

Screening of β -thalassemia trait and other hemoglobinopathies among blood donors in Punjab

Abstract

Rajesh Kumar,
Sonia Gupta,
Aikaj Jindal,
Shruti Kakkar¹,
Amarjit Kaur

Department of Immuno-
Hematology and Blood
Transfusion, ¹Paediatrics,
Dayanand Medical College and
Hospital, Ludhiana, Punjab, India

Address for the Correspondence:

Dr. Rajesh Kumar,
9942/1, Joshi Nagar,
Ludhiana - 141 001, Punjab, India.
parul_pulkit@yahoo.co.in

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Introduction: Hemoglobinopathies are common genetic disorders of hemoglobin, which can be prevented by population screening and offering genetic counseling. The cumulative gene frequency of hemoglobinopathies in India is 4.2%. The carrier state for β -thalassemia in India varies from 1% to 17% with an average of 3.2%. The present study was undertaken to find out the burden of hemoglobinopathies and spectrum of this disorders among the blood donors. **Materials and Methods:** The study includes 975 students between 18 and 25 years who donated blood, were screened for β -thalassemia trait and related hemoglobinopathies by high-performance liquid chromatography (HPLC) using Bio-Rad variant. Samples were also run on a hemato analyzer for red cell indices and peripheral smear for red cell morphology. **Results:** A total of 41 donors showed abnormal hemoglobin fractions in HPLC. Out of these, 32 (3.3%) were diagnosed with β -thalassemia trait, 8 (0.8%) with Hb-D Punjab and 1 (0.1%) with Hb-S trait. The frequency of β -thalassemia trait in the student from different geographical regions varied from 0.8% to 4.44%, being the highest in those from Punjab and frequency of β -thalassemia trait in different caste groups varied from 0 to 4.74%, being the highest in the student from Jatt Sikh community. **Conclusions:** A universal approach of screening for β -thalassemia trait should be included as a part of standard blood testing among the college students, premarital and of the extended family of thalassemics. Population group with high gene frequencies requires screening programs as well as increased awareness and education program to control the birth of thalassemia major.

Key words: Blood donor, hemoglobinopathies, high performance liquid chromatography, β -thalassemia trait

INTRODUCTION

Hemoglobinopathies are common genetic disorders of hemoglobin in which there is an abnormal production or structure of the hemoglobin molecule. These hereditary disorders are major public health problem in many parts of the world including South East Asia like India. The clinical spectrum of these disorders varies from asymptomatic conditions to serious disorders like thalassemia major that requires regular blood transfusions and widespread medical care.^[1,2] World Health Organization (WHO) figures estimate that 7% of the world population is carrier for hemoglobin disorders.^[3] The cumulative gene frequency of hemoglobinopathies in India is 4.2% with a population of over 1.2 billion and over 12,000 infants born each year with a clinically significant hemoglobinopathies.^[4,5] The carrier state for β -thalassemia in India varies from 1% to 17% with an average of 3.2%.^[6,7] Hemoglobin E- β -thalassemia and Hb-S (sickle cell anemia) are also a very common problem with high frequency in South East Asia and have been reported from different parts of India.^[8,9] Hemoglobin D-Punjab is one of the most commonly encountered abnormal hemoglobins worldwide.^[10] It is present in a large number of people in Pakistan and North-West India and has a high frequency in Punjab with an incidence of 2-3%.^[11,12]

India is a vast country with considerable regional and ethnic heterogeneity; various studies are being conducted in evaluating the prevalence of thalassemia trait and hemoglobinopathies in various

regions. Patients homozygous for β -thalassemia usually present with symptoms of the disease, whereas carriers for β -thalassemia trait can have varying degrees of anemia while some of them may have no symptoms. They are usually detected during examination of the relatives of severely affected patients as part of screening programs or during the investigation of mild iron refractory hypochromic anemia.^[13]

Accurate and timely screening of various Hb variants including β -thalassemia traits before marriages of couples at risk and prenatal diagnosis can prevent the occurrence of more serious disorders like thalassemia major in newborns substantially.^[14] Prospective prevention through population screening and genetic counseling is the best possible strategy in the prevention of these disorders. As the exact data pertaining to the prevalence of hemoglobinopathies in this region is scarce, we consider that it is important to find out the extent of burden of hemoglobinopathies in this region.

The aim of study was to find out the prevalence of β -thalassemia trait and other hemoglobinopathies in blood donors.

