The burden of haemoglobinopathies in India and the challenges ahead

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Among the inherited disorders of blood, haemoglobinopathy and thalassaemia constitute a major bulk of noncommunicable genetic diseases in India. They cause a high degree of morbidity, moderate to severe haemolytic anaemia among vulnerable segments of the society like infants and children, adolescent girls, pregnant women, etc. and several deaths in India. It has been estimated that with a population of 1000 million at the new millennium (2000) and a birth rate of 25 per thousand, there would be about 45 million carriers and about 15,000 infants born each year with haemoglobinopathies in India. The carrier frequency of haemoglobinopathy varies between 3 and 17% in different populations of India. The cumulative gene frequency of the three most predominant abnormal haemoglobins, i.e. sickle cell, haemoglobin D and haemoglobin E has been found to be 5.35% in India. This article provides glimpses of haemoglobinopathy and thalassaemia with special emphasis on the epidemiology, diagnosis and clinical profile, and hematological characteristics, distribution and prevalent mutations in India. The current status and challenges of haemoglobinopathies have also been highlighted and discussed with special reference to India.

The inherited disorders of blood include haemoglobinopathies as one of the major public health problems in India. It has been estimated that with a population of 1000 million at the millennium year 2000 and a birth rate of 25 per thousand, there would be about 45 million carriers and about 15,000 infants born each year with haemoglobinopathies in India. The carrier frequency of haemoglobinopathy varies from 3 to 17% in different population groups of India. The cumulative gene frequency of the three most predominant abnormal haemoglobins, i.e. sickle cell, haemoglobin D and haemoglobin E has been estimated to be 5.35% in India. Thus, there is a tremendous amount of burden of haemoglobinopathies in India.

This article is focused on the review of relevant literature along with providing a brief account of magnitude, epidemiology and diagnostic aspects of the most common public health problem of haemoglobinopathy in India.

Haemoglobinopathies

The haemoglobinopathies are characterized by the production of structurally defective haemoglobin due to abnormalities in the formation of the globin moiety of the molecule. When biological function is altered owing to a mutation in the haemoglobin, the condition is known as a haemoglobinopathy. Mutations in the genes that code for the alpha or beta chains potentially can affect the biological function of haemoglobin. Of the several hundred known human mutant haemoglobins (most of them extremely rare and benign), those in which biological function is altered have been briefly discussed here.

The globin moiety of haemoglobin (Hb) molecule is composed of seven different types of polypeptide chains, varying in number and arrangement of amino acids during different stages of human intra-uterine development and are designated by the Greek letters, alpha (α), beta (β), gamma (γ), delta (δ), epsilon (ε), and zeta (ζ). Epsilon, zeta and some alpha chains are synthesized in early embryonic life, alpha and gamma chains are in foetal life, and alpha, beta and delta chains predominate in the postnatal life. The polypeptide chains available determine the type of haemoglobin molecule produced. The beta, gamma and delta chains of human haemoglobins have highly conserved primary structures. In earliest embryonic (foetal) life, zeta and epsilon chains combine to form Hb Gower I (ζε), alpha and epsilon chains form Hb Gower II (αε), and zeta and gamma chains form Hb Portland (ζγ). By the end of first trimester, alpha chains have replaced zeta chains and gamma chains have replaced epsilon chains. In late foetal life, the predominant molecule is HbF (αγζ), which forms about 80–90% of the total haemoglobin at birth, whereas in the postnatal (postpartum) life, only traces are observed up to 6–12 months and foetal haemoglobin is replaced by the human adult haemoglobin HbA (α2β2) comprising about 97% and HbA2 (α2δ2), the latter constitutes about one-fortieth (1.5–3.5%) of the total adult haemoglobin. \( P_{50} \) is the partial pressure of oxygen that half-saturates a haemoglobin. For human HbA, \( P_{50} = 26 \text{ mm Hg} \); for HbF, \( P_{50} = 20 \text{ mm Hg} \) (ref. 3). This difference permits HbF to extract oxygen from the HbA of placental blood during the gestation period. Postpartum, however, HbF is unsuitable, since its high affinity for oxygen dictates that it can deliver less oxygen to the tissues. During development, there is a coordinated switching of haemoglobin synthesis, affecting both the site of erythropoiesis as well as the types of polypeptide chains. The different polypeptide chains manifest many similarities in their molecular structure, and on comparison of amino acid composition are clearly divided into two.

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groups, the alpha and zeta chains and the beta, gamma, delta and epsilon chains. Of these, one pair of alpha chains is of universal occurrence. The different globin chains have probably arisen by successive gene duplications from an ancestral alpha-like globin structure, and the close similarity between the structures of the beta and the delta chains suggests that they have diverged recently in terms of evolution. The alpha and zeta chains contain 141 amino acid residues and the beta, gamma, delta and epsilon chains contain 146 amino acid residues.

Classification

The hereditary disorders of haemoglobin may be classified into two broad groups, the haemoglobinopathies and the thalassaemias. They produce a massive public health problem in many countries including India. The haemoglobinopathies are characterized by the production of structurally defective haemoglobin due to abnormalities in the formation of the globin moiety of the molecule such as haemoglobins S, C, D, E, etc. The thalassaemias are characterized by reduced rate of production (synthesis) of normal haemoglobin due to absence or decrease in the synthesis of one or more types of globin polypeptide chains. Clinically, these disorders are known as the thalassaemia syndromes, resulting from both underproduction of haemoglobin and imbalanced globin chain synthesis, leading to a shortened red cell survival rate. The two principal types of thalassaemias, alpha and beta, are due to a reduced rate of synthesis of the respective chains.

