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Thalassaemia in Malaysia : A Strategy for Prevention

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Abstract

In Malaysia, α-thalassaemia, β-thalassaemia, haemoglobin (Hb) E, δβ-thalassaemia and Hb Constant Spring are prevalent. It has been estimated that 1 in 4 persons carries one of the above genetic abnormalities. In clinical practice, the major problems are: Hb Bart's hydrops fetalis (homozygous α^{o}-thalassaemia), homozygous β^{o}-thalassaemia, E-β thalassaemia and HbH disease. The laboratory procedures for diagnosis are standardised and the molecular basis of most of these genetic abnormalities are characterised. Thus it is possible to formulate a strategy for the detection and prevention of these disorders. The steps include the setting-up of population screening and genetic counselling service for the affected individuals, Society of Thalassaemias for public education and group support, and prenatal diagnosis with selective abortion of affected pregnancies. We embarked on such a programme between 1988 and 1992 in Kuala Lumpur General Hospital and hope to kindle similar effort in other state hospitals.

Key words: Haemoglobinopathy, prenatal diagnosis.

INTRODUCTION

Thalassaemia and related disorders are the most common genetic disorders seen in this country. As in other parts of South-East Asia[1] α-thalassaemia, β-thalassaemia, Haemoglobin E and Haemoglobin Constant Spring are prevalent. It is estimated that 1 in 4 persons carries the abnormal gene. The clinical problems are: Bart's hydrops fetalis, β-thalassaemia major, Haemoglobin E-β thalassaemia and Haemoglobin H disease. With recent success in economic growth and improved health care, genetic disorders vis-a-vis thalassaemias/ haemoglobinopathies have emerged as important causes of infant and childhood mortality and morbidity. Control strategies include prevention of new births of thalassaemias and proper treatment of existing disease. The latter involves use of iron chelators which are expensive and unaffordable by most patients. Between 1988 and 1992, with a grant secured under the Intensification of Research in Priority Areas (IRPA) mechanism from the Ministry of Science, Technology and Environment we embarked on a programme to identify the clinical problems and subsequently formulate a strategy for prevention. This resulted in the establishment of special laboratory procedures for screening and confirming the diagnosis of various thalassaemias/haemoglobinopathies as well as determining genetic abnormalities. We were able to carry out a survey to study the prevalence and do case detection to identify serious problems and at the same time establish a clinic for genetic counselling for the affected individuals. With collaboration of obstetricians trained in foetal-maternal medicine, a prenatal diagnosis programme was set up for the affected couples. For group support and public education, a national society for thalassaemias was formed.

Type and Prevalence

The thalassaemias/haemoglobinopathies that are commonly seen are α-thalassaemia, β-thalassaemia, Haemoglobin E, Haemoglobin Constant Spring and δβ-thalassaemia. A cross-sectional study of a Malay community in Tanjung Karang showed that out of 111 individuals screened by full blood counts, reticulocyte stain, haemoglobin electrophoresis, HbA2 and HbF estimations one in four persons carried one or more of these abnormal genes. A well-planned programme to establish the epidemiology of the disorders is required as part of the strategy for prevention. Establishing the frequency and the incidence of the different mutant genes helps to assess the magnitude of the problem besides studying the natural history and the clinical severity of each specific disorder.

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Clinical problems

The abnormal genes in different combinations lead to a number of syndromes. Clinically they are divided into thalassaemia minor, major and intermedia depending on the severity of the anaemia. Thalassaemia minor are heterozygotes or carriers and are asymptomatic. β-thalassaemia and α-thalassaemia 1 traits usually show abnormal red cell indices. Some of them may manifest themselves with mild pallor which could be mistaken for iron deficiency and thus are given iron medication unneccesarily. Thalassaemia major and intermedia are the serious forms of the disease which can cause considerable morbidity and mortality. In the homozygous α-thalassaemia 1 there is no α-globin chain production and this results in Hb Bart's hydrops fetalis. This not only causes perinatal death but is also associated with life-threatening maternal obstetric complications of severe eclampsia and difficult vaginal delivery. The problems of β-thalassaemia major are that of severe anaemia and its associated complications. Patients present with severe anaemia usually after one year of life and without transfusion early death in childhood occurs. With hypertransfusion regime without iron chelators patients die in early adulthood due to complications of iron overload. Iron chelators are not freely available in this country and because of their high cost are not affordable by most patients. Patients with thalassaemia intermedia are usually symptomless with normal physical development and no thalassaemic facies. Clinically there is mild to moderate anaemia, jaundice and hepatosplenomegaly. However, haemoglobin drops when there is associated infection and patient then requires proper treatment of infection and blood transfusion. The thalassaemia intermedia commonly seen in Malaysia are Hb H disease and Hb E-β thalasaaemia. Accurate diagnosis at an early age is important otherwise patients may be treated with frequent transfusions and end-up being transfusion dependent.

