

Profile of hemoglobin D trait in West Bengal, India

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Abstract

Hemoglobin (Hb) D Punjab is one of the most commonly observed abnormal hemoglobinopathy worldwide. There was no systematic large published study to investigate the characteristic of Hb D Punjab trait in India. This study was conducted in school and college students, newly married couples and pregnant women after proper counseling in the rural areas of West Bengal state in eastern India. Complete blood count was done by Sysmax Automated Hematology Analyzer KX 21 (Sysmex Corp., Kobe, Japan) and thalassaemia testing was done using high-performance liquid chromatography (Variant TM - Bio-Rad Lab., Hercules, CA, USA). A total of 46,139 individuals were screened for hemoglobinopathies. Hb D trait was found in 0.35%. Hypochromia rather than microcytosis is consistent finding in Hb D trait. Anisocytosis is absent in Hb D trait. In almost all (99.37%) cases, Hb D is within 40% of total hemoglobin. This data is likely to be helpful for screening of hemoglobinopathy in resource poor setting.

Introduction

Hemoglobin (Hb) D Punjab (Hb D Los Angeles) is a variant Hb. Hb D is due to amino acid substitution of glutamine for glutamic acid at codon 121 of the β -globin gene. Hb D trait, Hb D-Punjab disease, Hb D-Punjab/ β -thalassaemia and Hb D-Punjab/Hb S are the common form of Hb D. Hb D Punjab has an incidence of 2-3% among Sikhs in Punjab, 1% in Gujeratis, 0.37% in Bengalis in India and has also been found throughout the world with variable incidence.^{1,2}

Another tertiary centre study from North Western India, the prevalence of Hb D trait, Hb D-Punjab disease, Hb D-Punjab/ β -thalassaemia and Hb D-Punjab/Hb S was 0.33%, 0.13%, 0.05% and 0.02% respectively.³

There is at present no systematic, large published study that has surveyed the characteris-

tic of Hb D Punjab trait within West Bengal (WB) state in eastern India. The primary objective of this study was to determine the prevalence of hemoglobinopathies including Hb D Punjab trait in rural areas of WB that would be helpful in implementation of control programs.

Materials and Methods

This study was a part of a social service conducted by the Thalassaemia Society of Midnapur District, which is a non profit voluntary organization. The field survey was done under the direct guidance of the authors. The propagation of awareness on thalassaemia was done by regularly organized seminars in rural areas with help from local authorities such as principals of colleges and schools, political leaders, presidents of clubs, village heads, religious priests of all faiths and celebrities.

High school and college students, newly married couples and pregnant women were especially encouraged to come forward for testing after intensive awareness campaigns. Participation in this study was entirely voluntary. Blood samples were collected on a pre determined date from a designated centre. This study was conducted in the villages of the districts of Paschim Midnapur, Purba Midnapur, Purulia, Hooghly and Burdwan. These all are within the state of WB, India.

About 2.5 mL of venous blood were collected and transported to a laboratory under cool conditions and tested within 24 h of collection. Complete blood count were obtained with a Sysmax Automated Hematology Analyzer KX 21 (Sysmex Corp., Kobe, Japan) automated blood cell counter. Quantitative assessment of hemoglobin Hb F, Hb A₀, Hb A₂ and Hb D was performed using high-performance liquid chromatography (HPLC; Variant TM - Bio-Rad Lab., Hercules, CA, USA). Appropriate internal laboratory quality control procedures were performed on a regular basis. Serum ferritin was measured by dioxetane-based chemiluminescent (Lumi-Phos) techniques (Access 2[®] Immunoassay System, Beckman Coulter, Inc. Fullerton, CA, USA). α -thalassaemia was excluded by multiplex polymerase chain reaction methods.⁴

Results

A total of 46,139 samples were screened for hemoglobinopathies from June 2010 to July 2013. β -thalassaemia trait was found in 6.64%, E-trait 2.73%, sickle cell trait 0.55% and hereditary persistence of fetal hemoglo-

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bin heterozygous 0.074%. Hb D trait was found in 161 (0.35%) cases. Of these HbD traits, 59% were males and 41% were females. Majority of subjects are asymptomatic and few had mild anaemia with mean Hb 12.23 g/dL and red cell indices with mean corpuscular volume (MCV) 83.56 fL, mean corpuscular hemoglobin (MCH) 25.68 pg and mean corpuscular hemoglobin concentration (MCHC) 31.12g/dL. The mean red cell distribution width-coefficient of variation (RDW-CV) was 14.33%. On HPLC mean Hb D, Hb A₀, Hb A₂ and Hb F were 35%, 53.25%, 2.32% and 0.62% respectively. The hematological parameters are shown in Table 1.

