Prevalence Of Haemoglobinopathies In Gujarat, India: A Cross-Sectional Study


Citation

Abstract
Various haemoglobinopathies are major public health problem in Gujarat, a state located in the western part of India. The data pertaining to their occurrence and prevalence in the state of Gujarat are scarce and hence it was considered worthwhile to study the burden of haemoglobinopathies in Gujarat, India. A retrospective analysis of blood samples of 428 cases referred to the pathology laboratory from various private practitioners/Government hospitals for the workup of anemia or other blood related disorders was done by Bio-Rad D-10 instrument. 153 (35.7%) patients out of 428 had haemoglobinopathies. Thalassaemia minor (70 cases, 16.35 %), thalassaemia major (32 cases, 7.48%), sickle cell disease (22 cases, 5.14 %) and sickle cell trait (12 cases, 2.8%) were most common haemoglobinopathies. Less prevalent haemoglobinopathies were sickle-β-thalassaemia, α,β-thalassaemia heterozygote, Hb D trait, Hb E trait, Hb E- thalassaemia, Hb D disease, Hb E disease and sickle D disease. Our study indicates that almost all the common haemoglobinopathies are prevalent in Gujarat but sickle cell trait/anemia and β-thalassaemia are very common.

INTRODUCTION
Haemoglobinopathies are a group of genetic disorders of haemoglobin (Hb). Haemoglobin is a complex molecule contained within erythrocytes that binds to and transports oxygen and carbon dioxide in the body. Defects in genes of haemoglobin can produce abnormal haemoglobins and anemia, which leads to conditions, termed as “haemoglobinopathies”. Abnormal haemoglobins appear in one of two basic circumstances: decreased production of one of the globin chain e.g. thalassaemia, abnormal globin chain e.g. sickle cell disease 1,2,3.

β-thalassaemia is a heterogeneous group of inherited disorder of haemoglobin synthesis, characterized by a reduction of (β * ) or absence (β ) of synthesis of beta globin chains of haemoglobin. This results in an imbalanced chain synthesis, which determines the severity of the disease 4.

These hereditary disorders of haemoglobin pose a massive health problem in many countries including India 5. The distribution of specific disorders varies geographically and by community 6. WHO figures estimate that 5 % of the world population is carrier for haemoglobin disorders 7. They cause moderate to severe hemolytic anemia leading to high degree of morbidity and mortality. The frequency of β-thalassaemia in India ranges from 3.5 to 15 % in general population. Every year 10,000 children with thalassaemia major are born in India, which constitutes 10 % of the total numbers in the world 8. The overall β gene deletion frequency is 0.05 to 0.98 % but it is very high in India 9. In west central Gujarat, it is as high as 95 % 6.
Prevalence Of Haemoglobinopathies In Gujarat, India: A Cross-Sectional Study

The average frequency of haemoglobin S (Hb S) is 4.3 % in India. The range varies from 0-44 %. It is 0-18.5% in northeast zone, 0-33.5 % in west zone, 22.5-44.4 % in the central zone and 1-40 % in the southern zone. Sickle gene in India is mostly found amongst Dravidian and predravidian tribes. Haemoglobin E (Hb E) is mostly present in the northeastern states of India. Frequency of Hb E in Assam is 52 %, 7 % in Manipur and 3.33% in West Bengal. Hb E has also been documented in people from orissa, uttarpradesh, rajasthan, Bihar and Punjab.

The frequency of Haemoglobin D (Hb D) has been reported to be 0.5 to 3.1% in different castes of Uttar Pradesh. Hb D has also been reported from Bengal, Bihar, South India and Gujarat.

The main objective of the study was to know the prevalence of haemoglobinopathies in the state of Gujarat, located in the western part of India and to review various strategies that could be implemented for the effective control and prevention of these disorders.

MATERIALS AND METHODS

The present cross-sectional retrospective study included 428 patients referred for screening of haemoglobin disorders from September 2005 to April 2006 at Green Cross Pathology and RIA Laboratory, Ahmedabad - a reference laboratory which received various samples for testing and diagnosis from many small laboratories and clinicians, from all over Gujarat. Hence, the blood samples were collected from the patients who visited Green Cross Laboratory or alternatively the samples were collected and sent by other pathology laboratory/clinicians from Civil Hospital, Ahmedabad; Shingala Laboratories, Jamnagar; Guru Pathology Laboratory, Palanpur etc. for testing of parameters like hemogram, peripheral smear, haemoglobin analysis by HPLC (Bio-rad D-10, Bio-Rad Laboratories, USA) and sickling test. Hemograms were done on automated 5-part differential cell counter (cell dyne, Abbott Laboratories, USA). Haemoglobin analysis was done on Bio-rad D-10 using β-thalassaemia dual program. Haemoglobin analysis by Bio-rad D-10 is based on the principle of High Performance Liquid Chromatography (HPLC). Clinical history and physical findings were recorded as provided by the referring physician.