Molecular pathology

With the advent of recombinant DNA technology, the molecular defects of thalassaemias have been increasingly characterised over the last 15 years.[2] The development of rapid and easy methods of detection of known mutations has allowed the establishment of these techniques locally. This has enabled us to characterised the molecular defects in our thalassaemia population. The knowledge of pattern of mutations in different ethnic groups forms an important prerequsite for the setting up of a prenatal diagnosis programme. The molecular defects in both α and β-thalassaemias are heterogeneous but it is well known that the most common defect in α-thalassaemia is gene deletion whereas in β-thalassaemia the majority are due to point mutations.

A normal person has 4 α-globin genes with a duplicated pair located on each chromosome 16. The severest form of α-thalassaemia, α-thalassaemia 1 (α^0) involves deletion of the duplicated genes. Homozygosity for this defect (deletion of 4 α-globin genes) results in Hb Bart's hydrops fetalis. The milder form, α-thalassaemia 2 (α^+) has one α-gene left functioning with the most common abnormality being deletion of one α-globin gene. The next most common defect is a non-deletional α-globin gene abnormality where there is a point mutation at the termination at the termination codon of α2-globin gene resulting in an elongated unstable α-globin mRNA. The resultant product is a reduced amount of haemoglobin known as Hb Constant Spring (HbCS) and phenotypically behaves like an α-thalassaemia 2 trait. Co-inheritance of an α-thalassaaemia 1 and α-thalassaemia 2 genes causes Hb H disease. We examined 13 cases of Hb-H disease seen at the UKM adult Haematology Clinic and found that 6 cases were of genotype α-/– – (deletion of 3 α-globin genes), 4 cases were due to co-inheritence of HbCS and α-thalassaemia 1 ($\alpha^{CS}\alpha$/– –), 1 was homozygous for HbCS ($\alpha^{CS}\alpha/\alpha^{CS}\alpha$) and 2 showed co-inheritence of HbCS with another non-deletional single α-globin gene abnormality yet to be characterised. Single α-globin gene deletions have been shown to be of two types, the commonest being deletion of 3.7 kb of DNA and the less common type involves deletion of 4.2 kb of DNA.[3] We have observed the 3.7 kb α-globin gene deletion ($\alpha^{-3.7}$/) in all of the chromosomes with single α-globin gene deletion. The length of DNA deleted in α^0 thalassaemia is also known to be variable. The commonest type in this part of the world is that involving deletion of 20 kb of DNA ($--^{SEA}$ type).[3] We have examined 18 chromosomes carrying α^0 genes and found that they were all of the $--^{SEA}$ type.

Unlike α-thalassaemia, the gene abnormalities in β-thalassaemia are mainly point mutations or small gene deletions. Large gene deletions as seen in α-thalassaemia are uncommon, the well known ones being a 619 bp β-globin gene deletion

seen in certain parts of India and a 1.9 kb deletion seen in the Mediterranean.[2] To date more than 100 different β-thalassaemia mutations have been identified worldwide and they are shown to be population specific.[2] Most of the mutations occur in exons 1 and 2, intervening sequence 1 (IVS 1) and others in the flanking regions, intervening sequence 2 (IVS 2) and a few in exons 3 of the β-globin gene complex which is located on the short arm of chromosome 11. The mutations, most of which are single nucleotide substitutions produce defects in transcription, RNA splicing, RNA modification, translation via frameshift and nonsense codons, or they produce highly unstable β-globin. Most of these mutations cause β^0 thalassaemia.[2]

