Spectrum of Hemoglobinopathies in West Bengal, India: A CE-HPLC Study on 10407 Subjects

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Received: 8 October 2013 / Accepted: 11 March 2014 / Published online: 2 April 2014
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Abstract Hemoglobinopathies are common genetic disorders of haemoglobin. Identification of these disorders is immensely important epidemiologically and they can be prevented by population screening. The present study was carried out to evaluate the spectrum of hemoglobinopathies in the state of West Bengal by the cation-exchange high-performance liquid chromatography (CE-HPLC). A retrospective, single-center, cross-sectional study was conducted on consecutive 10,407 participants. Out of 10,407 subjects, 8,898 (85.5 %) were diagnosed as normal, 579 (5.6 %) were as β -thalassemia trait (BTT) and 522 (5.0 %) were detected as HbE carrier on HPLC study. Apart from BTT and HbE carrier ten additional variants were encountered. The present study showed that CE-HPLC is a convenient, high-throughput, labour-saving and objective screening tool for early detection and management of hemoglobinopathies.

Keywords β -Thalassemia trait · High performance liquid chromatography · Screening · Haemoglobin A₂ · West Bengal

Introduction

At present, approximately 250 million people constituting 4.5 % of the world population carry a potentially pathological haemoglobinopathy gene. Each year about 3 lacs infants are born with a major haemoglobinopathy [1]. Accurate and timely detection of various haemoglobin variants including β -thalassemia trait (BTT) can prevent occurrence of more serious disorders like thalassemia major in newborns [2]. Until recently the identification and quantification of haemoglobin variants required a sequence of tests each with inherent problems of reproducibility, accuracy, labour intensity and cost. The application of cation-exchange high-performance liquid chromatography (CE-HPLC) to separate and quantify various normal and abnormal haemoglobin fractions of clinical significance has solved many such problems and has been proposed for haemoglobinopathy screening [3, 4].

The present study was conducted to evaluate the spectrum and frequency of various hemoglobinopathies in the state of West Bengal by the CE-HPLC with the assessment of the common demographic factors and haematological parameters.

Materials and Methods

Study Design

The present single-center, retrospective, cross-sectional study was carried out on consecutive 10,407 subjects who

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were screened by CE-HPLC for hemoglobinopathies in the Thalassemia control unit (TCU) of the department of pathology of Institute of Post Graduate Medical Education and Research, Kolkata after obtaining the proper approval from ethical committee of the institution and informed consent from the patients. The institute serves mostly the people from the districts of south Bengal. Data of all the subjects during the study period of 3 years and 5 months (01.01.2010–31.05.2013) were retrieved from our database which was maintained and organized by the Linux-based Thalamon software (Venus IT Solutions). Although no absolute exclusion criteria were used but sampling was deferred for at least 4 weeks after or just before next transfusion in patients requiring blood transfusions. Common haematological parameters were measured with an automated haematology analyser (KX-21, Sysmex Corporation, Japan). Red cell morphology and platelet counts were crosschecked with well-prepared peripheral blood films. In addition to haematological profile, relevant demographic factors like age, sex, religion and respondent category, district wise distribution of all the subjects including the subjects with normal as well as with abnormal haemoglobin as analysed by HPLC were assessed. All the abnormal HPLC variants were corroborated with RBC morphology and indices, ethnicity, family history, HPLC study of the parents and/or other siblings.

Blood Sampling and HPLC Procedure

The evaluation was done in Bio-Rad Variant Haemoglobin Testing System (Hercules, California, USA) using variant β -thalassemia short program pack consisting of elution buffer 1 (sodium phosphate), elution buffer 2 (sodium phosphate), whole blood primer (lyophilized human red blood cell hemolysate with preservatives), haemolysis reagent (deionized water), wash solution (deionized water), HbA₂/F calibrator/diluent set (lyophilized human red blood cell hemolysate with deionized water), sample vials, ROM (read-only memory) card, cation exchange analytical cartridges and CD-ROM. The Bio-Rad Variant is a fully automated CE-HPLC system to separate and determine area percentages for haemoglobin A₂ and F and to provide qualitative determinations of abnormal haemoglobins.

1–2 ml of whole blood samples were collected in EDTA vials and were stored at 2–8 °C. EDTA anticoagulated 5 μ L whole blood samples were mixed with 1.0 ml of haemolysis reagent to each sample vial and were analysed in batches within 1 week. The prepared samples were injected sequentially into the analysis stream at 6.5-min intervals and separated by the cation exchange cartridge using a phosphate ion gradient generated by mixing two buffers of different ionic strengths to elute the different

haemoglobins. HbA₂/F calibrator and two level controls were analysed at the beginning of each run.

