Al Ameen J Med Sci 2013; 6(3):243-248 ● US National Library of Medicine enlisted journal ● ISSN 0974-1143

ORIGINAL ARTICLE CODEN: AAJMBG

Spectrum of hemoglobin variants in Eastern Indian population; a study of 14,145 cases

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Abstract: *Background:* Inherited disorders of hemoglobin are extremely common in Indian population ranging from near structurally normal hemoglobins to severe transfusion dependant hemoglobinopathies. Their detection is important epidemiologically and to prevent other more serious hemoglobinopathies in future generations. *Objectives:* This is a retrospective study, where our objectives are to analyze the different hemoglobin (Hb) variants in West Bengal (WB) population and adjacent areas and to find out which particular district is at risk to which particular hemoglobinopathy. *Materials & Methods:* The study was done by BIORAD VARIANT using beta thalassemia (thal) short programme using high performance liquid chromatography (HPLC) technique. Complete hemogram of all the surgical and other patients were available before HPLC study. Cases were referred mostly by surgeons and clinicians either after getting abnormal hemogram or for premarital counseling. *Results*: Out of 14,145 cases 74.35% (10,518 cases) showed normal Hb pattern on HPLC and rest 26.65% (3627 cases) showed some abnormality. Common hemoglobinopathies included beta thal trait, E trait, E beta thal, persistent fetal Hb, thal major, thal minor, HbS trait. Other disorders were rare (<0.01%). Complete hemogram was done in all cases & anisopoikilocytosis, hypochromia, and abnormal RDW were found in majority of cases. MCV was generally normal to very low. District wise HbS and beta thal were more common in western part of West Bengal and HbE is more prevalent in central and north-east part of West Bengal. *Conclusion:* Abnormal hemoglobins as HbE and beta thal is very common in West Bengal. However many other Hb variants including mixed patterns are also found HPLC provides a rapid and accurate method to analyze these Hb variants and by quantifying HbA2 level prevents occurrence of more serious hemoglobinopathies in future generations.

Keywords: HPLC, Hemoglobinopathy, Thalassemia.

Introduction

One of the commonest inherited disorders in humans is abnormality in hemoglobin (Hb) synthesis. Thalassemia syndromes cause major morbidity and mortality in India [1] and abroad [2], in human population. If premarital HPLC (High Performance Liquid Chromatography) blood examination or HPLC is done following an abnormal complete hemogram report or some other reasons, a good number of these silent or near asymptomatic carriers can be detected and more serious disorders like thal major and Eβ thal may be prevented in newborns [2]. Interactions between more than one abnormal Hb though not very common, pose diagnostic difficulties and therapeutic challenges [3]. So HPLC detects abnormal hemoglobins and also prevents occurrence of more serious hemoglobinopathies in future generations. Moreover as the population of West Bengal (WB) is heterogeneous, a district wise study for Hb variants may also be undertaken.

Aims & Objectives: In this retrospective study our objectives are to analyze the different Hb variants in the population of WB and adjacent areas and to find out which district is at risk to what particular hemoglobinopathies, so that definite plan of action may be undertaken by the community health personnel by detecting carriers to minimize more serious disorders in future generations.

Material and Methods

14,145 patients underwent HPLC examination of their blood in a private diagnostic centre in Kolkata for a eight-year long period. Many of these subjects came for a premarital check up

and many were referred by clinicians following an abnormal complete hemogram report such as anemia, low MCV, abnormal RDW, positive family history for thalassemia etc.

Our machine the BIORAD VARIANT, β thalassemia short programme, manufactured by BIORAD laboratories USA, uses the principle of High Performance Liquid Chromatography (HPLC). Calibrators were run before each examination. 5ml of venous blood was drawn from each patient and hematological parameters were done with help of cell counters before HPLC study. The HPLC graph was obtained from the machine and values were analyzed. Sickling test using sodium metabisulfite was done wherever needed. Family history was asked for in all cases though in a good number of cases a complete family history could not be obtained.

Exclusion criteria for patients:

1. Patients below 1 year of age- as significance of HbF(Fetal Hemoglobin) levels are hard to interpret.
2. Patients having recent blood transfusions- HPLC will not be able to distinguish between patients own cells and transfused cells.
3. Patients having unknown graph patterns on HPLC-as many of these blood samples are decomposed or molecular studies are not affordable/warranted for most of these patients.