Alpha-chain variant haemoglobins

Alpha chains are involved in the formation of HbA, HbA2, and HbF, and thus alpha-chain substitutions affect all these haemoglobins (Table 1). Adult heterozygotes for alpha-chain variants produce both normal and abnormal HbA, HbF and HbA2, the abnormal types having abnormal alpha chains in addition to the normal beta, gamma and delta chains. The major haemoglobin variant, i.e. HbA ranges from 15 to 45% of the total haemoglobin in the red cells. More than 100 alpha-chain variants have been described in the world.

Beta-chain variant haemoglobins

Beta chains take part in the formation of HbA only, and thus beta chain variants are all variants of HbA (Table 2). Heterozygous subjects synthesize both normal and abnormal beta chains, and the abnormal haemoglobin is usually about 30–40% of the total haemoglobin. Homozygous subjects synthesize the abnormal haemoglobin and the normal small amounts of HbA2, but no normal beta chains and, thus, no normal HbA. Heterozygotes for two beta-chain variants have equal amounts of the two abnormal haemoglobins and a small amount of HbA2 in their red cells. There are more than 457 such haemoglobin variants described throughout the world.

The delta–beta thalassaemia results from complete absence of both beta- and delta-chain synthesis, in most cases due to extensive deletion of DNA in the beta-globin gene complex. Homozygous condition is rare, but heterozygous form is common and resembles clinically as well as haematologically with beta-thalassaemia trait. HbA2 level is normal or reduced and HbF is elevated from 5 to 20%.

A low number of variants is on record for gamma and delta-chains also.

There is also hereditary persistence of foetal haemoglobin (HPFH) which represents extremely mild form of thalassaemia in adult life. In most cases the condition can be defined by hematological studies, haemoglobin electrophoresis and quantitation of haemoglobins A2 and F (ref. 5).

Abnormal haemoglobins are inherited as autosomal and co-dominant manner. The subjects who inherit one normal and one abnormal gene are heterozygotes, and those who have two identical abnormal genes are homozygotes. Double heterozygotes are those who have inherited two different abnormal genes. The homozygous state is usually referred to as the disease, and the heterozygous state as the trait, carrier or single dose.

Many of the haemoglobin variants do not interfere with the health of the individuals who remain asymptomatic and unaware of the abnormality within the red cell. Some variants might even provide advantages over normal haemoglobin in heterozygous condition against the fatal diseases like malaria, where they are less likely to sustain the very heavy load of parasitemia which is associated with overwhelming (cerebral) malaria.

Common clinical and laboratory features

The clinical severity and haematological diversity in both homozygous and heterozygous haemoglobinopathy dis-
orders show a wide spectrum, ranging from life-threatening complications and death in early childhood to transient symptoms or a relatively benign clinical course. The latter condition resembles the normal individuals, whereas the former sustains life by frequent blood transfusions. The clinical severity of homozygous sickle cell disease is highly variable in India and presents a wide spectrum of morbidity and mortality. The disease manifests as early as at three months of age or remains asymptomatic till adult life. In many patients, the clinical manifestations of the disease are extremely variable, ranging from completely asymptomatic to inexorable deterioration, distressing, crippling or life-threatening features often claiming life around 20 years of age. Symptoms are severe pain, bone necrosis and spleen infarction, among others. Death during childhood is related to infectious diseases like cough, influenza (pneumonia) or septicaemia, whereas that during adulthood is due to organ failure from repeated tissue destruction. Loss of splenic function, hand-foot syndrome, splenic sequestration syndrome, splenomegaly, hepatomegaly, etc. are the major causes of haemoglobinopathic morbidity and mortality in India.

A majority of the cases in India suffer from severe anaemia, i.e. haemoglobin level less than 10 g/dl, higher foetal haemoglobin level (2–20%), higher reticulocyte count (2–29%), lower mean cell volume (MCV) ranging from 60 to 113 flankolite (fl), lower red cell count (range 11.5–5.49 × 10¹²/L), lower mean cell haemoglobin (MCH) concentration (range 0.25–0.42 g/dl) and uncommon iron deficiency.

Diagnostic tests

The most common diagnostic tests performed for haemoglobinopathies in India include red cell indices, red cell morphology, reticulocyte count, naked eye single tube red cell osmotic fragility test (NESTROFT), sickling test (Figure 1), estimation of foetal haemoglobin (F) and adult A2 fraction, estimation of serum ferritin, iron binding capacity and bilirubin (both direct and indirect), haemoglobin electrophoresis (both alkaline and acidic), haemoglobin variant analysis or high pressure liquid chromatography (HPLC) and DNA mutations analysis, etc.

Haemoglobinopathies in India

In this section, a brief account of biochemical changes, genetics and diagnostic and haematological characteristics which are disease specific of common disorders of haemoglobin have been discussed, with special reference to India. These clinical and laboratory features are diagnostic in nature of the specific haemoglobinopathic disorder (Figure 1).

Alpha-thalassaemia syndromes

The deficiency of alpha chains leads to an excess of gamma chains in the foetus and that of beta chains in the adult. In the foetus, gamma chains form the tetramer Hb-Bart’s (γ4), and in the adult, the unstable beta chains precipitate and form Hb-H (β4). The presence of Hb-Bart’s and Hb-H diseases in the red cells has serious consequences as both haemoglobins have a high oxygen affinity and, thus are unsuitable to deliver adequate oxygen to the tissues.