The peripheral smear was stained with Leishman's stain (Merck, India). Grading of hypochromia, anisocytosis, microcytosis, macrocytosis and polychromasia was done according to the standard criterion. Inclusion bodies (basophilic stippling), sickle cells, target cells, nucleated red cells, spherocytes and schizocytes were noted in peripheral smear, when seen.

RESULTS

153 patients out of 428 cases studied had haemoglobinopathy. The patients ≤ 18 years of age were considered as pediatric patients. An Age and Sex wise distribution of patients with haemoglobinopathies is described in Table I.

Majority of the patients studied were Gujarati (native residents of the state of Gujarat) by origin. However, our study consisted mainly of hospital based case reports, which cannot be regarded as representative of a community or population. Majority of the patients had β-thalassaemia and sickle cell disease/trait. Five patients of sickle cell disease were diagnosed in adulthood although they were symptomatic from childhood.

Figure 1

Table I: Age and Sexwise distribution of Patients with different haemoglobinopathies

<table>
<thead>
<tr>
<th>Haemoglobinopathies</th>
<th>Pediatric</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Male</td>
</tr>
<tr>
<td>Thalassaemia Major</td>
<td>29</td>
<td>20(4.7%)</td>
</tr>
<tr>
<td>Thalassaemia Minor</td>
<td>22</td>
<td>15(3.5%)</td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>17</td>
<td>14(3.2%)</td>
</tr>
<tr>
<td>Sickle cell trait</td>
<td>3</td>
<td>3(0.7%)</td>
</tr>
<tr>
<td>Hb D disease</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hb D trait</td>
<td>1</td>
<td>0(0.2%)</td>
</tr>
<tr>
<td>Hb E trait</td>
<td>1</td>
<td>0(0.2%)</td>
</tr>
<tr>
<td>Hb E disease</td>
<td>1</td>
<td>0(0.2%)</td>
</tr>
<tr>
<td>Hb E-thalassaemia</td>
<td>2</td>
<td>1(0.3%)</td>
</tr>
<tr>
<td>Sickle thalassaemia</td>
<td>2</td>
<td>2(0.4%)</td>
</tr>
<tr>
<td>β-thalassaemia</td>
<td>2</td>
<td>1(0.3%)</td>
</tr>
<tr>
<td>Hb D-thalassaemia</td>
<td>1</td>
<td>0(0.2%)</td>
</tr>
<tr>
<td>Sickle D disease</td>
<td>1</td>
<td>0(0.2%)</td>
</tr>
</tbody>
</table>

Total No. of Patients: 153 (35.75%)

(No. of Patients with haemoglobinopathies = 153)

(Number of Patients in the parenthesis indicate the observed frequency (in %) of various disorders in the total numbers of samples studied (N=428)

13 patients out of 32 patients of thalassaemia major had
received blood transfusions before the investigations. Nearly 25.01\% patients were diagnosed late as shown in Table II (15.63\% between 3-10 years and 9.38 \% > 10 years).

**Figure 2**
Table II: Age wise Distribution of Patients with Thalassaemia major (n=32)

<table>
<thead>
<tr>
<th>Age</th>
<th>% of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 3 years</td>
<td>75.0%</td>
</tr>
<tr>
<td>3 - 10 years</td>
<td>15.65 %</td>
</tr>
<tr>
<td>11 - 20 years</td>
<td>9.38 %</td>
</tr>
</tbody>
</table>

Table III describes blood indices and haemoglobin analysis of patients with abnormal haemoglobin studied by HPLC method.