A dual-wavelength filter photometer analysed the haemoglobin elution from the cartridge by detecting the absorbance changes at 415 nm and the secondary filter at 690 nm corrected the baseline for effects caused by mixing buffers with different ionic strengths. Different peaks of different haemoglobins in defined windows with their retention time, relative percentage and area displayed in a chromatogram of absorbance versus time (Fig. 1). Total acceptable area of each analysis ranged from 1 to 3 million μ volt s. Subjects with HbA₂ level between 4.0 and 9.0 % were diagnosed as BTT.

Statistical analysis: Continuous variables were expressed as mean $\pm$ standard deviation (SD). Categorical variables were presented as frequencies and percentages. All the analyses were done using IBM SPSS statistics software, version 19 and MedCalc software, version 12.3.0.0.

Results

A total of 10,407 subjects were screened for BTT and other hemoglobinopathies during the period of 3 years and 5 months, of which 3,378 (32.5 %) were males and 7,029 (67.5 %) were females (Table 1). Mean age of the study population was 22.9 years, and most of the abnormal cases were first time presented in 2nd and 3rd decades. Among the 10,407 study participants, 8,451 (81.2 %) were Hindu and 1,898 (18.2 %) were Muslims. Study population was mostly comprised of antenatal mothers (33.3 %), premarital group (31.9 %), post marital group (16.0 %) and children (12.9 %). Majority of the normal as well as cases of hemoglobinopathies were from the districts of south Bengal namely Kolkata, Howrah, South 24 Parganas and Hooghly (Table 2). Out of 10,407 subjects, 8,898 (85.5 %) were diagnosed as normal, 579 (5.6 %) were as BTT and 522 (5.0 %) were detected as HbE carrier on HPLC study (Table 3). Apart from normal, BTT and HbE carrier ten additional variants were encountered. Sixty one cases (0.6 %) displayed the borderline HbA₂ levels i.e. between 3.5 and 4.0 %. The patients with borderline HbA₂ levels that could not be explained by iron status, family history, HPLC study of parents and other siblings, RBC morphology and indices as wells as the patients with unknown HPLC peaks and suspected variants requiring molecular analysis were included in inconclusive category. Haematological parameters displayed relatively higher RBC counts in BTT and HbD^{Punjab} disease, lower MCV in BTT, HbE carrier, HbE/ β -thalassemia, HbE disease, β thalassemia major and in HbD^{Punjab} disease, very high RDW in

Fig. 1 a CE-HPLC chromatogram of β Thalassemia Trait. b CE-HPLC chromatogram of β thalassemia Major. c CE-HPLC chromatogram of HbE carrier. d CE-HPLC chromatogram of HbE/ β -

thalassemia. e CE-HPLC chromatogram of HbE disease. f CE-HPLC chromatogram of HbS carrier. g CE-HPLC chromatogram of HbS disease. h CE-HPLC chromatogram of HbD^{Punjab} carrier

HbE/ β -thalassemia, β thalassemia major and in HbS/D double heterozygous cases.

Discussion

HPLC is a sensitive, specific, and reproducible alternative to electrophoresis. It appeared to be an appropriate candidate for direct provisional identification and sensitive quantification of major and minor, normal and abnormal haemoglobin fractions with a high degree of precision. On

the other hand, technical performance of electrophoresis depends on various factors like haemoglobin concentration, amperage, running temperature, and length of electrophoresis run. These variables can affect the quality of separation and relative positioning of the bands [5]. Limitations of HPLC are the possibilities of alpha thalassemia, normal A₂ beta thalassemia or other hemoglobinopathies that elute with similar retention values on HPLC, alteration of HbA₂ levels in cases with coexisting nutritional deficiency like iron deficiency anaemia cannot be ruled out by CE-HPLC [2, 6].

