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Screening of hemoglobinopathies in blood donors (A study of 800 cases)

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Abstract

Introduction: Haemoglobinopathies are common genetic disorders of haemoglobin in which there is an abnormal production or structure of the haemoglobin molecule. These hereditary disorders are major public health problems in many parts of the world including South East Asia like India. Prospective prevention through population screening and genetic counselling is the best possible strategy in the prevention of these disorders. 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.

Aim and Objectives: The aim of study is to find out the prevalence of β-thalassemia trait and other haemoglobinopathies in blood donors using the principle of High Performance Liquid Chromatography (HPLC). HPLC is considered as one of the best methods for screening and confirmation of various haemoglobinopathies with rapid, precise and reproducible results.

Material and Method: The study has been carried out as "Sample bound study" to find the pattern of *β*-Thalassemia and other Haemoglobinopathies in 800 blood donors, from April 2016 to November2016.The samples were then processed for sickle solubility test, HPLC and Hb electrophoresis for hemoglobinopathies in central clinical laboratory.

Result and Conclusion: In present study, out of 800 blood donors 25 were found to have β thalassemia or other haemoglobinopathy. Among 25 donors with haemoglobinopathies, 16 (61.64%) were having β-thalassemia which was most common followed by sickle cell trait (26.92%) and Hb D Punjab trait(11.54%).

Keywords: Hemoglobin, sickle cell trait, thalassemia minor, Hb D Punjab.

1. Introduction

Haemoglobinopathies are common genetic disorders of haemoglobin in which there is an abnormal production or structure of the haemoglobin molecule. 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.

World Health Organization (WHO) figures estimate that 7% of the world population is carrier for haemoglobin disorders.[1] The cumulative risk of gene frequency of haemoglobinopathies in India is 4.2% with a population of over 1.2 billion and over 12,000 infants born each year with clinically significant haemoglobinopathies. [2,3] The carrier state for β-thalassemia in India varies from 1% to 17% with an average of 3.2%.[4,5] Various studies are being conducted in evaluating the prevalence of thalassemia trait and haemoglobinopathies in various regions. Overall prevalence of β-Thalassemia Trait and Sickle cell trait in South Gujarat is 4.4% and 1.3% respectively. [6]

Patients homozygous for β-thalassemia usually present with symptoms of the disease, whereas carriers for β-thalassemia trait can have varying degrees of anaemia 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 investigations for mild anaemia. [7]

In the present study, donors who have donated blood in Blood bank, SSGH as well as blood donation

camps hosted by our Blood Bank are included. They are 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 investigations and counselling.

1.1 Aims and objectives

To find out the prevalence of β-thalassemia trait and other haemoglobinopathies in the blood donor population screened in the Department of Immunohematology and Blood Transfusion.

To spread the awareness about importance of thalassemia trait testing before marriage among donors.

To advice further studies to evaluate the effects of blood transfusions if prevalence of haemoglobinopathies is significant in blood donors.

2. Material & methods

The study has been carried out as "Sample bound study" to find the pattern of *β*-Thalassemia and other Haemoglobinopathies in 800 blood donors, from April 2016 to November 2016.

2.1 Inclusion criteria

All samples of blood donors who are declared fit to donate blood and donated blood in blood bank, S.S.G. Hospital or in blood donation camps hosted by the same.

2.2 Exclusion criteria

Inadequate sample, Clotted Blood, Haemolysed blood, Improper labelling

Screening for Haemoglobin was done by specific gravity method using $CuSO_4$ in blood bank. The samples were collected in EDTA vacuette. The samples were then processed for sickle solubility test, HPLC and Hb electrophoresis for hemoglobinopathies in central clinical laboratory.

3. Results and observations

Present study included investigations of blood samples of blood donors who have donated blood in Blood Bank, S.S.G. Hospital, Vadodara.

Table 1: Sex distribution (n=800)

Sex	Individual (%) (n=800)
Male	784 (98%)
Female	016 (2%)

In present study, 98% of the donors were male and only 2% of donors were female.