**Figure 3**
Table III: Blood indices and Hb analysis results of common haemoglobinopathies

<table>
<thead>
<tr>
<th>Laboratory Parameters</th>
<th>Thalassaemia Major</th>
<th>Thalassaemia Minor</th>
<th>Sickle Cell Disease</th>
<th>Sickle Cell Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (gm %)</td>
<td>5.5 (3.14–13.2)</td>
<td>10.14 (8.2–14.1)</td>
<td>7.76 (2.31–12.1)</td>
<td>9.77 (5.0–13.9)</td>
</tr>
<tr>
<td>RBC (mL/Venous)</td>
<td>2.6 (0.57–6.01)</td>
<td>5.37 (1.35–6.65)</td>
<td>3.35 (1.14–5.77)</td>
<td>4.51 (3.9–5.55)</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>66.9 (53.7–80)</td>
<td>63.1 (48.1–83.1)</td>
<td>75.98 (58.8–92.8)</td>
<td>73.98 (64.8–85)</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>20.43 (12.4–30.1)</td>
<td>16.75 (10.1–27)</td>
<td>22.66 (17.3–28)</td>
<td>22.32 (11.8–30.1)</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>29.93 (21.6–38.7)</td>
<td>29.92 (23.3–35.0)</td>
<td>29.42 (24.7–34.2)</td>
<td>31.19 (23.6–34.4)</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>15.04 (12.2–21.3)</td>
<td>15.59 (13.4–22.1)</td>
<td>16.43 (11.9–22.5)</td>
<td>15.45 (10.7–22.5)</td>
</tr>
<tr>
<td>HPLC - An (%)</td>
<td>2.96 (0.6–6.3)</td>
<td>6.69 (4.2–7.7)</td>
<td>3.51 (1.4–4.5)</td>
<td>5.58 (3.4–6.8)</td>
</tr>
<tr>
<td>F (%)</td>
<td>66.25 (48.0–74.5)</td>
<td>1.29 (0.3–1.8)</td>
<td>17.00 (4.7–20.5)</td>
<td>1.17 (0.4–2.2)</td>
</tr>
<tr>
<td>A (%)</td>
<td>14.95 (9.4–24.8)</td>
<td>81.83 (73.9–85.6)</td>
<td>5.89 (2.0–3.2)</td>
<td>55.83 (60.1–67.6)</td>
</tr>
<tr>
<td>R (%)</td>
<td>67.00 (28.4–69.6)</td>
<td>31.51 (20.5–47.5)</td>
<td>70.00 (19.5–47.5)</td>
<td>31.51 (20.5–47.5)</td>
</tr>
</tbody>
</table>

(Values in the parenthesis indicate the range of various parameters observed in the blood samples of patients with haemoglobinopathies)

Twenty-four patients (75\%) with thalassaemia major had severe anaemia at the time of diagnosis (< 7 gm % Hb). One patient had only 1.14 gm % of haemoglobin. Majority of the patients with sickle cell disease had blood indices and blood film suggestive of hypochromic microcytic anaemia (18 out of 22 patients, 81.8\%). One patient of sickle cell disease had received blood transfusion before investigations and hence had low Hb S (38.6\%) and high Hb A (33.2\%). Three patients (25\%) of sickle cell trait had severe anaemia (< 7 gm % Hb) due to associated problems. Six patients of sickle cell trait had blood indices and blood film suggestive of hypochromic microcytic anaemia. Seven patients (31.82\%) of sickle cell disease had very high Hb F (> 20\%). Eight patients (66.67\%) of sickle cell trait had very low Hb F (< 1\%). Six patients of sickle cell trait had < 30 \% of sickle haemoglobin. One patient of sickle cell disease had Hb F < 5 \% and one had Hb F < 10 \%.

All the patients with thalassaemia minor had low Mean Corpuscular Haemoglobin (MCH) (< 27 pg/dl), only one had Mean Corpuscular Volume (MCV) > 77 fl and 3 patients had high Red Cell Distribution Width (RDW). Four patients of thalassaemia minor had severe anaemia (< 7 gm % Hb).

**DISCUSSION**
The incidence of β-thalassaemia minor and major was 16.35 \% and 7.48 \% respectively in the present study. This incidence coincides with the previous reports

The frequency of sickle cell disease is 5.14 \% in our data. The average frequency in India is 4.3\%.

Hb E disease is most frequently found in Eastern and far Eastern parts of India. Hb E is not very common in Gujarat. The incidence of Hb E was very low (0.23\%) in this study.

The incidence of Hb D disease was low (0.23\%) in the present study which coincides with the previous reports.