Table 1 Distribution of the study population (n = 10,407) depending on various demographic factors

Parameters	All (n = 10,407; 100 %)	Normal (n = 8,898; 85.5 %)	BTT (n = 579; 5.6 %)	HbE carrier (n = 522; 5 %)	Other abnormalities (n = 408; 3.9 %)
I. Year of study					
2010	802 (7.7)	687 (7.7)	53 (9.2)	31 (5.9)	31 (7.6)
2011	2751 (26.4)	2,413 (27.1)	155 (26.8)	119 (22.8)	64 (15.7)
2012	4,758 (45.7)	4,013 (45.1)	260 (44.9)	260 (49.8)	225 (55.1)
2013	2,096 (20.1)	1,785 (20.1)	111 (19.2)	112 (21.5)	88 (21.6)
II. Age (year) (Mean $\pm$ SD, Minimum, Maximum)					
	22.9 $\pm$ 8.7	23.1 $\pm$ 8.3	24.8 $\pm$ 10.0	24.4 $\pm$ 10.1	14.0 $\pm$ 9.7
	Min: 1	Min: 1	Min: 1	Min: 1	Min: 1
	Max: 90	Max: 90	Max: 75	Max: 65	Max: 56
III. Sex					
Male	3,378 (32.5)	2,769 (31.1)	223 (38.5)	198 (37.9)	188 (46.1)
Female	7,029 (67.5)	6,129 (68.9)	356 (61.5)	324 (62.1)	220 (53.9)
IV. Religion					
Hindu	8,451 (81.2)	7,290 (81.9)	481 (83.1)	380 (72.8)	300 (73.5)
Muslim	1,898 (18.2)	1,561 (17.5)	96 (16.6)	136 (26.1)	105 (25.7)
Christian	40 (0.4)	33 (0.4)	2 (0.3)	3 (0.6)	2 (0.5)
Sikh	8 (0.1)	8 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Buddhist	4 (0.0)	0 (0.0)	0 (0.0)	3 (0.6)	1 (0.2)
Not specified/Others	6 (0.1)	6 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
V. Respondent category					
Antenatal mother	3,462 (33.3)	3,191 (35.9)	131 (22.6)	119 (22.8)	21 (5.1)
Premarital group	3,318 (31.9)	3,013 (33.9)	146 (25.2)	103 (19.7)	56 (13.7)
Post-marital	1,668 (16.0)	1,356 (15.2)	145 (25.0)	136 (26.1)	31 (7.6)
Children	1,345 (12.9)	963 (10.8)	66 (11.4)	65 (12.5)	251 (61.5)
Family members of affected person	447 (4.3)	247 (2.8)	83 (14.3)	92 (17.6)	25 (6.1)
Family members of carrier	8 (0.1)	5 (0.1)	1 (0.2)	2 (0.4)	0 (0.0)
Suspected patient	5 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	4 (1.0)
Family members of suspected patient	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)
Others	153 (1.5)	122 (1.4)	7 (1.2)	4 (0.8)	20 (4.9)

Figures in parentheses are
column percentages

SD standard deviation, Normal
normal hemoglobin analysis in
HPLC

Discrimination indices based on simple haematological parameters are useful tool in separating uncomplicated cases of BTT from iron deficiency anaemia but they are not generally applicable to pregnant women or children and are not useful in patients who have both iron deficiency and thalassemia trait [7].

Sachdev et al. [2] conducted an HPLC based study on northern India and observed abnormal haemoglobin fractions in 327 cases out of 2,600 subjects. They got 8.9 % prevalence of BTT. Rao et al. [6] found BTT in 18.1 % of cases and HbE carrier in 1.1 % of cases on HPLC screening of 800 samples. There are very few studies from West Bengal which evaluated and emphasized the role of HPLC for diagnosis of thalassemia and various hemoglobinopathies.

Dolai et al. [8] conducted a large HPLC based study on 35,413 participants from rural Bengal. BTT was found in 10.38 %, HbE carrier in 4.30 % and sickle cell trait in 1.12 % cases. In another study from West Bengal, Jain et al. [9] observed that overall 29.3 % of subjects were positive for hemoglobinopathies. BTT was appeared to be the most common haemoglobinopathy in their study followed by haemoglobin E heterozygous.

In the present study on 10,407 participants, 1,509 (14.5 %) cases had abnormal haemoglobin fractions. Although BTT was the commonest (579 cases, 5.6 %) haemoglobinopathy in this study group, it is very closely (522 cases, 5.0 %) followed by HbE carrier. These findings along with the results of haematological parameters are comparable with that of other studies.

