Original Article

Spectrum of Hemoglobin Variants in the Population of Northern Region of West Bengal: An Ethnogenetic Proposition

Bidyut Krishna Goswami, Raghunath Pramanik, Sudipta Chakrabarty, Partha Pratim Pal[1], Sarama Banerjee[2], Arghya Bandyopadhyay[3]

Departments of Pathology, and [1]Community Medicine, [2]Department of Immune-Hematology Medical Officer, Regional Blood Transfusion Centre, North Bengal Medical College, Dist. Darjeeling, [3]Department of Pathology, Burdwan Medical College, Burdwan, West Bengal, India

Abstract

Context: The birth of transfusion-dependent states of hemoglobinopathies including thalassemias is preventable by population screening and genetic counseling. Magnitude is not addressed in the Northern Region of West Bengal where many ethnic variants inhabit. **Aims and Objectives:** The aim of the following study is to find out the burden of different entities of hemoglobinopathies, their correlation with ethnicity and the "at risk" groups. **Subjects and Methods:** A descriptive study was conducted from the Hematology Unit of North Bengal Medical College over 1 year on the subjects underwent screening for hemoglobinopathies for detection of abnormal hemoglobin (Hb) variants by "cation-exchange high-performance liquid chromatography" principle along with other relevant tests. **Statistical Analysis:** Data was analyzed by frequency distribution and Chi-square test assuming *P* value as 95% of the level of significance using the SPSS version 16 (SPSS Inc., Chicago, Illinois, U.S.A). **Result:** Abnormal Hb variant was 47.5% among 1872. Hb E trait (34.4%) was most common followed by Hb E disease (25.3%) and others. Hb E disorders (92.7%) were observed mostly among Rajbangsi population while E-β-thalassemias (40%) in the Muslims and a heterogeneous pattern noted among tribal and mongoloid. **Conclusion:** Hb E hemoglobinopathies was high among Rajbangsi and Muslims with identification of some other hemoglobinopathies involving tribal and mongoloid.

Keywords: Ethnicity, hemoglobinopathies, high-performance liquid chromatography, Northern region of West Bengal, thalassemias

Introduction

Inherited hemoglobin (Hb) disorders which encompass the thalassemias, the structural variants and other rare entities due to failure to switch on globin chain synthesis are the major type of genetic disorders prevalent world-wide as well as in South-East Asia including many parts of India.[1-4] The clinical spectrum of the disorders varies from asymptomatic conditions to serious disorders like thalassemia major that requires regular blood transfusions and extensive medical care. World Health Organization figures estimates that 7% of the world population is a carrier for Hb disorders.[5] Prevention by population screening, prenatal diagnosis, genetic counseling and avoiding marriage between the carriers plays the most important role to avoid the dreaded consequences the affected family faces.[6] Community-based genetic screening of inherited Hb disorder has not been undertaken in the Northern districts of West Bengal also called North Bengal. North Bengal Medical College and Hospital is the only tertiary referral center of Northern Districts of West Bengal with facilities for thalassemias screening. As the blood bank of this institution has the facilities of component separation, almost all patients with hematological disorder are referred to this institution represent the pattern of abnormal Hb in this region. The present study was undertaken to find out the occurrence of hemoglobinopathies, to classify the pattern of inherited Hb disorders among the local population of the North Bengal and to identify those population groups "at risk" having high occurrence of such disorders.

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Address for correspondence: Dr. Bidyut Krishna Goswami, Department of Pathology, North Bengal Medical College, P.O. Sushrutanagar, Dist. Darjeeling, West Bengal, India.
E-mail: drbkg61@gmai.com

Subjects and Methods

The subjects included in this study were exclusively from the districts of North Bengal, comprising of six districts, i.e. Darjeeling, Jalpaiguri, Coochbehar, North Dinajpur, South Dinajpur and Malda. During the period from January 2010 to December 2010, a total of 1872 subjects who were referred to the Hematology Unit, North Bengal Medical College and Hospital for study of Hb variants from different hospitals as well as private clinics of the zone comprising the study group. Informed consent has been obtained from all the subjects who participated in this study. Subjects with incomplete information and with history of blood transfusion (up to 6 weeks) and babies less than 6 months of age and known cases of hematological malignancies are kept excluded. Patients with border-line value for Hb A_2 (3.6-4.0%) were also excluded from this study.