Normal subjects have two linked alpha gene loci on the short arm of chromosome 16, thus giving an alpha gene haplotype of αα and genotype of αα/αα. The alpha thal-
lассаemia are most common due to deletions of one or more of these genes. In most affected populations, two abnormal alpha gene haplotypes are found, namely $\alpha'^{-}$ which arises from deletion of one alpha gene and $\alpha^-\alpha^+$ from deletion of both alpha genes. The alpha thalassaemias in the world have been reviewed by Higgs and Weatherall.

Work on molecular genetics has provided clear evidence that the alpha-thalassaemia showing a complete absence of alpha chain production results in severe form of alpha thalassaemia, called $\alpha^0$ thalassaemia, whereas the mild form of alpha thalassaemia having only a partial deficit of alpha chain production is called $\alpha^+$ thalassaemia. Both $\alpha^0$ and $\alpha^+$ thalassaemias can result from several different molecular defects involving the alpha globin gene cluster. The $\alpha^0$ thalassaemias result from a series of gene deletions which involve both alpha globin genes. The $\alpha^+$ thalassaemias result from deletions of one of the linked pairs of alpha globin genes or from a series of non-deletion defects in which the alpha globin genes are present, but their output is reduced. There are common structural haemoglobin variants which are synthesized at a reduced rate and produce the clinical phenotype of $\alpha^+$ thalassaemia. The commonest of these are haemoglobin Constant Spring and Koya Dora which have been reported from the populations of Southeast Asia and Andhra Pradesh in India.

Alpha-thalassaemia trait: Alpha-thalassaemia trait is asymptomatic and is difficult to diagnose with certainty in adult life, except using gene-mapping studies.

Alpha-thalassaemia 2($\alpha^+\alpha^+$ thalassaemia): This represents the heterozygous state for $\alpha^+\alpha^-$ thalassaemia ($\alpha\alpha^+\alpha^-$) (Table 1). During neonatal period, affected infants may have 1–2% Hb-Bart’s which they gradually lose over the ensuing months. In adult life, the haemoglobin pattern is normal, and Hb-H inclusions are not found at any stage. Haemoglobin level and blood film are normal, although the mean cell volume (MCV) and mean cell haemoglobin (MCH) may be mildly reduced.

Alpha-thalassaemia 1($\alpha^0\alpha^+$ thalassaemia): This represents the heterozygous state for $\alpha^0\alpha^0\alpha^+$ or the homozygous state for $\alpha^0\alpha^0\alpha^-$ (Table 1). During neonatal period, 5–6% Hb-Bart’s is found, but the haemoglobin pattern is normal in later life. Hb-H inclusions are usually present in very small numbers. The haemoglobin level is normal or mildly reduced, but the red cells are usually mildly hypochromic and microcytic, and the MCV and MCH are reduced. HbA2 is reduced in some cases.

Haemoglobin-H disease: Interaction of the $\alpha^+$ determinant gives rise to this form of alpha-thalassaemia ($\alpha\alpha^+\alpha^-$) (Table 1). Clinically, Hb-H disease is characterized by a moderate anaemia with a haemoglobin level of 8–9 g/dl, mild jaundice and physical findings similar to that of beta-thalassaemia major. The reticulocyteosis ranges between 5 and 10%. After incubation of red cells with brilliant cresyl blue, many ragged inclusion bodies form due to the redox action of the dye, causing precipitation of HbH. The haemoglobin pattern consists of Hbs A, H and A2 with variable amounts of Hb Bart’s, the A2 being reduced to 1.5–2%. It is very unstable and likely to be precipitated at room temperature. On haemoglobin electrophoresis, Hb-H and Bart’s migrate more rapidly than HbA at an alkaline pH. The severity of anaemia fluctuates during pregnancy or intercurrent infection, etc. Splenomegaly is present in 85% of patients and cholelithiasis is also common.

Blood film shows marked red cell morphological changes, including severe hypochromia, microcytosis and target cell formation. Red cells are nucleated. There is a mild reticulocytosis. Numerous Hb-H inclusions with brilliant cresyl blue stain and large Heinz body-like inclusions are also present after the splenectomy.

The haemoglobin pattern consists of 2–40% Hb-H, the remainder being HbA, HbA2 (which is reduced) and HbF. HbH disease has a small amount of an alpha-chain variant, Hb Constant Spring. Neonates with Hb-H disease have 25% Hb-Bart’s. Haemoglobin Constant Spring

Figure 1. a. Thalassaemia major. Blood film showing poikilocytosis, target cells and hypochromic cells; b. Sickle cell disease. Blood film showing sickled and hypochromic cells.
occurs at extremely low levels in heterozygous carriers, usually less than 1% of the total haemoglobin. It migrates more slowly than HbA2 on alkaline haemoglobin electrophoresis and tends to break up into 2-3 separate bands. Family studies show the α2 thalassaemia trait in one parent and the Hb Constant Spring trait in the other. Individuals homozygous for Hb Constant Spring have slightly hypochromic red cells with normal MCV.