Inclusion criteria of patients:

1. Those patients who had a positive family history of Thalassemia.
2. Patients with abnormal hemograms suggestive of hemolytic anemia. (i.e, low Hb, low MCV, abnormal RDW, high RBC count.)
3. Patients coming for a premarital check up voluntarily.

Results and Analysis

In this retrospective study, a total of 14,145 cases were studied for an 8 year long period. Out of these patients, 10,518 (74.73%) had normal HPLC pattern and rest 3267 (25.6%) had some abnormality. β thal trait was the commonest abnormality encountered. 1362 patients (9.62%) had elevated HbA2 levels (HbA2 above 3.5%) and was therefore diagnosed as β-Thal trait. The highest number of these patients were from Midnapore district of WB; however other districts were also significantly affected. Peripheral blood showed microcytic hypochromic picture with an average Hb of 10.1gm/dl, MCV-67.4 fl and RDW was 16.1%, there was a slight increase of HbF leavels (average-1.3%) (table-1).

Table-1: Hemogram in β thalassemia

Name of disorder	Number of cases	Hb conc (gm/dl)	MCV (fl)	RDW (%)	HbF %	HbA%
β thal major	105	4.7	67.8	31.0	87.0	7.6
β thal intermedia	36	7.8	67.5	21.2	19.5	70.3
β thal trait	1362	10.1	67.4	16.1	1.3	80.9

There were 141 cases (0.72%) of thalassemia major (thal major). Patients with thal major presented with severe anemia during early childhood. Peripheral blood showed severe anemia, (average Hb – 4.7gm/dl), reduced MCV (average MCV- 67.8fl), and increased RDW (31.0%). HbF was the major Hb in this patients averaging 87% and HbA averaged only 7.6% (table-1).

Thalassemia intermedia, a clinical term, showed milder anemia, (Hb averaging 7.8%), HbF levels were modestly raised averaging 19.5%. These patients usually do not require frequent transfusions. Amongst our thal major cases 36(0.25%) patients had features tallying with intermedia. There were 258 patients of thal minor who presented with mildly raised HbF, and HbA2 level was also slightly raised (4-6% on average) These patients were asymptomatic, with mild microcytic hypochromic anemia (Hb around 8-9 gm/dl & MCV around 70-80 fl). Another abnormality that was commonly encountered was the HbE trait. This was particularly common in Malda & Murshidabad districts, though other parts of WB also showed presence of HbE in lesser amount. In this study there were 696 cases

(4.92%) of HbE trait (heterozygous) and 18 cases (0.127%) of homozygous HbE disease. In HPLC both HbA2 & HbE had similar elution time (average- 3.65 min). Patients with HbE trait had HbE concentration in 25-40% range, and those with homozygous HbE disease, had HbE concentration above 75%. It was seen that HbA2 values in thalassemia seldom rose above 10%. Patients with HbE trait presented with mild anemia, (average Hb –level 10.2gm/dl), low MCV (average MCV-75.6fl), and an average HbE conc of 30.2% table-2).

Table-2: Hemograms in heterozygous hemoglobinopathies

Name of disorder	Number of cases	Hb conc (gm/dl)	MCV (fl)	RDW%	% of abnormal Hb
S trait	39	11.1	76.7	15.2	31.0
D trait	12	10	81.8	16.9	34.5
E trait	696	10.2	75.6	16.0	30.2

Patients with homozygous HbE (i.e HbEE disease) showed greater anemia, (average Hb- 9.6 gm/dl), greater amount of microcytosis (average MCV- 63 fl), and an average HbE concentration of 91%. HbF was also raised averaging 3.4% (table-3).

Table-3: Hemogram in homozygous hemoglobinopathies

Name of disorder	Number of cases	Hb conc (gm/dl)	RDW %	% of abnormal Hb	HbF%
HbEE	18	9.6	17.6	91	3.4
HbSS	03	7.4	24.4	79	7.6

Of the 18 patients who were homozygous for HbE, 10 had positive family history for the disease and in the remaining 8 patients a family history could not be sought. Eβ thalassemia was the commonest severe hemoglobinopathy in WB requiring multiple transfusions. There were 570 cases of Eβ thal (4.03%). Characteristic features include raised HbE levels (in 30-75% range,) high HbF (15-45% range) with moderate to severe anemia (Hb-5-7 gm/dl) [4]. In our study the average Hb concentration of 570 patients were 6.7 gm/dl, MCV 66.9 fl. Average HbE and HbF concentration were 55% and 38.8% respectively (Table-4).