Epidemiological data of each individual pertaining to age, sex, caste, ethnicity, place of origin, consanguinity, etc., was recorded. Family history and treatment history were also taken. About 3-5 ml intravenous blood sample was collected after obtaining informed consent, using disposable syringe and needle with disodium salt of ethylenediaminetetraacetic acid (EDTA) as anticoagulant from each individual. Hematological indices were measured using Automated Cell Counter (Sysmax KX 21E-210, Japan) which was calibrated with commercially available controls (Bio-Rad Laboratories, USA). A vigilant examination of red blood cell morphology from Leishman stained direct smears were studied. Study of Hb variants were done following cation-exchange high-performance liquid chromatography principle, which is accepted to be the method of choice recently using Variant-1 (Biorad, USA) with β-thalassemias short program.[7] The retention time for Hb A_2 was 3.63 min and its normal level was 1.8-3.5%. Increased Hb A_2 is seen almost exclusively in β-thalassemias. It rarely reaches 7% and never more than 12% and thus any rise in Hb A_2 window >12% indicates a structural variant.[8] The retention times for Hb E is 3.69 min. The percentage Hb eluting in this window in cases of Hb E trait (heterozygous) is around 22-40% and in Hb E disease (homozygous) is around 70-90%. A value of in between 3.6% and 4.0% was considered "border-line" and parental study/deoxyribonucleic acid study was recommended for border-line values.[9]

Tests based on physico-chemical properties like the sickling test, using freshly prepared sodium metabisulfite solution as a reducing agent.[10] Fetal Hb was estimated by the method of Betke *et al*.[11] Osmotic fragility test, inclusion test for Hb H and Hb electrophoresis were carried out on cellulose acetate membrane in the tris-EDTA-borate buffer at pH 8.9 along with quantification of A2 fraction of adult Hb by elution method as when necessary.[10] Electrophoresis at pH 6.0 was also carried out to identify and confirm the presence of Hb D or E band in case of confusion.[7] Family screening with special importance to parental study was done in case of border-line values for β-thalassemia trait and all positive cases. All laboratory investigations were carried out following standard procedures after cross-checking for quality control from time to time.

The study population was classified on the basis of ethnic origin and linguistic affiliation in different population groups. "*Rajbanshis*" are hypothesized to be a mixture of different tribal groups (*Rabhas*, *Tiwas*, *Kacharis*, *Garos*, *Karbis*, etc.) that were converted to Hinduism and in the process became admixed with certain Caucasoid caste populations.[12] Other major ethnic groups encountered in the present studies are Caucasoid, mongoloid and tribes. The ethnic groups were further sub classified according to Dash.[13] The secondary data was entered into Microsoft Excel data sheet and subsequently imported to SPSS version 16 (SPSS Inc., Chicago, Illinois, U.S.A) for analyzing various descriptive statistic such as frequency distribution in percentages and Chi-square test assuming *P* value as 95% of the level of significance.

Results

Out of 1872 cases 47.5% had one or either type of hemoglobinopathies whereas 52.5% were found to be normal. Out of 890 abnormal cases 54.5% and 45.5% were males and females respectively.

In the spectrum of hemoglobinopathies in clinically asymptomatic, It was found that Hb E trait was the most common hemoglobinopathies (34.4%) followed by homozygous E (25.3%), β-thalassemia trait (17.8%), E-β-thalassemia (15.1%), β-thalassemia major (1.5%), sickle cell-β-thalassemia (3.4%), sickle cell trait (1.1%) and so on as shown in Table 1. It was also observed that approximately 40% of all the abnormal variants were contributed by homozygous Hb E hemoglobinopathies and compound heterozygous state for Hb E with β-thalassemia with a high morbidity.