The earliest case of Hb-H was recorded in a Bengalee subject from Calcutta12. Subsequently, five more such instances have been found in Bengalees (4%). The proportion of Hb-H in these varied from 12 to 25%. In none of the parents of these index patients was the anomaly detected, while Hb-H thalassaemia was found in the siblings13. Hb-H was reported by Swarup et al.14 in a 19-year-old Bengalee Hindu from India. Similar cases (1%) were reported from Mumbai15,16. Saha and Banerjee17 reported two cases of Hb-H traits among Malayalis, and one each among Tamils, Gujaratis and Sindhis in Singapore. Mishra et al.18 reported 15% haemoglobin Constant Spring (both heterozygotes and homozygotes) among the coastal people of Orissa.

Haemoglobin-Bart’s hydrops foetalis: The most severe manifestation of the alpha-thalassaemia gene is haemoglobin Bart’s hydrops foetalis. The compound heterozygous state for both αα thalassaemia and αα thalassaemia, or αα thalassaemia and Hb Constant Spring, results in Hb-H disease5. The homozygous state for αα thalassaemia causes a very mild anaemia with hypochromic red cells and no change in the haemoglobin pattern. The homozygous state for Hb Constant Spring is characterized by a mild haemolytic anaemia with splenomegaly. AFFECTED infants are homozygous for the αα determinant (−/α−/α), both parents having heterozygous αα-thalassaemia. There is total suppression of alpha-chain synthesis with a gross excess of gamma chains. The gamma-chain tetramer, Hb Bart’s, has a high oxygen affinity, and results in severe tissue hypoxia.

AFFECTED infants are either born dead (stillborn between 28 and 40 weeks gestation) or die within a few hours of birth19. They are underweight, pale (haemoglobin values in the range of 6–8 g/dl), mildly jaundiced, grossly edematous and have hepatosplenomegaly and ascites. The haemoglobin is around 6 g/dl, the blood film is grossly abnormal with anisopoiikilocytosis, hypochromia, target cells, polychromasia and a large number of nucleated red cells. The reticulocyte count is high and serum bilirubin elevated.

The haemoglobin pattern consists of 80–90% Hb-Bart’s, with a small amount of Hb-H and Hb-Portland. There is usually no HbA, HbA2 or Hbf.

In West Bengal, 4% of 100 cord blood samples obtained from newborns showed Hb Bart’s20. In one isolated case with Hb Bart’s at birth, Hb-H thalassaemia was recorded at the age of 2 years. Incidence of Hb Bart’s in 2% of cord blood samples was reported by Chouhan et al.11 and 4.2% by Vora et al.21 from Mumbai. Mishra22 reported 7.7% haemoglobin Bart’s in the cord blood samples among the people of north-western Orissa and, subsequently, 12.6% among the people of coastal Orissa9.

Alpha-thalassaemia mutations in India

In India, there have been sporadic reports of alpha-thalassaemia. Some cases of Hb-H disease have been found in West Bengal23 and screening of newborns in West Bengal and Mumbai showed that 2% and 4% of cord bloods contained Hb Bart’s15,23. Furthermore, 9.4% of a tribal population from East Godavari District in Andhra Pradesh were found to have the variant Hb Koya Dora, which is caused by an alpha globin chain termination mutant12,24. Using gene mapping analysis, alpha-thalassaemia has been diagnosed in a high proportion of a tribal (Toda) population in South India25 and in East India26, the latter study indicating an estimated gene frequency of 0.32 among patients with sickle cell disease in Orissa (Table 3).

The exact molecular basis of alpha-thalassaemia in India has not yet been described. The most common molecular basis for alpha-thalassaemia in India is the geographically widespread 3.7 kilobase (kb) and 4.2 kb deletions27. In one family, a novel 3.5 kb deletion removed the α1 globin gene with some of its flanking sequences. Most commonly alpha-thalassaemia results from deletions originating from unequal cross-over events in the alpha globin gene cluster. The occurrence of non-deletional forms of alpha-thalassaemia was suggested by low Hb S levels and red blood cell (RBC) indices in persons with the sickle cell trait27.

The prevalence of alpha-thalassaemia in India varies from one sub-geographical area to another: it is 71% in Behrampur and 42% in Jeypur (both in southern Orissa), and 11% in the nearby villages of (northern) Andhra Pradesh25. Labie et al.27 also found an almost equal number of rightward (−α22) and leftward (−α22) deletions,

<table>
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<th>Table 3. Percentage prevalence of alpha-thalassaemia mutations in sickle cell disease in India</th>
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<tr>
<td><strong>Ref. 27</strong></td>
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<td>Alpha-thalassaemia mutations</td>
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<tr>
<td>(n = 13)</td>
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<tr>
<td>αα/αα</td>
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<tr>
<td>αα/αα</td>
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<td>−α/αα</td>
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<td>−α/αα</td>
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<td>Gene frequency</td>
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*Data from ref. 32. **Calculated by the author.
even among the sickle cell homozygotes. The prevalence of alpha-thalassaemia was much higher in the other two regions, i.e. west central Gujarat (95%) and in Nilgiri hills in South India (85.7%), suggesting that the condition is almost genetically fixed in India. Interaction of alpha-thalassaemia for clinical severity and haematological expression of sickle cell anaemia has been implicated in western India and the synergistic role of co-inherited alpha-thalassaemia in ameliorating the severity of sickle cell disease in India has also been suggested.

When an unequal crossing-over event takes place between the two alpha-globin gene loci, it leads to one chromosome with a single alpha-globin gene and the other with triplicated alpha-globin genes. This event is rare in India. Usually, there is preferential selection of the single alpha-gene allele. Garewal and co-workers have observed 6 of 126 alpha-globin loci triplicated, giving a frequency of 0.05 (5%) in some populations in Punjab (Table 4). The combination of homozygous triplicated alpha-genes with beta-thalassaemia trait is more likely to cause a phenotype of thalassaemia intermedia as there would be a significant imbalance of alpha to beta-globin chain synthesis. Garewal and co-workers observed this phenomenon in two thalassaemia intermedia cases in Punjab (Table 4).