We have so far examined 134 chromosomes carrying β-thalassaemia mutations (55 Malays, 76 Chinese and 3 Indians) from both homozygous and heterozygous β-thalassaemia cases including HbE-β thalassaemia. By using established rapid methods of detection these patients were screened for known mutations. To date 80-90% of the β-thalassaemia genes have been characterised in the Malays and the Malaysian Chinese. Four previously described alleles were found in the Malays with 3 of the alleles accounting for 80% of the disease genes (Table 1). The commonest mutation among the Malays is IVS 1 nt-5 (G-C), a common mutation seen among the Indians in the Indian subcontinent and also the Melanesians.[1] The spectrum of β-thalassaemia alleles in Malaysian Chinese appear to be similar to those of Southern Chinese from Mainland China. 2 of the alleles account for almost 80% of the Chinese β-thalassaemia genes (Table 2). The commonest mutation seen among the Malaysian Chinese is

Table 1: Spectrum of β-Thalassaemia mutations in Malays

Mutations	No of chromosomes
IVS 1 nt-5 (G-C)	21
Frameshift 41-42 (-TCTT)	12
IVS 1 nt-1 (G-T)	4
Nonsense Codon 17 (A-T)	2
-28 (A-G)	1
Uncharacterised	9
Total	49

N.B. The chromosomes were not screened for nonsense codon 19 mutation, reported to occur in as high as 15% among the Malays in one series (Yang KG *et al*, Br J Haematol, 1989)

Table 2: Spectrum of β-Thalassaemia mutations in Malaysian Chinese

Mutations	No of Chromosomes
Frameshift 41-42 (-TCTT)	35
IVS 2 nt-654 (C-T)	26
-28 (A-G)	3
Nonsense Codon 17 (A-T)	2
Uncharacterised	10
Total	76

the frameshift abnormality at codon 41-42 (deletion of 4 nucleotides, -TCTT) with the next commonest being IVS 2 nt-654 (C-T). It is interesting to note that the frameshift abnormality at codon 41-42 is also commonly seen among the Malays and is reported to occur at high frequency among the Thais.[1] Most of these mutations are known to cause severe impairment of β-globin chain production. In Malaysia homozygosity or compound heterozygosity for most of these mutations result in transfusion-dependent β-thalassaemia major syndrome. Haemoglobin E, is essentially a β-globin structural variant resulting from a mutation in codon 26 (glu→lys) common among the Malays. This mutation also results in abnormal transcription leading to a reduction in the β-globin chains and hence demonstrating a β^+ thalassaemia effect. A compound heterozygous state with another β-thalassaemia mutation causes, in most cases, thalassaemia intermedia. Thalassaemia is rare among the Malaysian Indians. We examined 3 chromosomes from one homozygous and one heterozygous Indian individuals. Two of the alleles were found to have the mutations -28 (A-C) and -88 (C-T) with the other allele not characterised.

The detection of large gene deletions as in α-thalassaemias are carried out by Southern blot and hybridization technique. Genomic DNA prepared from peripheral blood cells is digested with restriction enzymes. DNA fragments are then separated according to size by electrophoresis on agarose gel and transferred to a nylon membrane by Southern blotting, followed by hybridization of membrane to P^{32}-labelled α-gene or zeta-gene specific probes and autoradigraphy. Results are interpreted by analysing the DNA fragments of specific sizes on x-ray film (Fig. 1). Point mutation defects as in β-thalassaemia and Hb Constant Spring are detected by polymerase chain reaction techniques. We adopted the Amplification Refractory

Mutation System, where mutations are detected by using primers designed to identify a specific allele.[4] The principle of the test is based on the fact that a single mismatch between primer and the patient's target gene sequence will not lead to any amplification of the target gene. Results are interpreted by presence or absence of DNA fragment of an expected size on agarose gel (Fig. 2).