Sex distribution

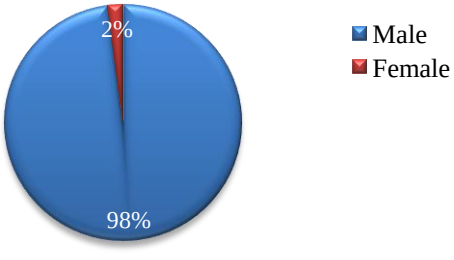

Figure 1: sex distribution

Table 2: Age distribution (n=800)

Age	Individual (%)
18-20	095 (11.88%)
21-30	354 (44.25%)
31-40	236 (29.50%)
>40	115 (14.37%)

In present study most of the individuals were in age group of 21-30 years (44.25%) followed by 29.5% in 21-30 years, 14.37% in more than 40 years of age and 11.88% in 18-20 years of age group. (Table 1)

Age distribution(approximate)

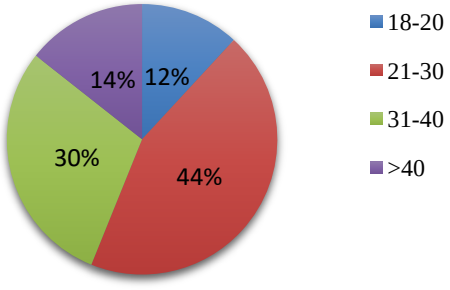

Figure 2: Age distribution

Table 3: Blood group distribution in blood donors (n=800)

Blood group	Individuals (%)
A+	195 (23.78%)
B+	253 (31.62%)
O+	250 (31.25%)
AB+	057 (07.13%)
A-	009 (01.13%)
B-	022 (02.75%)
O-	014 (01.75%)
AB-	000 (00.00%)

In present study among blood groups, B positive was commonest (31.62 %) followed by O positive (31.25%) (Table 3).

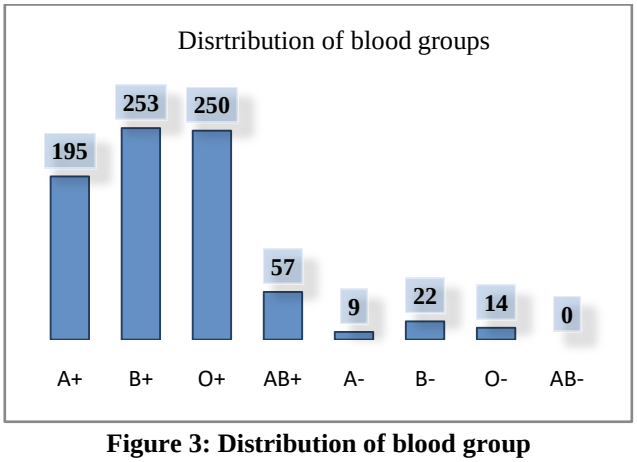

Figure 3: Distribution of blood group

Table 4: Approach to diagnosis of hemoglobinopathies with sickle solubility test, HPLC and Hb electrophoresis (n=800)

Sickle solubility test		Elusion pattern of HPLC	Elusion pattern of electrophoresis	Final diagnosis	Individual (%)
Positive	Negative				
00	774	A_0(774)	-	Normal	774 (96.75%)
07	000	A_0,S(7)	AS(7)	Sickle cell trait	007 (0.89%)
00	016	A_0,A_2(16)	A(16)	β Thalassemia Trait	016 (2.00%)
00	003	A_0,D(3)	AS(3)	Hb D Punjab trait	003 (0.38%)

Out of 800 samples 7 were positive for sickle solubility test. They were diagnosed as sickle cell trait using HPLC and haemoglobin electrophoresis (table 4). Out of 793 sickle solubility test negative samples 16 were having A_2window more than 4% and were diagnosed as β Thalassemia trait. Among 793 sickle solubility test negative samples, 3 samples showed D window in HPLC and diagnosed as HbD trait.

Bio-Rad CDM System **PATIENT REPORT**
Pathology dept, Medical College and SSG Hospital **V2_BThal**

Patient Data
Sample ID: BB 4676
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 04/05/2016 12:41:20
Injection Number: 6816R
Run Number: 685
Rack ID: 0009
Tube Number: 3
Report Generated: 28/07/2016 08:52:08
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.0	0.96	1062
F	0.3	---	1.09	6870
Unknown	---	0.7	1.26	19852
P2	---	2.3	1.36	65431
P3	---	2.7	1.78	75599
Unknown	---	2.3	2.12	64631
Ao	---	50.9	2.51	1440730
A2	2.3	---	3.66	72772
D-window	---	38.3	4.13	1083399