Beta-thalassaemias

Beta thalassaemias are also a complex heterogeneous group. Broadly, the beta-thalassaemia can be classified into the beta-thalassaemia, in which no beta globin chains are synthesized at all, and the beta-thalassaemia, in which there is a reduced rate of beta chain production. Usually, the homozygous state for beta-thalassaemia or the severe forms of beta-thalassaemia, or the compound heterozygous states for beta and severe beta-thalassaemia, are associated with transfusion-dependent anaemia from early life. Some patients run a milder course and the term thalassaemia intermedia is used for this condition.

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<th>No. of patients</th>
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<td>2</td>
<td>IVS 1 at (G-C)/cap + 1</td>
<td>αα/αα</td>
</tr>
<tr>
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<td>IVS 1 at (G-C)/cap + 1</td>
<td>α/αα</td>
</tr>
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<td>FS 8/9/cap + 1</td>
<td>α/αα</td>
</tr>
<tr>
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<td>FS 8/9/cap + 1</td>
<td>α/α-</td>
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<td>FS 41/42/cap + 1</td>
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<td>αα/αα</td>
</tr>
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<td>FS 8/9/Normal</td>
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<td>FS 8/9/Normal</td>
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<tr>
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<td>IVS 1 at (G-C)/88 (C-T)</td>
<td>αα/αα</td>
</tr>
<tr>
<td>1</td>
<td>FS 8/9-88 (C-T)</td>
<td>Not analysed</td>
</tr>
</tbody>
</table>

Table 4. Beta-thalassaemia intermedia in north-western India

Homozygous beta-thalassaemia or thalassaemia major: This condition usually presents with severe anaemia within the first year of life. Before the first transfusion, these patients show a variable degree of anaemia with marked variation in shape and size of their red cells with hypochromia and a mild reticulocytosis (Figure 1). The bone marrow shows erythroid hyperplasia and many of the normoblasts contain ragged inclusions after incubation of the marrow with methyl violet.

The haemoglobin pattern in this condition consists almost entirely of Hbf with variable Hba2 levels which may be reduced, normal or elevated. Haemoglobin A is completely absent. Both parents show the features of heterozygous beta-thalassaemia with elevated Hba2 levels.

Several hospital-based studies are available from different parts of India. Malhotra and Chhuttani reported a case of Cooley’s anaemia. Similar cases were reported by Narayanappa and Srinivasan et al. from South India. Mariswamy and Pierce reported 5 cases of thalassaemia major from Mysores. Sharma et al. recorded 80 cases of thalassaemia major in Mumbai and the regional distribution was as follows: Gujratis (35), Maharashtrians (21), Sindhis (12), Goanese (9), Bengalees (1) and Uttar Pradesh (1). Homozygous beta-thalassaemia has been encountered more frequently in Calcutta as well as reported from other parts of India.

Heterozygous beta-thalassaemia or thalassaemia minor

The heterozygous states for beta thalassaemia are associated with a mild but significant degree of anaemia. The haemoglobin values for males ranged from 9 to 15 g/dl with a mean value of 12–13 g/dl. In females, the range has been between 8 and 13 g/dl with mean value of 9–10 g/dl. The red cell indices are quite characteristic, with reduced MCH values in the 20–22 picogram (pg) range and reduced MCV values in the 60–70 picolitre (pl) range. It is most unusual to find a beta-thalassaemia carrier with an MCH above 25 pg or an MCV of more than 70 fl. The screening for beta-thalassaemia using an electronic cell counter is also effective these days in India.

The main diagnostic feature of this condition is an elevated Hba2 level in the 3.5–7.5% range. The condition is also called beta-thalassaemia trait. Some cases also show a slight elevation of Hbf.

Several cases of beta-thalassaemia trait (26%) have been reported by Swarup-Mitra from Calcutta. Flatz et al. had reported the beta-thalassaemia trait among Assamese (5%), Ahoms (1%), and Khasis (<1%) in Assam. Mishra et al. reported 8% prevalence of beta-thalassaemia trait among the coastal population of Orissa. Almost similar situation prevails in other parts of India.

Beta-thalassaemia mutations in India

In India, beta-thalassaemia comprises about 80–90% of the total thalassaemias reported. More than 200 beta-
thalassaemia mutations have been identified all over the world and of these about 28 mutations have been documented in Indian patients. Six mutations, 619 bp deletion at 3' end of beta-globin gene, IVS-1 nt 5 (G-C), IVS-1 nt 1 (G-T), frameshift mutations FS 8/9 (+G), codon 41/42 (-CTTT) and nonsense codon 15 account for 90-94% of the beta-thalassaemia mutations in India. There are mutations which are less frequent but have been observed in the Indian people: codon 15 (G-A), codon 16 (-C), codon 30 (G-C), IVS-1-110 (G-A), -88 (C-T), CAP+1 (A-C), codon 5 (-CT), FS 41/42 (-CTTT), codon 88 (+T), 25 bp deletion, IVS-2 nt 1 (G-A) and IVS-1 minus 1 (G-A). These mutations have been observed in patients in homozygous, heterozygous or in combination with other mutations (in double heterozygote form). There are few rare mutations which have either been detected in India or among the Indian people abroad but are yet uncharacterized (Table 5).