Age wise distribution of different hemoglobinopathies as identified in this study is presented in Table 2, which reveals

Table 1: Spectrum of hemoglobinopathies in Northern Districts of West Bengal *n*=890

Type of hemoglobinopathies	Sex		Total
	Female	Male	
β-thalassemia major	6 (1.5)	7 (1.4)	13 (1.5)
β-thalassemia trait	81 (20)	77 (15.9)	158 (17.8)
E-β-thalassemia	55 (13.6)	79 (16.3)	134 (15.1)
Hemoglobin E trait	136 (33.6)	170 (35)	306 (34.4)
Hemoglobin J trait	1 (0.2)	1 (0.2)	2 (0.2)
Hemoglobin D trait	4 (0.98)	3 (0.6)	7 (0.8)
Hemoglobin E disease	102 (25.2)	123 (25.4)	225 (25.3)
δ β-thalassemia	1 (0.2)	1 (0.2)	2 (0.2)
Sickle cell disease	2 (0.49)	0	2 (0.2)
Sickle cell β thalassemia	12 (2.96)	18 (3.7)	30 (3.4)
SD disease	0	1 (0.2)	1 (0.1)
Sickle cell trait	5 (1.2)	5 (1)	10 (1.1)
Total	405 (45)	485 (54.5)	890 (100)

Parenthesis indicates percentages

that most of the cases (64.1%) are contributed by less than 20 years age group followed by middle age group (32.2%) and few cases (3.7%) having age more than 50 years. The frequency was decreasing with the age advances. The trend was statistically highly significant $P < 0.05$ (χ^2 for linear trend 32.3).

The present study shows that the occurrence of hemoglobinopathies was highest (72.1%) among *Rajbanshis* population followed by Muslim group (54.9%) and in tribes like "*Santal*" and "*Oraon*" as shown in Table 3. and in Bengali Hindu and *Marwari/Behari*, approximately equal percentage (34%) observed while least belong to mongoloid like "*Nepalis*" and other 'Hill men' population (17.5%). Statistical analysis showed that *Rajbanshis* have significantly ($P < 0.05$, df 2) greater predilection for hemoglobinopathies than other ethnic groups in this region.

Hb E hemoglobinopathies were the most frequent pattern detected in *Rajbanshis* (92.7%) and Muslim (81.5%) in the form of heterozygous, homozygous or double heterozygous state with other variants as shown in Figure 1. In the tribes as well as in *Nepali* population groups one mixed distribution pattern was noted comprising of both Hb E hemoglobinopathies and β-thalassemias along with Hb S hemoglobinopathies [Figure 2].

Another interesting observation is identification of some cases of Hb S hemoglobinopathies with their compound heterozygous state. Altogether 43 cases were found of which contribution from tribal population was significant while in *Rajbanshis* it was very low. Though very few, some rare type of abnormal Hbs were also observed in this study, 2 cases of Hb J, 7 case of Hb D and one case of Double heterozygous state for Hb S and Hb D hemoglobinopathies [Table 1].

Discussion

The population of northern districts of West Bengal and its adjacent area is unique and admixture of different casts and tribes. Due to practice of non-random mating pattern for a long time, some particular mutations are restricted to some specific ethnic groups.[14] Hb E is found to be the most prevalent hemoglobinopathies in this area; the result is similar

Table 2: Age wise distribution of cases of different hemoglobinopathies *n*=890

Age groups	Hemoglobinopathies		Total
	Yes	No	
0-20	571 (64.1)	497 (50.7)	1068 (57)
21-50	287 (32.2)	432 (44)	719 (38.4)
51 and above	32 (3.7)	53 (5.3)	85 (4.6)
Total	890 (100)	982 (100)	1872 (100)

Parenthesis indicates percentage. χ^2 for linear trend 32.3. $P<0.05$

Table 3: Ethnicity wise distribution of hemoglobinopathies *n*=1872

Ethnic groups	Hemoglobinopathies		Total
	Yes	No	
Bengali Hindu	303 (34.5)	577 (65.5)	880 (47.0)
Hindi speaking, (Marwari, Behari)	24 (34.3)	46 (65.7)	70 (3.7)
Nepali	11 (17.5)	52 (82.5)	63 (3.4)
Muslim	119 (54.9)	98 (45.1)	217 (11.6)
Santals, Oraow	43 (42.6)	58 (57.4)	101 (5.4)
Rajbanshis*	390 (72.1)	15 1 (27.9)	541 (28.9)
Total	890 (47.5)	982 (52.5)	1872 (100)

Parenthesis indicate percentages. **Rajbanshis* population group was compared to other ethnic groups as a whole. χ^2=182.43, df=1, $P<0.05$

