

The prevalence and characterization of β -thalassemia trait by using high-performance liquid chromatography among the rural population in West Bengal, India

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Abstract

Hemoglobinopathies, common genetic disorders of hemoglobin, can be prevented by population screening and genetic counseling. Identification of these disorders is immensely important epidemiologically and aid in prevention of more serious hemoglobin disorders. Thalassemia is the commonest monogenic disorder in India, which belongs to the thalassemia belt of the world. The present study was undertaken to find out the characteristics of β -thalassemia trait and spectrum of this disorder among the rural population, screened under the hospital-based screening program in West Bengal, a state in eastern part of India. This study was carried out in school and college students, newly married couples and pregnant women after proper counseling in the rural areas of five southern districts of this state. Blood samples were tested by high-performance liquid chromatography. Total 1429 β -thalassemia traits were detected by random screening from this population. The mean value of HbA₂ of the study population, having β -thalassemia trait is 4.9%. The prevalence (10.5%) of β -thalassemia trait in West Bengal is higher than other parts of the country. These data are likely to help us in future planning for screening programmes in rural areas of West Bengal, India.

Introduction

Hemoglobinopathies are a group of genetic disorders of hemoglobin in which there is abnormal production or structure of the hemoglobin molecule. These hereditary disorders are major public health problem in many parts of the world including India. It is estimated that there are about 65,000-67,000 β -thalassemia

patients in our country with around 9000-10,000 cases being added every year.¹⁻⁵ 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 (WHO) estimates that 7% of world population is carrier for hemoglobin disorders.⁶ Inherited hemoglobin (Hb) disorders are caused by structural abnormalities and abnormal synthesis of globin chains and represent some of the most common monogenic disorders in the world. Clinical manifestations and severity of structural globin chain defects are caused by abnormal solubility, stability and oxygen carrying capacity, whereas, in thalassemia syndromes, these are related to the degree of reduction of α - or β -globin chains. India is in the thalassemia belt of the world.¹ The carrier rate for β -thalassemia gene varies from 1-3% in Southern India to 3-15% in Northern India. Certain communities in India, such as Sindhis and Punjabis from Northern India, Bhanushali's, Kutchis, Lohana's from Gujarat, Mahar's, Neobuddhist's, Koli's and Agri's from Maharashtra, Gowda's and Lingayat's from Karnataka etc. have a higher carrier rate.^{2,3} The estimated prevalence of the thalassemia carrier state in India is 3.3% of its total population.^{7,8} There is at present no systematic, large published study that has surveyed the prevalence rates of β -thalassemia carrier within West Bengal state in eastern India. Population screening has identified the prevalence of β -thalassemia trait as high as 17% in certain communities in India.⁹

Materials and Methods

This study was part of a social service scheme conducted by the Thalassemia Society of Midnapore District, which is a nonprofit voluntary organization. The field survey was done under direct guidance of the authors. The Thalassemia Society of Midnapur District regularly arranges camps in different districts of rural areas of West Bengal for screening of individuals for the thalassemia carrier state and for the propagation of awareness. Seminars on thalassemia are organized on a regular basis in rural areas with help from local administrative authorities, volunteers, institutional heads, representatives from clubs, village heads, religious leaders of all faiths and celebrities. Primary objective of this study was to determine the prevalence of β -thalassemia trait in rural areas of West Bengal that would be helpful in the implementation of control programmes in near future. Awareness camps were organized to encourage people especially the high school and college students, newly married couples and

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pregnant women to come forward for voluntary blood testing. This study was conducted in the villages of 5 (five) southern districts of Paschim Midnapur, Purba Midnapur, Purulia, Hooghly and Burdwan; within the state of West Bengal, India.

Blood collection

Two mL of venous blood were collected in tri-potassium ethylenediaminetetraacetic acid, vials from individuals after obtaining written consent. Blood samples were transported to the laboratory, stored at 2-8°C and tested within next 24 h.