Many patients of thalassaemia major had received blood transfusions (13 patients out of 32, 40.6\%) before the diagnosis and many patients were more than 3 years of age at the time of diagnosis (25.01\%). A thalassaemia intermedia is suspected when a patient presents after 3 years of age or needs fewer blood transfusions. Early splenectomy is helpful in thalassaemia intermedia. Some of our patients could have thalassaemia intermedia but since they were transfused before the diagnosis, it was difficult to differentiate between thalassaemia intermedia and thalassaemia major. Majority of the patients (75\%) of thalassaemia major had severe anaemia at the time of diagnosis indicating lack of awareness about the disease, in treating clinicians or tendency of the parents to seek advice of the doctors only as a last resort.

A patient with sickle cell disease or trait has normochromic normocytic anaemia but majority of the patients in this study had hypochromic microcytic anaemia (81.8\% and 50 \% of...
been demonstrated in many countries with diverse carrier-

Effective prevention approaches to thalassaemia have now

been reported from India.

Thus, haemoglobinopathies exert significant burden on

India, especially in the western part of the country. Adequate

measures and screening procedures should be adopted to

reduce this burden. Screening is affordable and an accessible

way to detect carriers, and can be offered in a range of

settings in different societies: in high school, before

marriage, or in antenatal clinics. Haemoglobinopathy testing

should be performed concurrently with ferritin, serum iron

and Total Iron Binding Capacity (TIBC) for:

- Pregnant woman with low red cell indices
- Pregnant woman from a high-risk ethnic background
- Partner of the pregnant woman should be tested at the
  same time as the pregnant woman
- Partners of individuals who are carriers for
  thalassaemia or a haemoglobin variant
- A family history of haemoglobinopathy or
  haemoglobinopathy carrier state
- Individuals from ethnic groups with a high
  prevalence of haemoglobinopathy
- Consanguinity

Effective prevention approaches to thalassaemia have now

been demonstrated in many countries with diverse carrier-

screening programmes. For example, in Cyprus, Greece, the

Islamic Republic of Iran and Italy, premarital screening for

thalassaemia is standard practice and national audit data are

available; most at-risk couples are identified in time to be

offered early diagnosis for the first pregnancy 26-27. The

majority of such couples use this service and produce

healthy offspring. In the United Kingdom of Great Britain

and Northern Ireland and other north-western European

countries where prenatal diagnosis is generally available,

screening is offered during pregnancy 7-28. Besides, such

screening programmes must be supported by public

education and regulatory structures so that individuals may

make informed decisions and that people are protected

against discrimination as a consequence of their test results

References

364:1343-1360
2. Mohanty D, Mukherjee MB. Sickle Cell Disease in India.
4. Majumder PP, Roy B, Balgir RS, Dash BP. Polymorphisms in
the beta-globin gene cluster in some ethnic populations of
5. Balgir RS. The genetic burden of hemoglobinopathies
with special reference to community health in India and the
challenges ahead. Indian J Hemat Blood Transfus 2002;
20:2-7.
6. Balgir RS. Genetic epidemiology of the three predominant
abnormal hemoglobins in India. J Assoc Physicians India
7. WHO- EXECUTIVE BOARD EB118/5, 118th Session
Report by the Secretariat on Thalassaemia and other
haemoglobinopathies : Prevalence of Haemoglobinopathies.
on the Indian subcontinent; the basis of prenatal diagnosis.
9. Das BM, Deka R, Das R. Haemoglobin E in six
10. Balgir RS, Sharma SK. Distribution of sickle cell
11. Menon A, Salim KA. Sickle cell disease in South India. J
Assoc Physicians India 1993; 41: 617.
12. Balgir RS. Aberrant heterosis in hemoglobinopathies
with special reference to - thalassemia and structurally
abnormal hemoglobins E and S in Orissa, India. J Clin
13. Deka R. Fertility and haemoglobin genotypes: A
population study in upper Assam (India). Hum Genet 1981;
59:172-174
14. Balgir RS. The spectrum of hemoglobin variants in two
scheduled tribes of Sundargarh district in Northwestern
Author Information

Jagruti Patel, Ph.D.
Department of Pharmacology, Institute of Pharmacy, Nirma University of Science & Technology

Ashwin Patel, M.D.
Consultant Hematologist, Narayan complex

Jigar Patel, M.Pharm.
Department of Pharmacology, Arihant School of Pharmacy

Amarjeet Kaur, M.D.
Consultant Pathologist, Green Cross pathology and RIA laboratory

Vinod Patel, MLT
Green Cross pathology and RIA laboratory