.36 | 24.18±2.63 | 13.64±2.59 [ |
| HBE Homozygous | 1 | 2 | 10±0.14 | 64±4.24 | 21±1.98 | 14.85±2.33 |
| HBD Heterozygous | 1 | 0 | 7.00 | 52.00 | 15.7 | 16.4 |
| Compound Heterozygous for HBE β-Thalassaemia | 0 | 3 | 7.10±0.20 | 60.67±3.51 | 17.83±1.04 | 19.30±2.49 |
| Compound Heterozygous for HBS β-Thalassaemia | 0 | 1 | 6.2 | 76 | 21.2 | 21.6 |
| HPFH Heterozygous | 1 | 2 | 12.33±2.89 | 81.47±10.31 | 26.23±3.00 | 14.10±5.05 |
| Total | 208 | 691 | | | | |

Values mentioned are mean±SD. Hb=Hemoglobin; β=Beta; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; RDW-SD=Red cell distribution width-standard deviation;

**Table-3:** Relevant Hematologic parameters of the patients stratified according to the hemoglobin patterns.

Heterozygous (1.56%).

Compound heterozygosity was found in four females (3 cases of HbE- β Thalassemia and 1 case of HbS- β Thalassemia). One case of Heterezygosity for Hb D was noted.

Analyzing the hematological parameters (Tables 2, 3) mean normal Hemoglobin level was 11.90± 2.37 g/dL, while the mean Hemoglobin in β trait was 10.68± 1.59 g/dL. In the four compound heterozygous cases Hemoglobin was significantly low compared to normal female mean.

In the major career state (β trait) reduced MCV, MCH, MCHC and increased RDW was seen.

## DISCUSSION

The Thalassemias are a group of congenital anemias that have a common, usually quantitative, deficient synthesis of one or more of the globin subunit of normal hemoglobin. But there are mutations resulting in structural variants produced at reduced rate (i.e., HbE, Hb Lepore) and mutations producing hyper unstable hemoglobin variants with a Thalassemia phenotype. (Thalassemia Hemoglobinopathies)[4]

Thalassemias are generally inherited as alleles of one or more of the globin genes located either on chromosome 11 (for β,γ, δ chains) or chromosome 16 (for α chains).[5]

Career states for thalassemias are usually clinically silent other than a mild anemia. However, when two careers plan a family, there is a 25% chance each for a normal child and a child with homozygous state, while the chance of offspring being a career is 50%, according to Mendelian law of inheritance.

Abnormal Hemoglobin pattern seen in the present study was 13.69%, while it was 11.62% and 11.43%, respectively in two other large population based studies in West Bengal done by Mandal et. al.[6] and Mondal et. al.[7] In our study, career state for β thalassemia was the most common abnormality (8.34% of the subjects), similar to the finding in the two above mentioned studies. The distribution of beta thalassemia gene is not uniform in Indian subcontinent varying from 1% to 17% in different populations in India as revealed by many studies.[8-10]

Yet another study with 1726 cases of tribal population (including 463 cases from West Bengal) of Eastern and North-eastern India showed prevalence of β thalassemia career as 5.18%.[11] Comparing the hematological parameters of β thalassemia trait (Hb%, MCV, MCH) to another study in West Bengal (Mondal et. al.[7]) yielded almost similar results.

In the present study the second most common hemoglobinopathy found was Sickle cell disease (Trait in 2.22% and homozygous in 0.33%). 3 cases of HPFH trait was also found.

In a recent study from Orisha[12] sickle cell trait was found in as high as 29.8% cases. However Mandal et al.[6] reported prevalence of only 0.56% of such cases. The area of present study is close to Orisha so population migration and heterogenecity may explain this higher prevalence rate in our study.

Hb E disease (Trait and homozygous) prevalence in our study population is 1.89%, which is more or less similar prevalence of 2.83% reported by Mandal et al.[6]

Other hemoglobinopathies detected in our study was compound heterozygous for Hb E and beta thalassemia and HbS- beta thalassemia.

HPLC has been considered till now as an accurate, cost effective screening tool for identification of hemoglobin variants. However chromatogram should be interpreted along with clinical history, family history and hematogram including PBS. Additional tests like PCR ARMS must be done to establish diagnosis in difficult cases.

The limitation of this study as perceived by us was inability to do family screening and follow up of difficult cases which were referred for confirmatory tests. Comparing to other population studies was not strictly justified as ours was a study limited to antenatal mothers and premarital men and women.

## CONCLUSION

The present cross-sectional study closely follows thalassemic gene burden in similar studies done in this area previously. Moreover during the study period, a number of premarital men and women came voluntarily for Hb-HPLC indicating increasing social awareness. With increasing awareness and with this simple screening procedure, it may be possible to eradicate or at least lessen the present community burden of thalassemia in foreseeable future.

## ACKNOWLEDGEMENT

The authors sincerely acknowledge the contribution of Asgar Ali and Susanta Patra for technical help and data entry.

## REFERENCE

1. Madan N, Sharma S, Sood SK, Colah R, Bhatia LH. Frequency of beta-thalassemia trait and other hemoglobinopathies in northern and western India. Indian J Hum Genet. 2010;16:16-25.
2. 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.
3. Collaborative study on Thalassemia. Eds Sood SK, Madan N, Colah R, Sharma S, Apte SV. Report of ICMR TASK Force Study, Indian Council of Medical Research (1993).
4. Borgna-Pignatti C, Galanello R. Thalassemias and Related Disorders: Quantitative Disorders of Hemoglobin Synthesis. In: Greer JP, et al. editors. Wintrobe's clinical hematology, Philadelphia: Lippincott Williams & Wilkins; 2014. 13th Ed. p. 862.
5. Wild BJ, Bain BJ. Investigation of abnormal haemoglobins and thalassaemia. In: Bain BJ. et al. Eds. Dacie and Lewis practical haematology. 11th ed. Churchill Livingstone; 2011.p302-332.
6. Mandal PK, Maji SK, Dolai TK. Present scenario of Hemoglobinopathies in West Bengal, India: An analysis of a large population. International Journal of Medicine and Public Health 2014;4: 496-9.
7. Mondal SK, Dasgupta S, Mondal S, Das N. Spectrum of Thalassemias and Hemoglobinopathies in West Bengal: A study of 90, 210 cases by cation exchange

high performance liquid chromatography method over a period of 8 years. Journal of Applied Haematology 2014; 5: 91-95.
8. Sharma RS, Parekh JG, Shah KM. Hemoglobinopathies in western India. J Assoc Physicians India 1963;11:969-73.
9. Misra RC, Ram B, Mohapatra BC, Das SN, Misra SC. High prevalence and heterogenicity of thalassemias in Orissa. Indian J Med Res 1991;94:391-4.
10. Dash S. Beta thalssemia trait in the Punjab (North India). Br J Haematol 1985;61:185-6.
11. De M, Halder A, Podder S, Sen R, Chakrabarty S, Sengupta B et al. Anemia and hemoglobinopathies in tribal population of Eastern and North-eastern India. Hematology 2006;11:371-3.
12. Balgir RS. Spectrum of Hemoglobinopathies in the State of Orissa, India: A Ten Years Cohort Study. JAPI 2005;53:1021-6.

**Source of Support:** Nil; **Conflict of Interest:** None

**Submitted:** 12-05-2019; **Accepted:** 01-06-2019; **Published:** 23-06-2019