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Study of spectrum of hemoglobinopathies and thalassemia by HPLC in South Bengal.

[1.] Dr. Soma Ghosh, [2.] Dr. Sharmistha Chakraborti
[1]*Associate Professor, Dept. of Pathology Burdwan Medical College, Burdwan.*
[2]*Medical officer In Charge, Thalassemia Care Unit, Burdwan Medical College, Burdwan.*
Corresponding Author: Dr. Soma Ghosh, Bahir Sarbomangala Road,
Near Carnival Marriage Hall / IIHT Computer Centre, Burdwan -713101.

Abstract:
INTRODUCTION *: Hemoglobinopathies are a group of inherited disorders with qualitative and quantitative defects .Their diagnosis relies on clinical examination, family history and laboratory investigations.*
OBJECTIVE *: The objective of present study was to find out the pattern of distribution of thalassemia and other various hemoglobinopathies in anemic patients attending outpatient department of tertiary healthcare institution, antenatal female unit and those with positive family history.*
MATERIAL & METHODS *: A total of 46,566 cases were screened for hemoglobinopathies by biorad variant - HPLC system (Biorad – Hercules,CA). The retention times, proportion of hemoglobin and the peak characteristics of all hemoglobin fractions recorded.*
RESULTS*: 81.7% of subjects were without any abnormal findings ; 5,977 (12.84%) demonstrated heterozygous variant(trait) ; 892 (1.92%) were either homozygote or compound heterozygote of different hemoglobinopathies.Females predominated in the study population. Beta thalassemia trait was found in 7.59% subjects followed by HbE trait in (4.21%), Eβthalassemia disease in (1.03%) and thalassemia major in (0.68%). Other variants detected were Sβdisease (0.056%), sickle cell disease(0.03%),sickle cell trait(0.49%), carrier of HbD variant (0.16%), HPFH(0.04%), β thalassemia with high fetal haemoglobin (0.21%), Hb Lepore(0.04%), Leporeβ(0.004%) and δβ thalassemia (0.002%). Double heterozygote forms of E/D, E/S and S/D were also detected.*
CONCLUSION*: Diagnosing both diseased form and trait of thalassemia is considered mandatory so that management strategies can get planned and preventive measures undertaken. HPLC is considered an accurate technique for diagnosis.*
Key words: *Hemoglobinopathy, Thalassemia, HPLC, prevalence.*

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I. Introduction

Hemoglobinopathies are a group of diseases characterized by both quantitative and qualitative abnormalities in the production of hemoglobin. Plethora of haemoglobin variants is prevalent in India owing to ethnic diversity of its population with minimal to major clinical significance. Being recessively inherited from the parents, thalassemia and thalassemic hemoglobinopathies pose serious health problem leading to severe morbidity and mortality in Indian population.[1] Detection of asymptomatic carriers by reliable laboratory methods is the cornerstone of prevention of this serious health problem alongwith various awareness programmes and counselling.[1]

Cation exchange high performance liquid chromatography (CE-HPLC) has become the preferred technique suitable in Indian scenario, as it can detect most of the clinically significant variants.The simplicity of the automated system with internal sample preparation, superior resolution, rapid assay time, and accurate quantification of haemoglobin (Hb)fractions makes this an ideal methodology for the routine clinical laboratory .[1,2] With increasing global awareness and mass screening programs undertaken at various levels by health care system,the responsibility for laboratory personnel has greatly enhanced in detection and prevention of this problem. Every year, there are over 42 million carriers and more than 12,000 infants born with a major and clinically significant hemoglobinopathy. In India, the cumulative gene frequency of hemoglobinopathies is around 4.2%. [1,2]Worldwide migration of human population, relatively higher frequency of consanguineous marriages has equally contributed to the increased burden of hemoglobinopathies.Thalassemia is characterized by a reduced rate of synthesis of normal Hb due to absence or decrease in the synthesis of one or more types of globin polypeptide chains. Clinically, these disorders are known as the thalassemia syndromes, resulting from

both the underproduction of Hb and imbalanced globin chain synthesis, leading to a shortened red cell survival rate. [3]

The prevalence of beta‑thalassemia trait and sickle cell in various regions of India is around 3%–17% and 1%–44%, respectively, because of consanguinity, caste, and area endogamy. Every year, around ten thousand children with beta‑thalassemia major are born in India, which constitutes about 10% of the total global load of beta‑thalassemia.The clinical spectrum of these disorders varies from asymptomatic conditions (beta‑thalassemia minor) to serious disorders such as thalassemia major that require regular blood transfusions and extensive medical care. Accurate and timely detection of various known and unknown Hb variants can prevent the occurrence of serious Hb disorders such as thalassemia major in the newborns. The objective of present study was to determine the pattern of distribution of various hemoglobinopathies and thalassemia in the study population .[3,4]

II. Material & Methods :

This study was conducted in the department of thalassemia care unit of a tertiary care teaching medical institution of South Bengal, India over a period of 7 years following clearance of instituitional ethics committee .