Table-4: Hemogram in mixed hemoglobinopathies

Name of disorder	Number of cases	Hb conc (gm/dl)	MCV (fl)	RDW %	% of abnormal Hb	HbF%
HbE with β thal	570	6.7	66.9	26.0	55.0	38.8
HbS with β thal	135	8.9	72.8	18.5	65.0	19.0
HbD with β thal	120	8.0	66.0	20.8	77.5	20.2

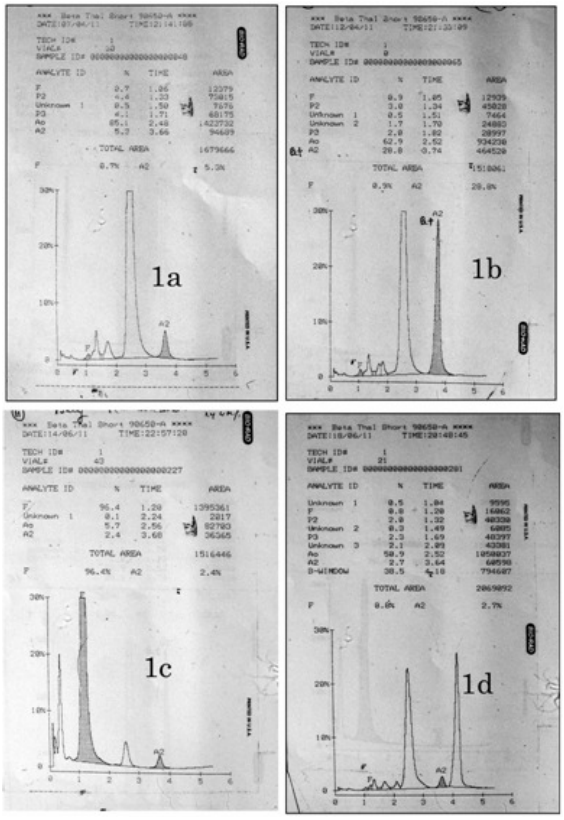
In 269 patients (1.9% cases), there were high HbF levels despite other normal blood parameters. These patients possibly had hereditary persistence of fetal Hb, and molecular studies are recommended in these cases.

In the HbS spectrum as per HPLC graph analysis, there were 3 patients (0.021%) with homozygous HbS disease, and 39 cases (0.276%) with HbS trait. Sickling test with metabisulfite was done in most of these patients. Patients with HbS trait had mild anemia, (average Hb-11.1 gm/dl) mild microcytosis (average MCV- 76.7fl) and an average HbS concentration of 31% (table-2). Patients who were homozygous for HbS (i.e HbSS), had greater anemia (average Hb-9.6gm/dl), similar microcytosis (average MCV 76.8 fl) and an average HbS conc of 91% (table-3). These patients also had a modest elevation of HbF averaging 7.6% (table-3). Mixed hemoglobinopathy, i.e Sβ thal was seen in 0.95% cases, (in 135 pts)

showing increased levels of both HbS and HbF. In this study, patients with Sβ thal had an average Hb concentration of 8.9 gm/dl, an average MCV of 72.8 fl and an average HbF concentration of 19% (table-4).