The prevalence of different mutations varies significantly in different regions of India. The IVS-1-5 mutation is the commonest mutation found in the Indian populations and its prevalence varies from 22.8 to 81.4% in different regions of India, being the highest in Tamil Nadu in south-eastern India. In the north-western part of India (including the states of Punjab, Haryana, Uttar Pradesh and Rajasthan, adjoining Delhi), the 619 bp deletion mutation is the commonest beta-thalassaemia mutation observed in patients originating from Sindh, Gujarat or among the families migrated from Pakistan during partition of the country in 1947. Data have been reported from Punjab, Delhi, Haryana and Rajasthan, Uttar Pradesh, Madhya Pradesh and Bihar, North-Eastern region, Maharashtra and South India. The difference in prevalence of DNA mutations in beta-thalassaemia from different regions of India reflects the ethnic and genetic diversity of populations in India. The heterogeneous populations belonging to the Indian subcontinent origin (Pakistan, Sindh, Punjab, Gujarat, Tamil Nadu, Maharashtra, West Bengal, Andhra Pradesh, Kerala) were studied abroad by a number of investigators.

Table 5 presents the mutation studies on Indian patients belonging to Punjab, Haryana, Delhi, Uttar Pradesh, Rajasthan, Gujarat, Maharashtra, Madhya Pradesh, Bihar, West Bengal, Tamil Nadu and South India.

**Thalassaemia (delta-beta) intermedia**

This thalassaemia results from complete absence of both beta- and delta-chain synthesis, in most cases due to extensive deletion of DNA in the beta-globin gene complex. The high level of gamma chain production leads to a relatively mild degree of globin chain imbalance and hence these conditions are much milder than the beta thalassaemias. Homozygous condition is rare, but heterozygous form is common and resembles clinically as well as haematologically with beta-thalassaemia trait. HbA2 level is normal or reduced and HbF is elevated from 5 to 20%. The Hb Lepore disorders are forms of delta-beta thalassaemia. These conditions result from the production of delta-beta fusion genes which direct the synthesis of delta-beta fusion chains. They arise by unequal crossing over between the delta and beta globin genes, depending upon the position and structure of abnormal crossing over.

Agarwal and Mehta have reported 36 cases of delta-beta-thalassaemia from Mumbai. Similarly, Brittenham et al. showed interaction of delta-beta genes in South India.

Dash et al. after surveying 300 cord blood samples had identified a foetal haemoglobin variant of the gamma-chain with an electrophoretic mobility intermediate between Hbf and Hba2, in a Punjabi Hindu newborn at Chandigarh. This abnormal haemoglobin made up 15% of the total foetal haemoglobin.

**Hereditary persistence of foetal haemoglobin**

The term hereditary persistence of foetal haemoglobin (HPFH) is used to describe a group of conditions in which there is persistent foetal haemoglobin synthesis into adult

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<td>IVS at 1-5 (G-C)</td>
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<td>22.5</td>
<td>35.4</td>
<td>38.3</td>
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<td>67.5</td>
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<td>20.6</td>
<td>22.7</td>
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<td>16.4</td>
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<td>12.0</td>
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<td>FS 41/42 (-CTTT)</td>
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<td>11.8</td>
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<td>CD 16 (C-C)</td>
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<td>Total chromosomes</td>
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<td>80</td>
<td>156</td>
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life in the absence of any major hematologic abnormality. This is confirmed by the study of family members (pedigrees).

This is a relatively benign condition where production of Hbf continues in adult life. Instances of hereditary persistence of Hbf have been reported in 13 cases in heterozygous form, out of which in 3, double heterozygous state, i.e. Hbf and thalassaemia major by Sukumar and co-workers, interaction of persistent Hbf and thalassaemia genes was documented in two Bengalee families by Chatterjee and by Parekh et al. in Mumbai and Bird et al. at Pune in Western India. Recently, Desai et al. reported a family with hereditary persistence of Hbf and haemoglobin D and E in Kolhapur district of Maharashtra.

The presence of HPFH in the heterozygous state γ-glycine (136) and γ-alanine (136) in 6 persons was reported from four Indian families. Further, Sukumar et al. reported the homozygous state of HPFH γ-glycine (136) in two Indian families.

Abnormal structural variants of haemoglobin

The Indian peninsula is a vast reservoir of abnormal haemoglobins as well as thalassaemias. Most of the abnormal haemoglobins either have first been detected in India or among the individuals of Indian origin abroad. The abnormal haemoglobins so far detected in India include Hb D, E, H, J, K, L, M, Q, S, Lepore, Norfolk, Koya Dora, Chandigarh and the hereditary persistence of Hbf.

Several reviews are available in India on haemoglobinopathy and thalassaemia, sickle cell haemoglobin, haemoglobin E, haemoglobin D, haemoglobin double heterozygosity and various mutations detected in India. A map depicting distribution of cases of major forms of haemoglobinopathies in India is given in Figure 2.

Since the most commonly found abnormal haemoglobins in India, i.e. sickle cell haemoglobin (S), haemoglobin-E and haemoglobin-D have recently been extensively reviewed elsewhere, they have been excluded from the present consideration. A brief account of other important variants of haemoglobin is presented here.