Table 2 Distribution of the study population (n = 10,407) in the districts of West Bengal

District	All	Normal	BTT	HbE Carrier	Other abnormalities
Kolkata	3,495 (33.6)	3,108 (34.9)	157 (27.1)	141 (27.0)	89 (21.8)
Howrah	2,267 (21.8)	2,003 (22.5)	99 (17.1)	92 (17.6)	73 (17.9)
South 24 Parganas	1,718(16.5)	1,354 (15.2)	117 (20.2)	124 (23.8)	123 (30.1)
Hooghly	1,036 (10.0)	913 (10.3)	66 (11.4)	38 (7.3)	19 (4.7)
North 24 Parganas	723 (6.9)	634 (7.1)	33 (5.7)	34 (6.5)	22 (5.4)
East Medinipur	228 (2.2)	171 (1.9)	28 (4.8)	17 (3.3)	12 (2.9)
West Medinipur	190 (1.8)	153 (1.7)	21 (3.6)	2 (0.4)	14 (3.4)
Murshidabad	167 (1.6)	117 (1.3)	15 (2.6)	18 (3.4)	17 (4.2)
Bardhaman	161 (1.5)	130 (1.5)	15 (2.6)	7 (1.3)	9 (2.2)
Nadia	121 (1.2)	98 (1.1)	7 (1.2)	8 (1.5)	8 (2.0)
Malda	73 (0.7)	48 (0.5)	5 (0.9)	11 (2.1)	9 (2.2)
Birbhum	62 (0.6)	50 (0.6)	8 (1.4)	4 (0.8)	0 (0.0)
Bankura	59 (0.6)	50 (0.6)	4 (0.7)	3 (0.6)	2 (0.5)
Cooch Behar	30 (0.3)	18 (0.2)	0 (0.0)	11 (2.1)	1 (0.2)
North Dinajpur	23 (0.2)	11 (0.1)	1 (0.2)	5 (1.0)	6 (1.5)
Purulia	18 (0.2)	14 (0.2)	2 (0.3)	0 (0.0)	2 (0.5)
South Dinajpur	17 (0.2)	12 (0.1)	0 (0.0)	4 (0.8)	1 (0.2)
Jalpaiguri	16 (0.2)	12 (0.1)	1 (0.2)	2 (0.4)	1 (0.2)
Darjeeling	3 (0.0)	2 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)
Total	10,407 (100.0)	8,898 (100.0)	579 (100.0)	522 (100.0)	408 (100.0)

Figures in parentheses are column percentages
Normal normal hemoglobin analysis in HPLC

Table 3 Assessment of hematological parameters in different groups of patients and normal subjects

Diagnosis (n = 10,407)	No. of Cases (%)	Hb (g/dL)	Hematocrit (%)	RBC ($\times 10^6/\text{mm}^3$)	MCV (fl)	MCH (pg)	MCHC (g/dL)	RDW (%)
1. Normal	8,898 (85.5)	12.2 $\pm$ 2.0 Min: 7.8 Max: 16.9	37.7 $\pm$ 5.8 Min: 24.3 Max: 53.7	4.50 $\pm$ 1.81 Min: 2.20 Max: 6.98	86.2 $\pm$ 10.7 Min: 42.8 Max: 110.2	27.9 $\pm$ 3.0 Min: 14.8 Max: 35.6	32.3 $\pm$ 1.7 Min: 23.7 Max: 36.4	14.8 $\pm$ 5.0 Min: 11.9 Max: 33.7
2. β Thalassemia Trait	579 (5.6)	10.7 $\pm$ 1.8 Min: 1.7 Max: 13.8	35.4 $\pm$ 5.8 Min: 6.2 Max: 44.8	5.30 $\pm$ 0.95 Min: 2.98 Max: 7.24	67.4 $\pm$ 5.8 Min: 52.0 Max: 94.9	20.4 $\pm$ 2.2 Min: 13.3 Max: 31.2	30.2 $\pm$ 1.3 Min: 19.8 Max: 34.4	16.9 $\pm$ 4.0 Min: 12.3 Max: 41.2
3. HbE carrier	522 (5.0)	11.8 $\pm$ 1.9 Min: 3.5 Max: 16.2	36.7 $\pm$ 5.7 Min: 10.4 Max: 50.4	4.77 $\pm$ 0.74 Min: 1.42 Max: 6.86	77.0 $\pm$ 5.5 Min: 27.4 Max: 100.0	24.8 $\pm$ 2.3 Min: 18.1 Max: 31.6	32.1 $\pm$ 1.7 Min: 24.1 Max: 35.2	15.8 $\pm$ 6.0 Min: 11.6 Max: 37.7
4. HbE/ β -thalassemia	92 (0.9)	6.3 $\pm$ 1.7 Min: 3.1 Max: 11.4	21.8 $\pm$ 5.0 Min: 11.6 Max: 34.4	3.45 $\pm$ 0.89 Min: 1.84 Max: 5.30	64.3 $\pm$ 9.0 Min: 49.0 Max: 106.3	18.4 $\pm$ 2.9 Min: 13.4 Max: 30.4	28.6 $\pm$ 1.9 Min: 24.1 Max: 33.9	29.3 $\pm$ 5.9 Min: 12.4 Max: 51.7
5. HbS carrier	37 (0.4)	12.1 $\pm$ 1.8 Min: 7.9 Max: 16.9	37.9 $\pm$ 8.7 Min: 22.1 Max: 53.2	4.61 $\pm$ 0.76 Min: 2.98 Max: 6.98	80.5 $\pm$ 7.0 Min: 65.0 Max: 91.2	26.6 $\pm$ 3.1 Min: 19.9 Max: 30.5	32.9 $\pm$ 1.4 Min: 30.1 Max: 35.7	14.7 $\pm$ 1.8 Min: 12.3 Max: 19.0
6. HbE disease	32 (0.3)	9.1 $\pm$ 2.3 Min: 3.7 Max: 13.2	29.4 $\pm$ 6.5 Min: 13.0 Max: 40.9	4.79 $\pm$ 1.05 Min: 1.90 Max: 6.83	61.8 $\pm$ 6.2 Min: 49.4 Max: 80.7	19.1 $\pm$ 2.6 Min: 13.1 Max: 25.9	30.7 $\pm$ 2.0 Min: 23.0 Max: 33.7	19.7 $\pm$ 4.0 Min: 15.6 Max: 33.6
7. β Thalassemia Major	11 (0.1)	6.7 $\pm$ 4.6 Min: 3.0 Max: 11.8	22.5 $\pm$ 14.5 Min: 10.7 Max: 46.2	2.95 $\pm$ 1.47 Min: 1.28 Max: 5.78	73.3 $\pm$ 12.4 Min: 56.4 Max: 100.1	21.9 $\pm$ 5.0 Min: 16.6 Max: 35.0	29.4 $\pm$ 1.1 Min: 27.6 Max: 31.2	31.4 $\pm$ 7.1 Min: 16.3 Max: 38.4