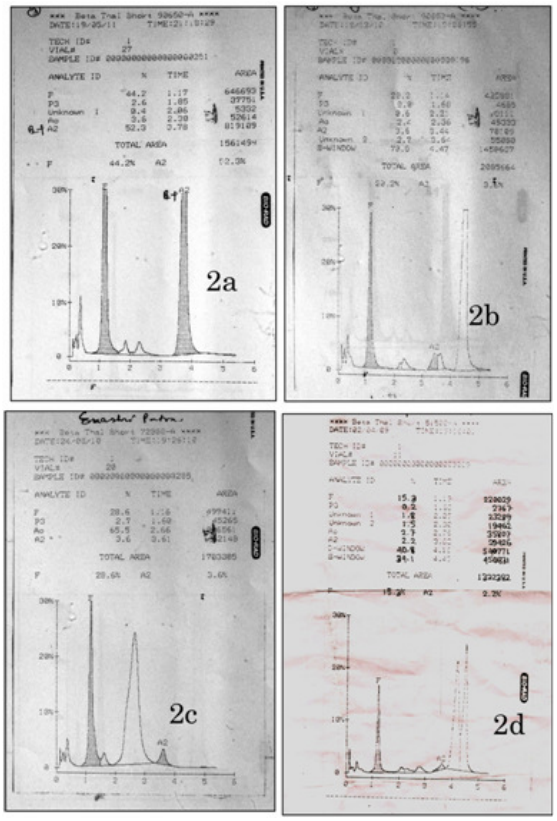
There were 12 cases of HbD trait (0.085%) and 0.85% patients were having HbDβ thal. Patients with HbD with beta thal inherited one gene of beta thalassemia and one gene of HbD trait from both parents. They usually present with a microcytic (MCV ranging 60-70 fl) and hypochromia (Hb concentration 7.5-10 gm/dl). HbA2 is also raised (range 5-10%) (table-4). Those with HbD trait had milder anemia (average Hb concentration 10.0 gm/dl) and average HbD concentration of 34.5% (table-2) and MCV was low normal (81.8fl) (Table-2). There were one each of HbSD disease and Hb lepore. In SD disease the patient receives two traits (i.e HbS trait and HbD trait) and has elevated concentration of all HbS, HbD, and HbF. This is the rarest of all known hemoglobinopathies. In our case the concentration of HbF, HbS, HbD were 15.3%, 34.1% & 40.8% respectively. In Hb lepore trait, there is modest (3-5%) elevation of HbF and Hb lepore elutes concurrently with HbA2, at about 3.5 minutes thereby appearing with the HbA2 peak.

One sample HPLC curve have been given below representing the following traits as they appear in the legends to pictures, (Picture-1 & 2). Picture-3 depicts the peripheral smears of few common hemoglobinopathies (beta trait, HbS trait, E Beta thal, Thalassemia E), we encountered in the study.



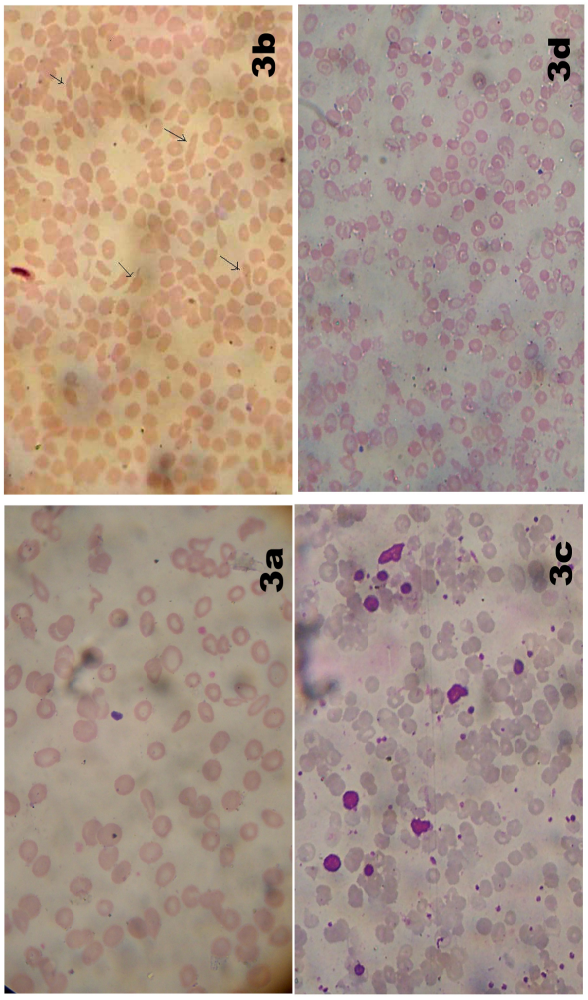
Picture-1:

a. Beta thalassemia trait showing increased HbA2 levels of 5.3%
b. Thalassemia E trait (HbA2 + HbE = 28.8%)
c. Thalassemia Major with >90% HbF levels.
d. HbS trait, with HbS levels increased to 38.5%.



Picture-2:

a. E Beta thalassemia, with increased HbF levels (44.2%) & (HbA2 + Hb E = 52.3%).
b. S Beta thalassemia, showing increase of both HbF & HbS upto 19.0% & 65.1% respectively.
c. Thalassemia intermedia, with increased HbF concentration of 28.6%.
d. Hemoglobin SD disease, with HbF, HbS, HbD increased upto 15.3%, 34.1%, 40.8%.