Laboratory analysis

Red blood cell indices were obtained with a Sysmex-1800i model automated blood cell counter (Sysmex Corp., Kobe, Japan). Appropriate internal laboratory quality control procedures were performed on a regular basis. Samples were tested on a VARIANT™ (Bio-Rad Lab., Hercules, CA, USA) high-performance liquid chromatography (HPLC) machine.¹⁰ An Hb A₂ value is of 3.9 to 9.0% were classed as β -thalassemia trait and values of 3.3 to 3.8% were considered as borderline.^{9,11} HbA₂ values below 2% were labeled as low A₂ and molecular analyses to detect α -gene defects planned for these cases.¹⁰ Samples showing borderline Hb

A₂ value have been submitted for genetic analysis of β -thalassemia mutations by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).¹²

Results

Altogether 13,609 samples (male, 64% and female, 36%) were screened to evaluate the prevalence and characterization of beta thalassaemia trait among the rural population by using HPLC in West Bengal, India from March 2011 to February 2013. Total 1429 patients (10.5%; 737 male and 692 female) were detected as β -thalassaemia traits of which 1374 patients (10.10%; 702 male and 672 female) showed mean HbA₂ value of 4.9% (3.9-9.0%) (Table 1) and 55 patients (0.40%; 35 male and 20 female) with borderline HbA₂ values of 3.7% (Table 2).

The values of red cell indices like Hb%, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width-coefficient of variation (RDW-CV), HbF, HbA₂ and HbA₀ of 55 patients with borderline HbA₂ values are 11.0 g/dL (2.2-14.8), 37.6 L/L (12.8-56.2), 71.4 fL (52.4-107.9), 21.2 pg (14.6-30.1), 15.3% (8.8-22.4), 0.8% (0-4.7), 3.7% (3.5-3.8), 75.8% (66.7-88.5) respectively, whereas the mean values of different parameters of 1374 samples (Table 2), are Hb 10.6 g/dL (4.5-21.2), Hct 36 L/L (12.4-75), MCV 66.5 fL (44.7-104), MCH 20 pg (9.3-53.2), RDW 15.2% (9.2-30.8), HbF 0.7% (0-12.2), HbA₂ 4.8% (3.9-10) and HbA₀ 84.6% (49.3-95.3). Patients with borderline HbA₂ values (n=55) were further tested by ARMS-PCR and five cases showed presence of different beta thalassaemia mutations prevalent in this part of the country. The mutations were IVS1-5 (G→C) in 3 cases, codon 30 (G→C) in one case and codon 15 (G→A) in one case.

Total of 1277 patients (92.93%) showed HbA₂ value in the range of 4.1-6.0 (Table 1). The mean values of red cell indices of normal individuals in the study population showed Hb-13.01 g/dL, Hct 39.41 L/L, MCV 82.2 fL, MCH 28.3 pg, RDW 14.9%, HbF 0.8%(0-12.2) and HbA₂ 2.61%.

Discussion

Thalassemias are inherited disorders characterized by abnormal production of hemoglobin and associated with low hemoglobin production and excessive destruction of red blood cells. It is recognized as the most prevent genetic blood disorder in the world. However β -thalassaemia, the most common abnormal single gene disorder worldwide, found in more than 60 countries with a carrier population of

up to 150 million.¹ The distribution and prevalence rates of different hemoglobinopathies in rural areas of the state of West Bengal where the majority of the population reside, is not known with certainty due to lack of comprehensive surveys. Since proper planning and implementation of thalassaemia control projects in rural areas will depend on data of actual prevalence of carrier states in these areas, the current study addresses this burning issue. Target population in this study was secondary school children and college students due to their future reproductive potential, and newly married couples or antenatal mothers. The groups considered fairly representative of the young general population of these rural areas and were more easily accessible for investigation and counseling.