Total Area: 2,830,346

F Concentration = 0.3 %
A2 Concentration = 2.3 %

Analysis comments:

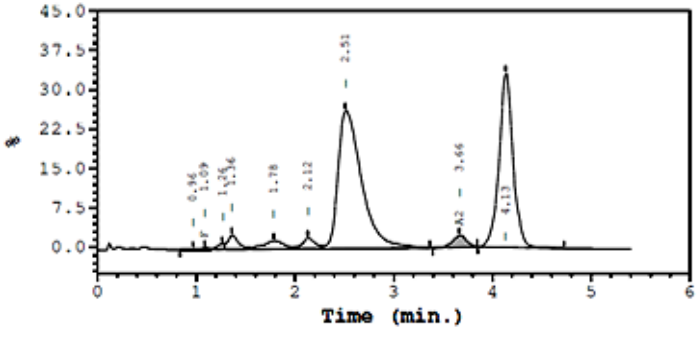

Figure 5: HPLC report of HbD Punjab trait

Bio-Rad CDM System **PATIENT REPORT**
Pathology dept, Medical College and SSG Hospital **V2_BThal**

Patient Data		**Analysis Data**	
Sample ID:	**BB 4700**	Analysis Performed:	04/05/2016 17:25:48
Patient ID:		Injection Number:	6840R
Name:		Run Number:	686
Physician:		Rack ID:	0009
Sex:		Tube Number:	9
DOB:		Report Generated:	28/07/2016 08:54:47
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.60	2233
F	0.5	---	1.10	13661
Unknown	---	1.5	1.25	45493
P2	---	7.0	1.37	211437
P3	---	4.2	1.80	128307
Unknown	---	1.2	2.14	36446
Ao	---	48.9	2.54	1479102
A2	3.4*	---	3.69	114855
S-window	---	32.9	4.39	995019

Total Area: 3,026,553*

F Concentration = 0.5 %
A2 Concentration = 3.4* %

*Values outside of expected ranges

Analysis comments:

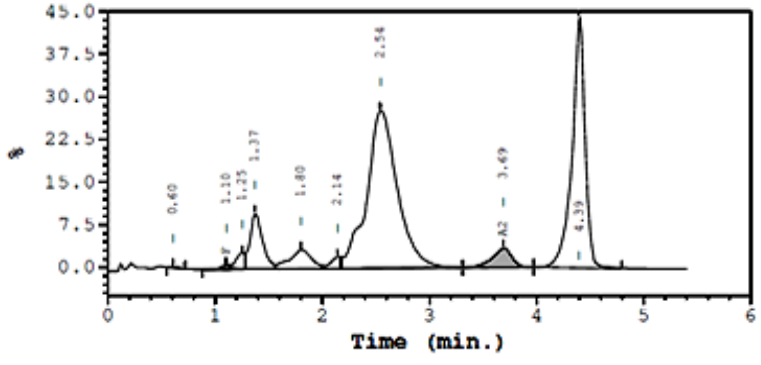

Figure 6: HPLC report of sickle cell trait.

Bio-Rad CDM System **PATIENT REPORT**
Pathology dept, Medical College and SSG Hospital **V2_BThal**

Patient Data
Sample ID: 4858
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 04/06/2016 03:51:21
Injection Number: 7386R
Run Number: 726
Rack ID: 0009
Tube Number: 7
Report Generated: 13/10/2016 18:58:35
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.0	0.97	1121
F	0.4	---	1.07	8689
Unknown	---	1.1	1.23	26844
P2	---	4.3	1.35	106766
P3	---	4.2	1.79	103143
Unknown	---	0.2	2.14	4926
Ao	---	83.6	2.56	2069427
A2	5.2*	---	3.70	153149

Total Area: 2,474,065

F Concentration = 0.4 %
A2 Concentration = 5.2* %

*Values outside of expected ranges

Analysis comments:

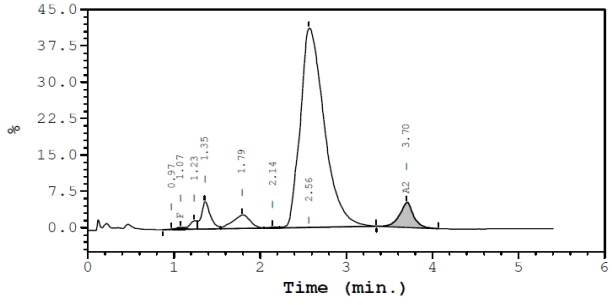

Figure 7: HPLC report of β thalassemia minor.