The sickle cell haemoglobin is widely distributed all over India, after screening 3000 subjects belonging to various ethnic groups of Jammu region in the state of Jammu and Kashmir, detected 39 cases of HbAD trait and 3 cases of homozygous HbD disease. Agarwal et al. recorded 16 (1.5%) cases of haemoglobin-D trait in Lucknow, Uttar Pradesh, out of 1098 unrelated individuals who were tested. In their study, the prevalence of HbD trait in Khatis was 3.1%, compared to 0.5% in other Hindus. Balbir detected three families of haemoglobin-D in Orissa.

Subedar et al. reported one case of Hb-J in a scheduled caste family from Nagpur.

Labie et al. recorded 3 cases of Hb-K among 114 Hindus of lower caste and another of unknown identity in Pondicherry. De Traverse et al. demonstrated 3 instances of Hb-K among 101 South Indians in Chennai. Trincao et al. reported 2 instances of Hb-K in a survey of 1843 Indians in Goa. Verma et al. detected 18 cases of HbAK trait among the Hindus migrated from Poonch and Mussafarabad area of West Pakistan in Jammu.

Sukumar et al. demonstrated 8 instances of Hb-L in three Gujarati-speaking Lohana families in Mumbai.

Only one family with haemoglobin-M has so far been detected in a Punjabi family from Amritsar. Three members of the family were found to have Hb-M levels of 7%, 33% and 50%. In alpha-chain haemoglobin-M variants, the R-T equilibrium favours the T form. Oxygen affinity is reduced, and the Bohr effect is absent. Beta-chain haemoglobin-M variants exhibit R-T switching, and the Bohr effect is, therefore, present. In practice, these defects are known as heterozygotes. The blood is dark in colour, the affected individuals are cyanosed in appearance, but they survive into old age without difficulty.

Trincao et al. detected 4 instances of Hb-Q in a survey of 1843 Indians in Goa. Sukumar et al. recorded a new Hb-Q (ref. 64) (aspartic acid → histidine), or Hb-Q (India), in two Sindhi families in Mumbai.

Figure 2. Map of India (not to scale) showing distribution of major forms of haemoglobinopathies (Hbs D, E, J, K, L, M, Q and S) in the different states.
Recently, a new beta-chain variant, haemoglobin Chandigarh has been detected by Dash et al.\textsuperscript{99}.

**Thalassaemia and other haemoglobinopathies:** Alpha-thalassaemia is found in association with alpha-chain haemoglobin variants, e.g. Hb-Q and Hb-I; beta-chain variants, e.g. HbE, Hbs; and with beta thalassaemia.

**S-thalassaemia:** Chatterjea\textsuperscript{67} reported 15 cases of S-thalassaemia, 8 in Oriya Hindus, 1 each in Bengalee Hindus and Muslims, and 1 in South Indian Hindus and 2 in Tamil Muslims. Mital et al.\textsuperscript{100} recorded a high incidence of S-thalassaemia among Sorathis in Palghar (3.7%). Lele et al.\textsuperscript{101} identified one family of S-thalassaemia in a survey of 100 students belonging to scheduled caste in Aurangabad.

**E-thalassaemia:** Chatterjea\textsuperscript{67} detected 526 cases of E-thalassaemia investigated in Calcutta among Indian Hindus and the regional distribution was as follows: Bengalees (508), Oriahs (10), Biharis (4), Assamese (2), Punjabis (1), South Indians (1), and 48 cases among Bengalee Muslims and one in Bihari Muslims. Sarkar et al.\textsuperscript{102} detected 14 cases of E-thalassaemia from Calcutta. Kochhar and Kathpalia\textsuperscript{103} and Prarahar\textsuperscript{104} reported solitary instances of E-thalassaemia in a Kannada and an Oriah family. Dash et al.\textsuperscript{105} demonstrated a case of E-thalassaemia in Punjab. Ghosh et al.\textsuperscript{106} described 7 cases of E-beta-thalassaemia from Punjab and one case from Rajasthan. High prevalence of haemoglobin-E in ten populations of Assam (20–60%) and in three populations of West Bengal (12–61%) has been studied by Deka et al.\textsuperscript{107} and Das et al.\textsuperscript{108}, respectively, in North-Eastern India. DNA haplotypes analysis showed a common origin of haemoglobin E mutation in Assam and in South-East Asia\textsuperscript{109}.

**D-thalassaemia:** Chatterjea\textsuperscript{67} recorded 9 cases of D-thalassaemia: 6 from Bengal, and one each from Bihar, Punjab and South India. Occasional cases of D-thalassaemia have been reported in and around Delhi\textsuperscript{110}. Lele et al.\textsuperscript{101} detected one case in a Kunbi family from Aurangabad. Sukumaran et al.\textsuperscript{111} reported one case each in a Sindhi and Gujarati-Lohana family. One case of Hb-D trait with thalassaemia was detected in a Muslim girl from Lucknow, Uttar Pradesh, by Agarwal et al.\textsuperscript{92}.

**J-thalassaemia:** Sanghvi et al.\textsuperscript{112} recorded one case of J-thalassaemia in a Gujarati-speaking Lohana. Svarup et al.\textsuperscript{113} reported 4 cases of J-thalassaemia in Bengalee Hindus.

**K-thalassaemia:** Svarup et al.\textsuperscript{114} reported an interaction of Hb E and K with thalassaemia in a Bengalee family of Calcutta.

**Q-thalassaemia:** Sukumaran et al.\textsuperscript{115} recorded one case of Q-thalassaemia major and 2 cases of Q-thalassaemia minor in Sindh families in Mumbai.