MATERIALS AND METHODS

The present study attempted to find out the prevalence of β -thalassemia trait and other hemoglobinopathies in the blood donor population screened during the period from September 2013 to March 2014 in the Department of Immunohematology and Blood Transfusion. An attempt was made to spread the awareness about importance of thalassemia trait testing before marriage among donors. A total of 975 students of Punjab Agriculture University and Guru Angad Dev Veterinary and Animal Science University, with age group ranging from 18 to 25 years who donated blood were included in the study and screened for various hemoglobinopathies. All the blood donors were from the different geographical region like Punjab, Haryana, Himchal Pradesh and Jammu Kashmir. Samples were run on LH 750 analyzer (Beckman Coulter) for red Cell indices, and peripheral smear for red cell morphology were analyzed. The samples having mean corpuscular volume (MCV) ≤ 76 fl and mean corpuscular hemoglobin (MCH) ≤ 27 pg were further quantified for HbA₂ using Bio-Rad "Variant," USA that utilized the principal of high-performance liquid chromatography (HPLC). It is a sensitive and precise method for quantification of HbA₂, HbF and abnormal hemoglobin fraction.^[15]

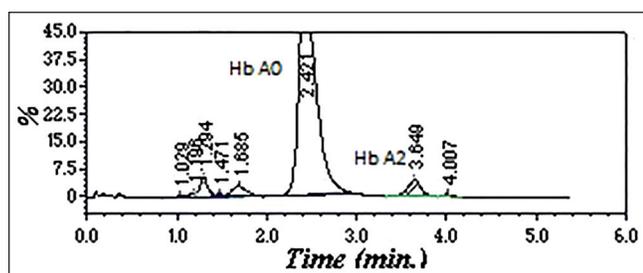


Figure 1: Chromatogram showing beta-thalassemia trait

RESULTS

A total of 975 blood donors were screened for β -thalassemia trait and other hemoglobinopathies. It included 830 males and 145 females with ages between 18 and 25 years. The mean age was 22.5 year and male-female ratio is 5.7:1. Of these, 41 (4.2%) donors showed abnormal hemoglobin fraction in HPLC. The major abnormalities observed were of high Hb-A₂ level and a cut-off of 3.5% was taken for diagnosis of b-thalassemia trait. A total of 32 donors (3.3%) were diagnosed with b-thalassemia trait. It was found that the majority of blood donors had Hb 12.5 g% or above including those who tested positive for hemoglobinopathies, but Hb-F levels are within normal limits with the variable reduction in Hb-A₁ [Figure 1]. MCV and MCH were below normal limits with raised RBCs count and serum ferritin levels are within normal limits. Peripheral blood film (PBF) showed mild anisopoikilocytosis and microcytic hypochromic blood picture. Serum ferritin and red cell indices of all the remaining blood donors were within the normal limits.

Eight (0.8%) donors showed the presence of Hb-D Punjab trait on HPLC displayed a D window with variant percentage ranging from 30% to 33%. The blood parameters were essentially normal in these donors [Figure 2]. One donor had high Hb-A₂ along with slightly increased Hb-S, blood picture showed target cells and was diagnosed as Hb-S trait. The diagnosis of Hb-D Punjab or Hb-S trait was not confirmed by molecular testing owing to high cost.

Prevalence of b-thalassemia trait and other hemoglobinopathies (Hb-D Punjab, Hb-S trait) are shown in Table 1. Majority of donors screened were from Punjab (383), Haryana (298), Himachal (175) and Jammu and Kashmir (119). The frequency of the b-thalassemia trait in donors of different states is 0.8% to 4.44%, being highest (4.44%) in Punjab as shown in Table 2. Students from castes group showed the presence of β -thalassemia trait with a frequency ranging from 0% to 4.74%, being highest (4.74%) in Jatt Sikh as shown in Table 3.

DISCUSSION

HPLC is considered as one of the best methods for screening and detection of various hemoglobinopathies with rapid, reproducible and precise results. It is recommended for detection of β -thalassemia trait in population and necessary for genetic counseling to reduce the incidence and burden of thalassemia major in the society.^[16,17] The present study included predominately students of two universities

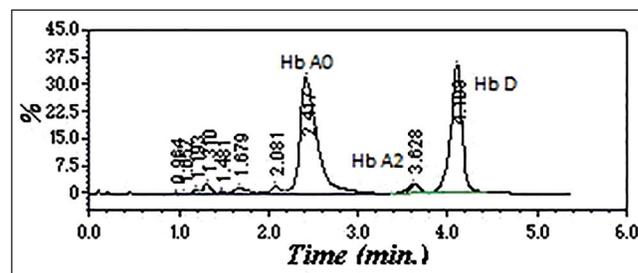


Figure 2: Chromatogram showing Hb D-Punjab

Table 1: Prevalence of β -thalassemia trait and related hemoglobinopathies in blood donors

Hemoglobinopathies	Donors screened (Total)	No. of positive donors	Percentage (Total)
β -thalassemia trait	975	32	3.3
Hb-D Punjab	975	8	0.8
Hb-S Trait	975	1	0.1

Table 2: Frequency of β -thalassemia trait in different geographical regions

State	Donors screened (Total)	Donors with β -thalassemia trait	Percentage (Total)
Punjab	383	17	4.44
Haryana	298	8	2.68
Himachal Pradesh	175	5	2.86
Jammu and Kashmir	119	1	0.8