Subjects who came for voluntary premarital checkup , patients with peripheral blood smear and complete hemogram suggestive of hemolytic anemia attending outpatient departments, and those who had a positive family history of thalassemia were included in the study. Subjects with age more than one year were included. Subjects suffering from chronic medical illness and with a history of recent blood transfusion were excluded. A signed consent form obtained from all subjects included in the study coupled with their detailed clinical and family history . Parents signed the consent form in subjects below eighteen years of age. Five milliliters of blood was collected in dipotassium ethylenediaminetetraacetic acid vacutainers and run in Sysmex autoanalyzer for hemogram and red cell indices followed by HPLC.

The samples were run on instrument manufactured by Bio-Rad Laboratories utilising the principle of HPLC where HbA2F calibrator with two levels of control were analyzed at beginning of each run. The positively charged Hb fractions were separated based on their ionic interactions with a negatively charged stationary phase in a chromatography column followed by their elution by a mobile phase with phosphate buffers differing in pH and ionic strength. The adsorbed positively charged Hb molecules were eluted from the column into the liquid phase at a rate related to their affinity for the stationary phase.

Hemoglobinopathies were identified by retention time and quantified by computing the area under corresponding peak in the elution profile. Based on retention time and proportion of Hb variants, different hemoglobinopathies were diagnosed and their spectrum analyzed according to manufacturer assigned windows for Bio-Rad variant HPLC system .For each subject, a peripheral blood smear (PBS) was stained with Leishman stain and observed microscopically for red cell morphology .

III. Results

This was a cross sectional observational study for a period of seven years comprising a population of 46,566 subjects; out of which 38,057 (81.7%) showed normal results ; 8,509 (18.3%) showed abnormal haemoglobin fractions on HPLC. Of the hemoglobinopathies, 5,977 (12.84%) were of heterozygous type (trait) ; 892 (1.92%) were either homozygote or compound heterozygote for different types of hemoglobinopathies.

However, 1600 (3.52%) demonstrated inconclusive results and they failed to get categorised in any established type of disorder. Females dominated the study population where the ratio of females to males was 2:1. The age of subjects ranged from 1year and 3 months to 55 years.

Thalassemia minor / ß thalassemia trait predominated in the study and was demonstrated in 3,534 subjects (7.59%) followed by HbEtrait (HbAE) in 1,958 cases (4.21%) . (Table-1)

In the diseased group; HbE- β thalassemia predominated in 481 cases (1.03%) followed by thalassemia major/ β thalassemia disease in 317 cases (0.68%)(Table-1)

Carrier of sickle cell /sickle cell trait (HbAS) was reported in 228 cases (0.49%) ; ß thalassemia with high fetal haemoglobin in 96 cases (0.21%) ; HbD carrier in 74 cases (0.16%) ; HbE disease in 50 cases (0.11%) ; Sickle cell disease with ß thalassemia in 26 cases (0.056%); Hereditary persistence of fetal haemoglobin (HPFH) in 21 cases (0.04 %) ; Sickle cell anemia in 15 cases (0.03%); Hb Lepore in 19 cases (0.04%). Other hemoglobinopathies reported in low numbers were double heterozygote forms of HbE/D in 05 cases ; HbE/S in 07 cases; HbS/D in 02 cases; lepore/ ß thalassemia in 02 cases; δ/β thalassemia in 01 case and

α thalassemia in 03 cases. The rest 30 cases displayed abnormal haemoglobin variants of heterozygote forms and were placed in the carrier category in any other hemoglobinopathy apart from the discussed ones.

Table : 1 :The frequency of heterozygote (trait / carrier) forms of hemoglobinopathies (n= 46,566)

Pattern of hemoglobinopathy	Number	Percentage (%)
β thalassemia trait	3,534	7.59
HbAE trait	1,958	4.21
HbAS trait	228	0.49
HbD trait	74	0.16
HPFH	21	0.04
β thalassemia with high FH	96	0.21
Delta-Beta thalassemia	01	0.002
HbALepore	19	0.04
E/D, double heterozygous	05	0.01
S/D, double heterozygous	02	0.004
E/S, double heterozygous	07	0.02
Lepore –Beta thalassemia	02	0.004
Any other hemoglobinopathy	30	0.06
Total	5,977	12.8

Table : 2 : The frequency of homozygote / compound heterozygote (disease) form of hemoglobinopathies (n=46,566)

Pattern of hemoglobinopathy	Number	Percentage (%)
Thalassemia Major	317	0.68
E-ß thalassemia	481	1.03
S-ß thalassemia	26	0.056
HbS (sickle cell disease)	15	0.03
HbE disease	50	0.11
Alpha thalassemia	03	0.006
Total	892	1.92

IV. Discussion

Thalassemia and other hemoglobinopathies are autosomal recessive inherited disorders. They are mainly confined to certain areas, religions, castes, tribes, particularly with endogamous norms of marriages and are now widely prevalent all over the world. This is because of migration of various races over the ages and hence, being home to an assortment of sociocultural, linguistic, and ethnically diverse people.[1]

This study was conducted in a population of South Bengal, India . In the present cross-sectional study, the prevalence of Hb disorders was found to be 18.3 %. This was lesser than a similar study conducted in the southern part of West Bengal where the prevalence of such disorders was reported to be 25% and it was reported as 12.17 % in another study conducted in part of West Bengal .[3,5] The high incidence of thalassemia trait calls for need of antenatal screening and screening of marriageable age groups. This would help in the prevention of thalassemia major in the offsprings. The most common Hb abnormality detected in this study was that of β thalassemia trait (7.59%); compared to the study by Mondal S et al where they found βthalassemia trait in 4.60% of cases .