Table 5: Distribution of haemoglobinopathies in present study (n=26)

Haemoglobinopathy	Individual	Prevalence (n=800)	Proportion of total haemoglobinopathies (n=26)
Sickle cell trait	07	0.89%	26.92%
β Thalassemia Trait	16	2.00%	61.64%
Hb D Punjab trait	03	0.38%	11.54%

In present study, out of 800 blood donors 25 were found to have β thalassemia or other haemoglobinopathy. Among 25 donors with haemoglobinopathies, 16 (61.64%) were having β-thalassemia which was most common followed by sickle cell trait (26.92%) and Hb D Punjab trait(11.54%).

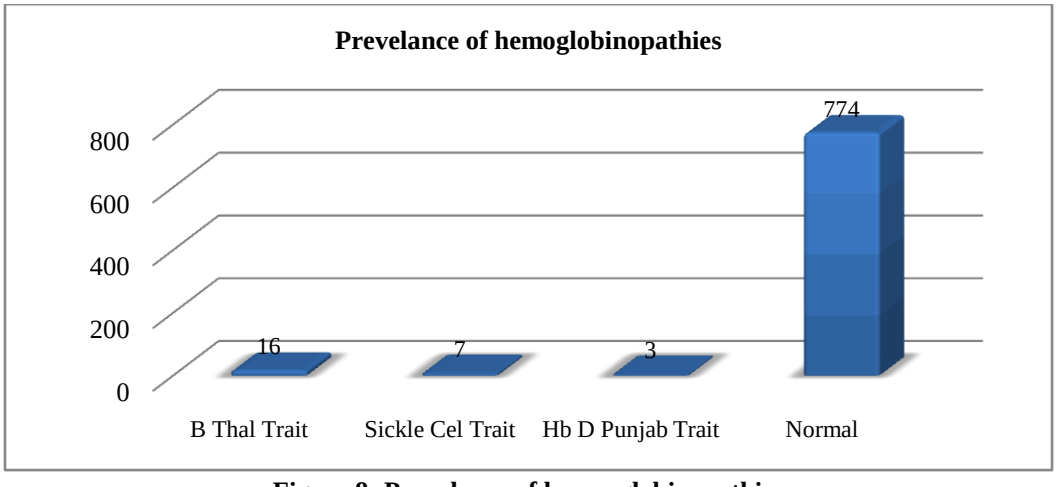

Figure 8: Prevalence of haemoglobinopathies

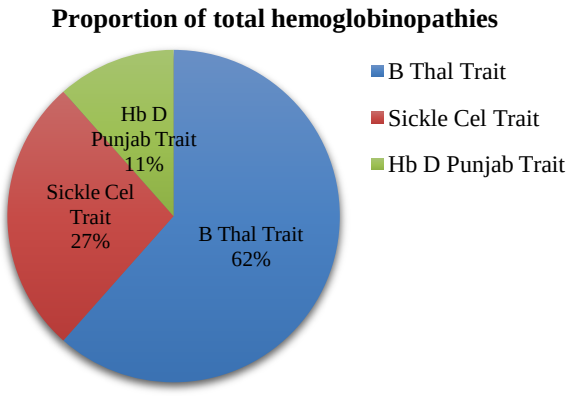

Figure 9: Proportion of total haemoglobinopathies

Table 6: Blood group among β Thalassemia trait positives (n=16)

Blood group	β Thalassemia trait (n=16)
A+	02 (12.5%)
B+	04 (8.7%)
O+	06 (37.5%)
AB+	01 (6.3%)
B-	01 (6.3%)
O-	02 (12.5%)