Table 3: Frequency of β -thalassemia trait in donors of different caste groups

Caste group	Donors screened (Total)	Donors with β -thalassemia trait	Percentage (Total)
Jatt Sikh	295	14	4.74
Jain	125	4	3.2
Brahmin	149	3	2
Baniya	120	4	3.3
Khatri	136	3	2.2
Jaat	120	3	2.5
Kayastha	30	0	0

who donated blood in voluntary blood donation camps; they were considered to be fair representative of all sections of the population and also comprised of that group of population which could be easily accessed for further investigation and counseling. There was in total 4.2% hemoglobin variant detected, β -thalassemia trait formed the largest subgroup of abnormal hemoglobin (3.3%) followed by Hb-D Punjab (0.8%) and Hb-S trait (0.1%). Majority of the donors were males (85.12%) and low percentage of female donors could be due to low weight and anemia that barred them from donating blood. β -thalassemia trait is probably the most common inherited hemoglobin disorder in the Indian sub-continent. This study on students established that β -thalassemia trait is the most frequent hemoglobinopathies of clinical importance although it varies considerably in the region and in different caste groups. The incidence was higher in Punjab being 4.44% in our study as compared to 6.75% in West Bengal,^[18] 5.5% in Delhi,^[19] 2.8% in Eastern Uttar Pradesh^[20] and 2.7% in Mumbai.^[19] Prevalence rate of β -thalassemia trait in different caste groups varied from 0% to 4.74% in our study. It was 4.74% in Jatt Sikh, 3.3% in Baniya, 3.2% in Jain, 2.5% in Jatt, 2.2% in Khatri, and 2.1% in Brahmin. Similar results have reported a prevalence of β -thalassemia trait in different caste group as 2.7% in Baniya, 4.8% in Jain, 3.8% in Khatri and 5.3% in Brahmin.^[19] In the neighboring country of Pakistan, the frequency of β -thalassemia trait has been reported to be 5.6% similar to our

results with a largely Punjabi population.^[21] Hb-D Punjab (0.8%) was seen in students which were Punjabi as compared to 0.08% in Eastern Uttar Pradesh,^[22] 0.7% and 1.1% in Delhi and Mumbai, respectively.^[19] Hb-S trait (0.1%) was seen in a student who belonged to Jammu Kashmir probably due to the greater influx of population from tribal area and present 0.08%, 0.2% and 0% in Eastern Uttar Pradesh,^[22] Mumbai and Delhi.^[19] Hb-S is more frequently observed in tribal population, Hb-E in the eastern region, Hb-D in Punjab and β -thalassemia trait to varying degree in almost all the population groups. However, with the migration of population across the country often for the purpose of employment, likely to be cause of the presence of unexpected hemoglobin in the population.^[23,24]

Using the overall carrier frequency of β IT of 3.3% found in this study, the birth incidence of thalassemia can be calculated using the Hardy-Weinberg equation for recessively inherited single gene disorders. With current estimates of the Indian population being approximately 1.25 billion and a birth rate of 23/1000, the approximate estimate of homozygous births would be 10,879 per year. This gives a fairly accurate assessment of the thalassaemic load. The observed incidence in students of different regions and caste groups will permit National Programs for Thalassemia to be planned and carried out with greater precision and assurance. Several Mediterranean and western countries have achieved a significant change in the homozygote population since last two decades after the implementation of Thalassemia Control Programs.^[25] However, few antenatal screening programmes are going on but more centre are require to screened the population with follow-up to reduce the birth of β -thalassemia major.

A universal approach of screening of college students, premarital and of the extended family of thalassaemic along with antenatal diagnosis needs to be considered for this vast and ethnically diverse country. Education and awareness regarding thalassemia need to be accelerated urgently among medical practitioners, paramedics, thalassaemic and general population to reduce the morbidity and mortality and the financial and socio-psychological burden of the thalassaemic families. Extended family screening of thalassaemic allows identification of large majority of population at risk by screening only 13% of the population.^[26] It has been estimated that the lifetime cost of healthcare, premature mortality and lost earnings versus a national screening program including antenatal diagnosis in Israel^[27] gives a cost-benefit ratio of 4.2:1 and adding a societal perspective 6.01:1. A recent report from Hong Kong^[28] offers an almost similar cost — benefit ratio.

To conclude, our study had 3.3% β -thalassemia trait and early detection of traits will prevent the occurrence of thalassemia major in offsprings. Detection of other variants becomes important due to complex interactions in cases with double heterozygous and homozygous states, which may lead to severe hematological abnormalities. Findings must be supplemented with hemogram findings, family/sibling studies, other confirmatory techniques and molecular studies based on HPLC findings. This is especially important in view of high incidence of β -thalassemia trait in the Indian subcontinent. Screening for

thalassemia trait should be included as part of a standard blood testing before blood donation.

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