**Haemoglobin Lepore:** Chouhan et al.\textsuperscript{116} reported the only case of Hb Lepore in an Indian family from Koondapur of Karnataka.

These hospital-based reports indicate that thalassaemia is not only common throughout India but also prevalent in high frequency, in association with abnormal haemoglobins in different population groups in different geographical regions of India. Although the association of alpha-thalassaemia with abnormal haemoglobin reduces the severity of the disease in patients, yet it poses a major challenge to the pediatricians, gynaecologists, clinicians, geneticists and general practitioners from the public health point of view in India.

Inherited haemoglobinopathies are major public health problems especially in the Mediterranean Sea region, Middle East, the Indian subcontinent, South-East Asia and Tropical Africa (Figure 3). It has been estimated that about 250 million people (4.5% of the world population) are heterozygous for these disorders and at least 3,000,000 lethally affected homozygotes are born annually throughout the world\textsuperscript{117}. Some 60–70% of all births of children with a major haemoglobin disorder, especially the sickle cell or Hb-C, occur in Africa. The frequency of beta-thalassaemia is close to 10% in Iran. In Pakistan, 8% beta-thalassaemia trait in Pathans and 3.3% in Punjabis has been reported apart, from the sporadic cases of Hb-D and Hb-J. In Thailand, about 40% of the population carries the major disorders of haemoglobinopathy like homozygous Hb-E disease, homozygous beta-thalassaemia, alpha-thalassaemia and Hb-H disease\textsuperscript{117}.

![Figure 3. Geographical distribution of haemoglobinopathies belt.](image-url)
Thus it is concluded that sickle cell and Hb-C are prevalent in tropical Africa in black populations, Hb-E is common in South-East Asia, India, Bangladesh, Myanmar and Sri Lanka, and Hb-D, Hb-J, Hb-K, Hb-L and Hb-M in the Indian subcontinent, especially in north-western India, Pakistan and Iran. Incidence of abnormal haemoglobins, alpha- and beta-thalassaemia in India is comparable with the neighbouring Asian countries and the other countries in the same geographical belt (Figure 3). However, the sickle cell haplotypes prevalent in Eastern Saudi Arabia and Asia, especially in India, are different from the African haplotypes and are linked with milder clinical manifestations of the sickle cell disease in India. Indian sickle cell haplotypes produce high level of foetal haemoglobin and are also linked with the interaction with alpha-thalassaemia in comparison with the African mutations and hence present a milder clinical course of the disease in India.

**Impact of haemoglobinopathies on Indian society**

The situation of haemoglobinopathy and thalassaemia causing hereditary haemolytic anaemia is very grim in India. Unfortunately, there is no attempt at the national level to enlarge the epidemiological database, establishing specific programmes for screening populations at risk, imparting genetic counselling and establishing special treatment centres to alleviate the sufferings of dwindling masses in India. Only piecemeal and sporadic studies have been carried out in some communities in India. Hospital-based case reports are available, but without having a central registry.

The exact magnitude of the problem in India is still obscure. Only hospital-based data are available, which cannot be regarded as representative of the community or population. There is a genetic, ethnic and regional diversity of the haemoglobin variants as well as of the mutations in India which emphasizes to tackle the problem at a regional level. The generation of infra-structural diagnostic facilities at regional level, health education and prevention are needed for rehabilitation and amelioration of the affected masses in the country (Figure 4). Consanguineous marriages further compound the complexity by increasing the homozygosity in the community. Thus these genetic disorders of blood should be tackled at individual, family, community and national levels with full strength and sincerity.

Most of the patients of haemoglobinopathies have a high morbidity rate, intercurrent infections being unusually common, suffer from high economic burden, terminate fatally in childhood, and, have emotional and psychological trauma including the family members. The most effective approach to reduce the burden of the society (Figure 4) is to reduce the incidence by implementation of a carrier screening programme offering genetic counselling, prenatal diagnosis and selective termination of pregnancy of the affected foetuses in India.

There is an urgent need for making the people aware of this lethal malady. Health education is an important component of the preventive genetic programmes. With improving environmental and socio-economic conditions, better public health care and medical facilities, effective malarial prophylaxis and better nutrition, children suffering from thalassaemia and haemoglobinopathy can be better managed and rehabilitated in India. This requires proper health education and adequate sensitization to the individual, family or community to accept

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**Figure 4. Flow chart for approach to diagnosis and control of haemoglobinopathies in India.**
these preventive remedial measures. Thus, thalassaemia and haemoglobinopathy, which are prevalent throughout India are heritable, treatable, curable and preventable disorders (Figure 4). We must make efforts to eliminate these from our society. In this way, we may be able to alleviate and ameliorate the sufferings of the affected masses in our country. High cost of treatment, repeated blood transfusion and chelation therapy, and economic burden on family resources, all suggest that prevention is better than cure. Thus a joint venture of antenatal and inductive screening seems to be the most fruitful strategy for haemoglobinopathy in India.

For human gene therapy, there are genetic disorders that involve treatment of bone marrow cells, these include the genetic disorders of haemoglobin–sickle cell anaemia and thalassaemia. These represent more formidable problems because haemoglobin is the product of more than one gene, and its expression must be limited to a small sub-fraction of bone marrow cells, called the stem cells, which are the progenitors not only of erythrocytes but of granulocytes, macrophages and platelets. Potential treatment of some more common genetic disorders must await development of methods to remove and replace cells from other tissues. The prospect of curing such diseases holds great potential for alleviating human suffering in India.