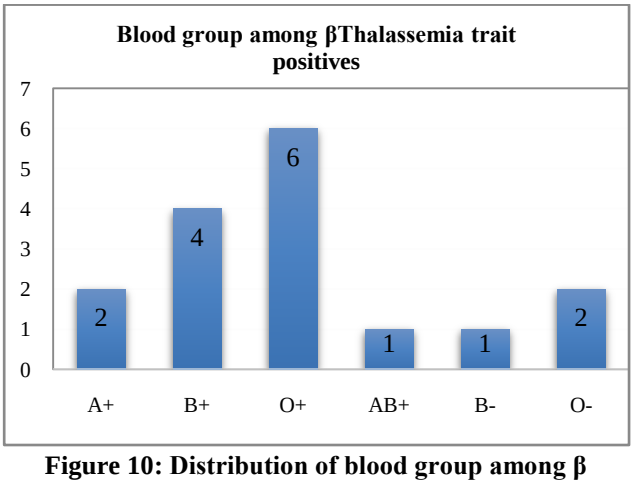

Figure 10: Distribution of blood group among β thalassemia Trait

Analysis:

In present study, following points were observed:

1. Male and female ratio was 98% and 2% respectively (Table 1).
2. Most of the individuals were in age group of 21-30 yrs. (Table 2).
3. Among the 800 blood donors 26 (3.26%) found positive for haemoglobinopathies. Out of these 26, β Thalassemia was most common (61.64%) followed by sickle cells trait (26.92%) and Hb D Punjab trait (11.54%). (Table 5).

4. Discussion

HPLC (high performance liquid chromatography) is considered as one of the best methods for screening and detection of various haemoglobinopathies 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. [8,9]

Majority of donors were males (98%). Low number of female donors could be because of local social factors and physical health factors like anemia barring them from blood donation (Table 1). Majority of blood donors under this study were in reproductive age group (21-30 years) of life (Table 2). However, since this study has included only blood donors (need to be adult as per law), age distribution may not be true representation. But this study concludes the importance of screening for haemoglobinopathies among so called healthy blood donors.

Among the blood groups, B positive was commonest (31.62 %) followed by O positive (31.25%) and is the usual population concentration of groups in this part of the country (Table 3). Distribution of blood groups is comparable to the study done by Meena *et al* in Uttar Pradesh which was also showing B positive and O positive as most common blood groups (Table 7).

Table 7: Comparison blood group distribution of present study with other similar study

Blood group	Meena *et al*. (2012)	Present study
O+	448 (37.33%)	250 (31.25%)
A+	97 (8.08%)	195 (23.78%)
B+	529 (44.08%)	253 (31.62%)
AB+	29 (2.42%)	57 (7.13%)
A-	34 (2.83%)	9 (1.13%)
B-	42 (3.50%)	22 (2.75%)
O-	17 (1.42%)	14 (1.75%)
AB-	04 (0.33%)	0 (0%)

Among 800 blood donors 26 (3.27%) tested positive for β thalassemia trait and other haemoglobinopathies. Among this 26 β thalassemia trait was most common (61.64%) followed by sickle cell trait (26.92%) and Hb D Punjab (11.54%). This study is similar to the study done in Uttar Pradesh by Meena, *et al* and in Punjab by Kumar, *et al* in regards of percentage (Table 8).[10,11]

Table 8: Comparison of present study with other similar studies

Haemoglobinopathy	Meena, *et al*[10]	Kumar *et al*[11]	Present study
B Thalassemia trait	12 (1.0%)	32 (3.3%)	16 (2.0%)
Sickle cell trait	01 (0.08%)	01 (0.1%)	07 (0.9%)
Hb D Punjab Trait	01 (0.08%)	08 (0.8%)	03 (0.4%)
	1200	975	800

Thus, present study is correlated well with other studies in terms of prevalence of β thalassemia trait. However, prevalence of sickle cell trait was seen higher in present study and prevalence of HB D Punjab was higher in study done by Kumar *et al* in Punjab. This indicates variation in geographic distribution of different ethnic population.

WHO Working Group recommends the population survey of the prevalence of haemoglobinopathies and thalassemia, because it allows the identification of heterozygous and homozygous individuals. Moreover, these programs also have a responsibility to clarify and educate patients.[12] These people should have the knowledge of their genetic condition, regarding high risk of bearing children with β thalassemia and other severe conditions.

5. Conclusion

To conclude, our study had prevalence of 3.25% of thalassemia and other haemoglobinopathies and early detection of these traits will prevent the occurrence of thalassemia major and other severe haematological diseases in offsprings. Detection of other variants is important due to complex interactions in case with double heterozygous and homozygous states, which may lead to severe haematological abnormalities.

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