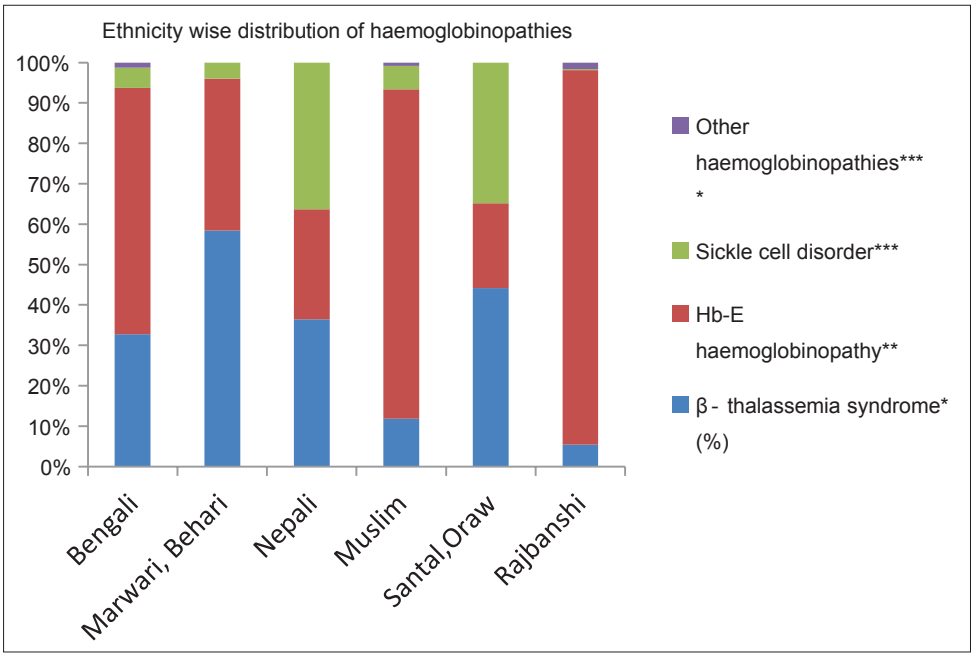

Figure 1: Distribution of hemoglobinopathies in different ethnic groups (*n* = 890). *β-thalassemia includes β-thalassemia major, β-thalassemia trait, **Hb E hemoglobinopathy includes Hb E trait, Hb E disease, E-β-thalassemia, ***Sickle cell disorders include sickle cell trait, sickle cell disease, sickle cell β-thalassemia, ****Others includes Hb J trait, Hb D trait, δ β-thalassemia