Microcytic anemia (MCV \leq 80 fL) was seen in 18.63% of cases and hypochromic anaemia in 94.40% of Hb D trait cases (Table 2). Serum ferritin values were normal in all patients. Hypochromia rather than microcytosis was seen in Hb D trait patients. Normal RDW-CV was seen in all cases and suggested that anisocytosis is absent in Hb D trait. Hb D within 40% of total hemoglobin was seen in 99.37% of cases.

Discussion

India is in the thalassemia belt of the world. There are marked regional differences in the incidence of hemoglobinopathies in India and is true for Hb D Punjab trait also. Due to lack of comprehensive surveys in rural part of WB, exact prevalence of Hb D trait was not known. The current study addresses this burning issue for detection of carrier and profile of Hb D trait in rural people to institute appropriate screening procedures, designed not to miss the carriers by HPLC methods. The groups in this study

Table 1. Hematological profile in Hb D trait (n=161).

Parameter	Mean	Range
Hb g/dL	12.23	8.7-17.8
Hct %	40.56	27.7-56.1
MCV fL	83.56	65.4-98.5
MCH pg	25.68	17.2-30.6
MCHC g/dL	31.12	22.6-37.4
RDW-CV %	14.33	11.6-16.5
Hb A ₀ %	53.25	50.1-60.5
Hb A ₂ %	2.32	1.2-3
Hb F %	0.62	0-3.1
HbD %	35	26.3-40.5

Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution cell-coefficient of variation.

Table 2. Mean corpuscular volume and mean corpuscular hemoglobin values in Hb D trait (n=161).

RBC indices	Number	Percentage
MCV <80 fL	30	18.63
MCV ≥80 fL	131	81.36
MCH ≤28 pg	152	94.40
MCH >28 pg	9	5.60

RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin.

were also considered fairly representative of the general population of these rural areas and were also more easily accessible for investigation and counseling.

In our study, the prevalence of Hb D trait was 0.35% in the rural areas of Bengal. This is different from a previous study in the city of Lucknow from Uttar Pradesh, India that showed the incidence of Hb D trait was 1.5% and the prevalence of Hb-D trait in Khatri was 3.1% compared to 0.5 % in other Hindus.⁵

There are no detailed reports on the clinical and hematological profile of Hb D except a few published case reports.⁶ This is the first large systematic study of Hb D trait patients in rural India. Apart from mild anaemia in few cases, rest of our patients of Hb D trait is asymptomatic. In a small study, six out of the 30 patients of Hb D trait were clinically symptomatic and presented with anaemia, jaundice, pallor, weaknesses and the disease behaviour was like cases of thalassemia intermedia.⁷

MCV >80 fl was seen in 81.36%, MCH >28 pg was in 5.60% and normal RDW-CV was seen in all cases of Hb D trait. However, in a study of 11 patients of Hb D trait showed normal clinical and blood count parameters.⁸

On HPLC, Hb A₀ was always more than 50% of total hemoglobin but Hb D was within 40% of total hemoglobin and is seen in 99.37% of cases in our study population.

α -thalassemia and iron deficiency anaemia were excluded in cases where marked microcytosis was observed. In few cases, low value of Hb D was observed. In such atypical cases further investigations may be required to exclude association of other hemoglobinopathies.

In heterozygous state, hemoglobin D is somewhat less than 50% of total hemoglobin as described by Bain¹ and even coexisting α -thalassemia may reduce the percentage of the variant.⁹

The thalassemia carrier rate in WB is high and varies from area to area.² Knowledge of local prevalence of Hb D trait will help in planning and implementing appropriate screening programs. The emphasis is to be given on health education and awareness programs in communities clearly explaining the consequences of marriage between two carriers.

Conclusions

Large number of samples was screened for hemoglobinopathies from June 2010 to July 2013. Prevalence rates of Hb D trait show regional variations. The incidence of Hb D trait in rural areas of WB is much lower than Punjab. Hb D trait is one of the important differential diagnoses in patients who present with persistent hypochromic anemia. In these contexts, we suggest both red cell indices and HPLC are sufficient for screening of Hb D Punjab trait in resource poor setting.

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