Table 3 continued

Diagnosis (n = 10,407)	No. of Cases (%)	Hb (g/dL)	Hematocrit (%)	RBC ($\times 10^6/\text{mm}^3$)	MCV (fl)	MCH (pg)	MCHC (g/dL)	RDW (%)
8. HPFH trait	9 (0.1)	11.1 $\pm$ 3.8 Min: 4.1 Max: 15.4	34.3 $\pm$ 11.9 Min: 12.2 Max: 47.5	4.09 $\pm$ 1.44 Min: 2.20 Max: 5.56	85.4 $\pm$ 11.5 Min: 69.3 Max: 101.9	27.9 $\pm$ 4.4 Min: 21.2 Max: 34.2	32.6 $\pm$ 1.2 Min: 30.6 Max: 34.6	16.2 $\pm$ 1.6 Min: 13.6 Max: 21.3
9. HbD ^{Punjab} carrier	7 (0.1)	11.7 $\pm$ 2.3 Min: 6.9 Max: 13.6	35.3 $\pm$ 6.5 Min: 22.1 Max: 41.0	4.14 $\pm$ 0.94 Min: 2.60 Max: 5.10	85.9 $\pm$ 4.1 Min: 80.4 Max: 92.9	28.5 $\pm$ 1.5 Min: 26.7 Max: 30.4	33.2 $\pm$ 1.3 Min: 31.2 Max: 35.2	19.5 $\pm$ 11.6 Min: 12.5 Max: 33.4
10. HbS disease	7 (0.1)	8.2 $\pm$ 1.3 Min: 6.4 Max: 10.1	25.8 $\pm$ 3.0 Min: 22.1 Max: 31.0	3.15 $\pm$ 0.36 Min: 2.50 Max: 3.60	82.1 $\pm$ 7.4 Min: 73.3 Max: 91.4	25.9 $\pm$ 3.3 Min: 21.9 Max: 30.1	31.6 $\pm$ 2.0 Min: 29.0 Max: 34.5	21.3 $\pm$ 7.0 Min: 15.4 Max: 36.3
11. HbS- β thalassemia*	4 (0.0)	8.2 $\pm$ 1.0 Min: 6.9 Max: 9.1	26.9 $\pm$ 4.0 Min: 22.6 Max: 31.3	3.63 $\pm$ 0.56 Min: 3.03 Max: 4.38	74.2 $\pm$ 4.2 Min: 70.6 Max: 79.9	22.7 $\pm$ 1.4 Min: 20.8 Max: 24.2	30.5 $\pm$ 1.3 Min: 29.1 Max: 32.2	22.8 $\pm$ 5.3 Min: 17.2 Max: 29.2
12. HbD ^{Punjab} disease*	3 (0.0)	11.3 $\pm$ 1.8 Min: 10.0 Max: 13.3	34.9 $\pm$ 3.9 Min: 32.5 Max: 39.5	5.32 $\pm$ 0.57 Min: 4.76 Max: 5.90	66.7 $\pm$ 14.4 Min: 55.8 Max: 83.0	21.6 $\pm$ 5.5 Min: 18.0 Max: 27.9	32.2 $\pm$ 1.5 Min: 30.8 Max: 33.7	16.2 $\pm$ 3.7 Min: 12.8 Max: 20.1
13. HbS/D double heterozygous*	1 (0.0)	5.3	18.4	2.68	68.7	19.8	28.8	30.8
14. Inconclusive	205 (2.0)	10.4 $\pm$ 2.3 Min: 3.5 Max: 14.6	32.1 $\pm$ 6.8 Min: 11.9 Max: 44.6	3.89 $\pm$ 0.67 Min: 1.41 Max: 5.77	82.7 $\pm$ 10.4 Min: 55.4 Max: 106.9	26.7 $\pm$ 3.9 Min: 17.1 Max: 36.1	32.3 $\pm$ 1.7 Min: 25.3 Max: 38.8	20.6 $\pm$ 10.2 Min: 11.6 Max: 49.4