A study by Madan N etal in north and western India found βthalassemia trait in 4.05% of cases ; 3.5% of cases showed β thalassemia trait in study by Baruah MK et al and 6.61% cases showed βthalassemia trait in study by Mandal PK et al. [3,6,7,8] Colah R et al. reported carriers of β thalassemia in nearly 1.5% of the world's population .[9]

Several studies present β thalassemia trait as the commonest Hb disorder in most parts of India ; similar to that found in present study population . The prevalence of β thalassemia trait has been reported to be as high as 10.38% in the rural parts of West Bengal in a study by Dolai TK et al . In central India, it was found to be 9·59% in the study by Chatterjee N et al. [10,11] Incidence of HbE β thalassemia was 1.03% in the present study compared to 1.16% in the study by Mondal S et al and 1.25% in study by Mondal B et al. [3,12] Present study found E trait (HbAE) in 4.21% of cases ; Mondal S et al found the same in 3.02% of cases and Mondal B et al found HbAE in 3.86 % of cases. 2.78% cases showed HbE trait in study by Mandal PK et al. [8,12]

It is said that worldwide approximately 50% of severe beta thalassemia major patients are of HbE β thalassemia.[13]These patients presented with a variable clinical picture ranging from a condition indistinguishable from thalassemia major requiring blood transfusions from infancy to mild form of thalassemia intermedia presenting with mild asymptomatic anemia. The Indian Council of Medical Research (ICMR) study reported a

higher incidence of 1.44% in the general population.[14] In Odisha, sickle-cell trait was the most common abnormality found.[2] In the present study, sickle-cell trait was found in 0.49% cases compared to 0.38% cases in the study by Mondal S et al.[3]

Patel et al detected sickle cell anemia in 4.89% of cases and thalassemia major in 5.63% which was too high compared to present study where HbS was detected in a mere 0.03% of cases and thalassemia major in 0.68% of cases.[15]
Other variants detected in this study were Hb E disease (0.11 %), sickle-cell disease, sickle β thalassemia (0.056%) , HbD-Punjab trait, , α-thal trait, double heterozygous state of HbS and HbE, HbS and HbD, HbE and HbD , β thalassemia with high fetal haemoglobin, HPFH, delta β-thal trait , Hb Lepore (0.04%) and Lepore β (0.004 %). Rao S et al. reported three cases of HbQ-India, which eluted in an unknown window (retention time: 4-4.9 min).[16] δβ thalassemia is a rare cause of elevated fetal Hb. Very few cases have been reported from India.[17] HPFH has also been reported infrequently from different regions of India.[2] The ICMR multicenter study found an incidence of 0.73% of δβ thalassemia and 0.18% of HPFH; whereas present study found δβ thalassemia in 0.002 % cases, HPFH in 0.04 % of cases and β thalassemia with high fetal haemoglobin in 0.21% of cases.HbD trait was detected in 74 (0.16%) patients.

In India, the gene frequency of HbD is relatively low with a tendency to cluster toward the northwestern part of the country. [4] Alpha thalassemia is a common problem of Southeast Asian countries with gene frequencies reaching up to 30-40% in some parts of Thailand. [18] An incidence of 3.84% in some Assam tribesmen has been reported previously. [19] In the current study, α-thal trait was not detected . J chain hemoglobinopathy is also a rare finding in previous studies. Srinivas U et al. in their study reported seven cases from New Delhi , India.[20].

HPLC has been established as a sensitive, specific, and accurate technique for identification and quantification of different Hb fractions. However, it should be kept in mind that HPLC is limited by its inability to detect α thalassemia and normal HbA2 β thalassemia. Hb variants that elute with same retention time also cannot be separately identified by HPLC and during interpretation of chromatograms, nutritional anemias must always be taken into account.

A low level of HbA2 may be induced by iron deficiency, thus masking β thalassemia trait. Similarly, cobalamin or folate deficiency may raise HbA2 level, leading to false diagnosis of thalassemia trait [21] . Whenever necessary, HPLC must be followed by molecular studies, such as polymerase chain reaction (PCR), amplification refractory mutation system (ARMS), and other similar tests to determine specific mutations responsible for the Hb disorder[22] Herein lies the limitation of the study where HPLC fails to diagnose some types of hemoglobinopathies which were categorised as any other hemoglobinopathy or at times results were inconclusive.

V. Conclusion

HPLC forms a rapid, accurate, and reproducible tool for early detection and management of hemoglobinopathies with variants which is especially important in view of high incidence of β thalassemia trait . Premarital and antenatal screening should be made mandatory to prevent the birth of offspring with β thalassemia major .Prior knowledge of common Hb patterns in a particular region helps to formulate appropriate preventive and therapeutic strategies.

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