Many communities in India have a high prevalence of the β -thalassaemia gene.¹³ It varies between 1% and 17%, with a mean prevalence of about 3.3%.^{14,15} In a multi-centric study conducted recently by the Task Force of the Indian Council of Medical Research (ICMR), New Delhi, among schoolchildren aged 11-18 years

in Mumbai, New Delhi and Calcutta showed a β -thalassaemia carrier rate of 2.6%, 5.5% and 10.2%, respectively, in these three cities.¹⁶

The collected 13,609 samples were examined by using HPLC to study the prevalence and characterization of β -thalassaemia trait and to evaluate the mean values of different biological parameter among the rural population in West Bengal. In the present study, the prevalence of the β -thalassaemia trait using the HPLC test among the people residing in the tribal areas who voluntarily attended the organizing camps was 10.5%; which is similar to the other observation.^{9,14} Total of 1277 patients (92.93%) showed HbA₂ value in the range of 4.1-6.0 (Table 1). In our observation, borderline HbA₂ values were found in 0.40% (n=55) of the studied population. And the patients with borderline HbA₂ values (n=55) who were further tested by ARMS-PCR showed presence of different β -thalassaemia mutations prevalent in this part of the country in a total of 5 cases. In all these, further testing for iron profile revealed concomitant iron deficiency anemia; which could explain the reason

Table 1. HbA₂ values of β -thalassaemia trait patients (n=1374) showing mean HbA₂ value of 4.9% (range: 3.9-9.0).

HbA ₂ value (range)	No. of patients	Percentage (%)
3.9-4.0	62	4.51
4.1-5.0	710	51.67
5.1-6.0	567	41.26
6.1-7.0	24	1.74
7.1-8.0	5	0.34
8.1-9.0	6	0.43

Table 2. Mean values of red blood cells indices of patients showing borderline (3.3-3.8) HbA₂ values and high HbA₂ values of 3.9-9.0%.

Parameters	Patients (n=55) showing border line HbA ₂ values		Patients (n=1374) showing HbA ₂ values of 3.9-9.0%	
	Mean values	Range (minimum and maximum value)	Mean values	Range (minimum and maximum value)
Hb (g/dL)	11.0	2.2-14.8	10.6	4.5-21.2
RBC (x 10 ¹² /L)	5.24	2.46-6.74	5.32	1.91-11.09
Hct (L/L)	37.6	12.8-56.2	36	12.4-75
MCV (fL)	71.4	52.4-107.9	66.5	44.7-104
MCH (pg)	21.2	14.6-30.1	20	9.3-53.2
MCHC (g/dL)	29.1	21.1-40.3	30.4	12.7- 45.7
RDW%	15.3	8.8-22.4	15.2	9.2-30.8
HbF%	0.8	0-4.7	0.7	0-12.2
HbA ₂ %	3.7	3.5-3.8	4.9	3.9-10
HbA ₀ %	75.8	66.7- 88.5	84.6	49.3-95.3

Hb, hemoglobin; RBC, red blood cells; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width.

behind low/borderline Hb A₂. A low HbA₂ value may indicate α -thalassemia, if iron deficiency is excluded.¹⁷ However, the possibility of δ -thalassemia should also be considered.¹⁸

The inherited hemoglobin disorders cause considerable pain and suffering to the patients and their families and are a major drain on health resources in the country. Screening in antenatal clinics is the best way to identify couples at immediate risk of having an affected child. Counselors should be aware that couples at risk of having a child with β -thalassemia major, Hb S β -thalassemia, HbE β -thalassemia, $\delta\beta$ - β -thalassemia, should be given the option of prenatal diagnosis to avoid the birth of a child with a severe disorder.¹⁹

Premarital screening programs for thalassemia may be more effective and culturally acceptable in the reduction of pregnancies with thalassemia major and provide insights into culturally congruent educational interventions to reach out diverse socio-demographic and ethnic communities to increase knowledge and cultivate positive attitudes toward prevention of thalassaemia.²⁰

To conclude, though the number of borderline HbA₂ cases is low or apparently may seem insignificant, this is very important in antenatal cases, especially if the other partner is β -thalassemia or HbE trait. In these contexts, mutation analysis is mandatory in such cases for characterization and confirmation of the diagnosis.¹³

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