HPFH hereditary persistence of fetal hemoglobin; continuous variables presented as mean $\pm$ SD, *Min* minimum, *Max* maximum, *Normal* normal hemoglobin analysis in HPLC

* Values may be unreliable due to the small number of observations

Conclusions

The present study revealed that CE-HPLC is a labour-saving and objective screening tool for early detection and management of hemoglobinopathies. Although the interpretation of HPLC requires diagnostic expertise and extensive training over a period of time it is easily amenable to quality control. It is diagnostic in most of the cases and only a few inconclusive or silent cases and cases with coexisting nutritional deficiency require other modalities like DNA analysis and iron study. BTT and HbE carrier are the most prevalent abnormalities in this region and more intensified HPLC screening program should be established to find out these hidden carriers.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Angastiniotis M, Modell B, Englezos P, Boulyjenkov V (1995) Prevention and control of haemoglobinopathies. *Bull World Health Organ* 73:375–386
- Sachdev R, Dam AR, Tyagi G (2010) Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: report of 2600 cases. *Indian J Pathol Microbiol* 53:57–62
- Waters HM, Howarth JE, Hyde K, Goldstone S, Cinkotai KI, Kadkhodaei-Elyaderani M, Richards JT (1998) An evaluation of the bio-rad variant haemoglobin testing system for the detection of haemoglobinopathies. *Clin Lab Haematol* 20:31–40
- Riou J, Godart C, Hurtrel D, Mathis M, Bimet C, Bardakdjian-Michau J, Préhu C, Wajcman H, Galactéros F (1997) Cation-exchange HPLC evaluated for presumptive identification of hemoglobin variants. *Clin Chem* 43:34–39
- Joutovsky A, Hadzi-Nesic J, Nardi MA (2004) HPLC retention time as a diagnostic tool for hemoglobin variants and hemoglobinopathies: a study of 60,000 samples in a clinical diagnostic laboratory. *Clin Chem* 50:1736–1747
- Rao S, Kar R, Gupta SK, Chopra A, Saxena R (2010) Spectrum of haemoglobinopathies diagnosed by cation exchange-HPLC and modulating effects of nutritional deficiency anaemias from north India. *Indian J Med Res* 132:513–519
- Bain BJ (2006) Disorders of red cells and platelets. In: Bain BJ (ed) *Blood cells: a practical guide*, 4th edn. Blackwell, Oxford, pp 283–397
- Dolai TK, Dutta S, Bhattacharyya M, Ghosh MK (2012) Prevalence of hemoglobinopathies in rural Bengal, India. *Hemoglobin* 36:57–63
- Jain BB, Roy RN, Ghosh S, Ghosh T, Banerjee U, Bhattacharyya SK (2012) Screening for thalassemia and other hemoglobinopathies in a tertiary care hospital of West Bengal: implications for population screening. *Indian J Public Health* 56:297–300