Picture-3:

a. A case of beta trait, showing hypochromia, anisopoikilocytosis & tear drop cells.
b. A case of HbS trait showing sickle cells (arrowed).
c. E Beta thalassemia showing anisopoikilocytosis & plenty of nucleated RBC's.
d. A patient of thalassemia E showing severe anisopoikilocytosis & many target cells.

Discussion

This study was done primarily with patients of WB and nearby areas. In this study β- thal trait was the commonest disorder (9.62%), followed by HbE trait (4.92%), E β thal (3.67%) and persistent foetal Hb (1.9%). Other hemoglobinopathies were relatively rare (<1%). The frequency of β thal has been reported from 8.9 % [5] to as high as 37.9% [6] by various authors. Madan N et al reported the frequency of beta thal to be 5.47% and 2.68% in schoolchildren of Delhi and Mumbai [7]. which is lower than what we found. In WB, β thal is mainly prevalent in its western part in the districts of Purulia and Midnapore. The frequency of HbS and HbD in this study was 0.297% and 0.085% which is lower than the national average of 4.3% and 0.86% respectively [1]. HbS is mainly prevalent in central part of India and HbD near Punjab and Uttarpradesh (UP). The prevalence of HbD in UP is about 0.5 to 3.1% [8]. These hemoglobins are not so prevalent in WB. The average frequency of HbS in central India is about 5.7% in children [9]. E β thal is the commonest severe hemoglobinopathy in WB (4.03%) requiring multiple transfusions. The frequency of E β thal is reported from 0.7% [5] to 4.6% [1] in different studies. In WB Eβ thal is particularly prevalent in the state of Malda and Murshidabad overlapping with the HbE belt. In north western India however (Tripura, Arunachal etc) HbE is the commonest abnormal Hb [10].

The average frequency of HbE gene in Indian population is about 10.9% [1]. The frequency of thal major in this study was 0.47%which is much lower than that of Orissa i.e 5.3% [5], our adjacent state. Another study done by Sachdev R in patients of northern India showed the prevalence of thal major to be 0.67% [11]. Another abnormal hemoglobin, HbS is not very common in WB (0.276% of HbS trait). HbS is commonest in Purulia district of WB, which is adjacent to Orissa as in Orissa, however, HbS is very common, making it the commonest hemoglobinopathy in that state [5].

There were one each of Hb Lepore and HbSD disease. These are very rare hemoglobinopathies seldom encountered anywhere and such findings are accidental in any study irrespective of geography and population. Hemogram was done in all the cases and the lowest average Hb (4.7 gm/dl) was seen with thal major. Anisopoikilocytosis was also greatest giving the highest RDW of 31% in thal major. Among the homozygous hemoglobinopathies, HbSS had the lowest average Hb level of 7.4 gm/dl. Amongst the heterozygous hemoglobinopathies, HbD trait had the lowest average Hb level of 10 gm/dl. This may be due to the highest concentration of abnormal Hb in HbD trait (34.5%) as

compared to the abnormal Hb concentration in HbS trait (31.0%). Finally among the mixed hemoglobinopathies Eβ thal had lower Hb concentration than Sβ thal (6.7 gm/dl vs 8.9 gm/dl). So Eβ thal requires more frequent transfusions than Sβ thal. MCV was also lesser and HbF higher in Eβ thal as compared to Sβ thal.

Thus to conclude HbS, HbE, & HbD, are the major abnormal hemoglobins in India [1] and HPLC study with hemogram analysis is a quick and accurate method to detect various Hb variants in the community [12]. As the incidence of thalassemia in our country and in the state of WB is still unacceptably high, such studies [13] as ours (particularly pre-marital testing) will prevent or reduce the number of more serious hemoglobinopathies like thalassemia major in future generations.

Acknowledgements

We are grateful to DR. K N Poddar, Msc, PhD of Clinical Laboratory, Kolkata, for all the HPLC data used in this study.

Grand chart of abnormal hemoglobin variants		
Name of the disorder	**Number of cases**	**Percentage of cases**
β thal major	105	0.47%
β thal intermedia	36	0.25%
β thal minor	258	1.82%
β thal trait	1362	9.62%
Persistent fetal hemoglobin	269	1.9%
HbE trait	696	4.92%
HbS trait	39	0.276%
HbD trait	12	0.085%
HbEE(E disease)	18	0.127%
HbSS(S disease)	03	0.021%
HbE with β thal	570	3.67%
HbS with β thal	135	0.95%
HbD with β thal	120	0.85%
Hemoglobin lepore	01	0.007%
Hemoglobin SD disease	01	0.007%

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