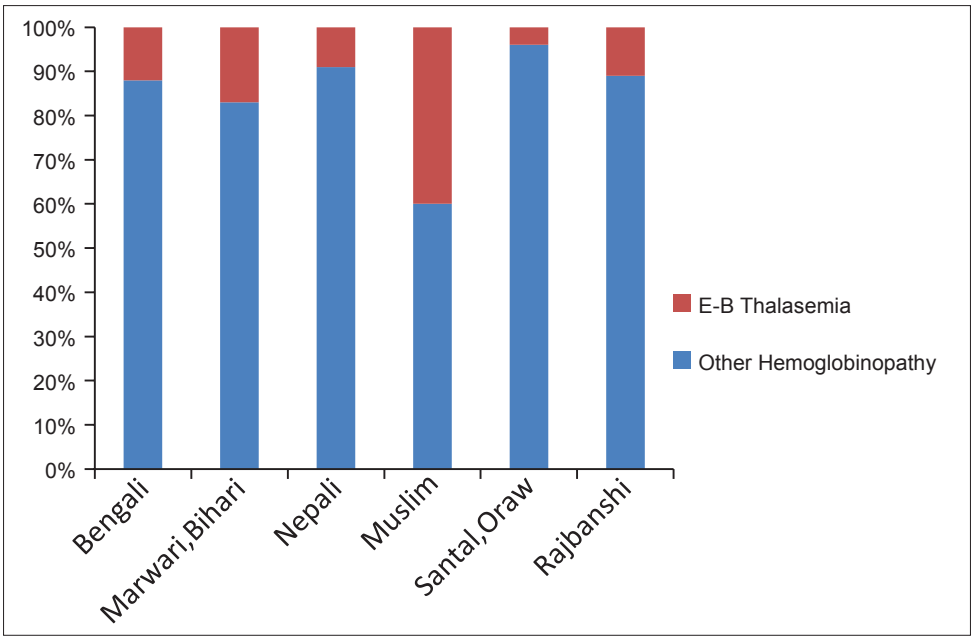

Figure 2: Distribution of β-thalassemias in various ethnic groups

to other studies published from eastern India.[2,15] As such Hb E hemoglobinopathies, especially Hb E trait may not be of clinical significance, but interaction of Hb E and thalassemias produces variable phenotype.[16] *Rajbanshis* have been described as the predominant race in the Sub-Himalayan West Bengal and the survivors of an aboriginal race.[11] They have a high occurrence of hemoglobinopathies and most of them suffer from Hb E (92.7%) trait or disease. This high occurrence of Hb E hemoglobinopathies among *Rajbanshis* reflects a lineage simulation between *Assames* and *Rajbanshis* of this zone.[15]

Anemia, specially Iron-deficiency anemia is considered to be the most common form of health problem which present morphologically as microcytic and hypochromic anemia, a great simulator of Hb E hemoglobinopathies specially if present as double heterozygous state with ß-thalassemias.[17] This is a common practice at the basic level of health care as well as recommendations from different nutritional deficiency control program to prescribe Iron-supplement in anemia detected clinically or at random. Pathogenically Iron-supplement has no role in anemia due to Hb E hemoglobinopathies until and unless there is Iron-deficiency, rather this is detrimental especially in case of Double heterozygosisty with ß-thalassemias. In the present study, this is well-documented that Hb E hemoglobinopathies with their Double heterozygous state with ß-thalassemia are the predominant type of Hb-variants among Rajbangshi and Muslim population groups, of which Rajbangshis being the most prevalent, putting a question mark on the random use of Iron-supplements in this region. It also emphasizes the importance of routine Screening procedure for Hb variants even in primary health care. Tribal population of this region (*Oraws*, *Mundas*, *Santals*) migrated into the northern districts of west Bengal for tea cultivation from the Chota Nagpur plateau.[13] They have high occurrence of sickle cell disease (34.8%) among them. The results are similar to the other studies published from central east coast of India.[18] The high occurrence of Hb E hemoglobinopathies among Muslim groups may be related to the facts that these Muslims are mostly converted from regional aborigine where *Rajbanshis* and other similar populations are prevalent.[19] Another new finding in this study is that though as whole hemoglobinopathies are lowest (17.5%) among mongoloids, they show heterogeneous distribution similar to tribal (*Santhal/Oraw*) groups, may be related to inter-community marriage among *Nepalis*/hill men and tribals for last centuries, due to cohabitation at same places. Pattern of hemoglobinopathies among Bengali population is quite different from studies published from Kolkata and its adjacent areas. Hb E is the most prevalent hemoglobinopathies in this region compare to β-thalassemia syndrome in other parts of West Bengal.[7] It may be due to narrowing of differences between Hindu Bengali and *Rajbanshis* population groups, so far socio-economic background and geographical positioning is concerned, leading to high marriage rate between Bengalis and *Rajbanshis*.

This study is limited by the lack of facilities like DNA studies, the inherent problem of a remotely placed tertiary care center.

The usual geographical clustering of inherited Hb disorders due to various factors (e.g. comparative small population size, caste endogamy, consanguinity and virtual lack of medical facilities and natural barriers such as forests, river etc., have been changed due to changing life-pattern and communication system, which has compounded the complexity of β-thalassemia syndrome and Hb E disorder in the northern districts of West Bengal. The prevention and control of hemoglobinopathies in this region is an uphill task for the planners, policy makers and medical and health care machinery of the state with development of awareness among people. This study provides for the first time a

comprehensive database on the spectrum of hemoglobinopathies in the remotely placed Northern Districts of West Bengal.

Conclusion

This present study has identified high occurrence of Hb E hemoglobinopathies among *Rajbanshis* and Muslim population groups with identification of some other hemoglobinopathies involving tribal and mongoloid ethnicity. The present study unveils the unique and comprehensive spectrum of hemoglobinopathies in the Northern Districts of West Bengal which is essential for planning of screening and awareness program of different ethnic group in this region and to prevent birth of transfusion-dependent Hb E-β-thalassemia babies it also suggest its relevance in primary health care as well.

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