Prenatal Diagnosis

Prevention of new births forms an important step in any strategy of genetic diseases. The single most effective step in the prevention of severe forms of thalassaemias and related problems is selective abortion of afflicted fetuses diagnosed prenatally. The availability of rapid methods for detection of mutation together with the collaboration of obstetricians trained in foetal-maternal medicine has made the setting up of prenatal diagnosis services locally posssible. With the availability of procedures for chorionic villus sampling prenatal diagnosis can be performed as early as 8-9 weeks of pregnancy. We successfully performed our first case using chorionic villus sampling in August 1991 and a healthy baby girl

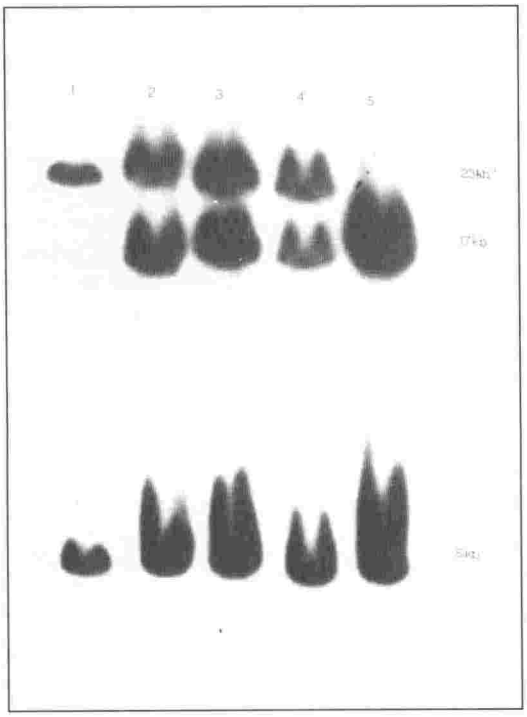

FIG. 1: Autoradiograph of Southern blot using P^{32}-labelled zeta-gene probe on DNA digested with restriction enzyme Eco RI in the diagnosis of α-thalasaemia

Lane 1 : normal individual (αα/αα) showing a 23 kb and 5 kb DNA band.

Lanes 2,3,4 : DNA from father, mother and a known α-thalassaemia 1 trait (αα/– –) showing 23, 17 and 5 kb DNA bands.

Lane 5 : Chorionic villus sample (CVS) showing only 17 and 5 kb bands. The absence of the 23 kb with presence of 17 kb DNA fragment in the CVS confirms the foetal genotype to be (– –/– –) .

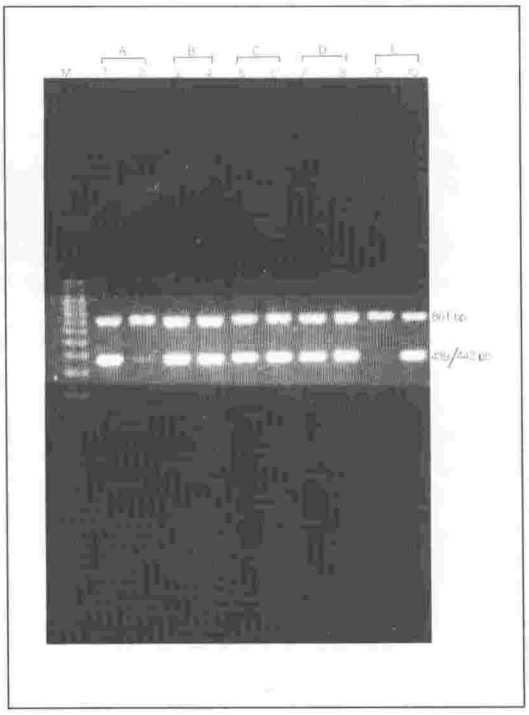

Fig. 2: DNA analysis of chorionic villus sample (CVS) by the ARMS technique to detect β-thalassaemia mutation frameshift 41-42 (-TCTT).

Lane M contains the X174 marker

Lanes 1,3,5,7,9 : using primers to detect normal allele, a 443 bp DNA fragment

Lanes 2,4,6,8,10 : using primers to detect mutant allele, a 439 bp DNA fragment

The upper band in each lane is the 861 bp fragment of another part of the β-globin gene used as internal control

A : normal DNA showing amplification only with normal primers

B,C,D : DNA from father, mother and fetus respectively, showing amplification with both normal and mutant primers indicating their heterozygous state for this mutation

E : DNA of a known homozygous Fr 41-42 showing amplification only with mutant primers

was born at 38th weeks gestation who was confirmed to have β-thalassaemia trait as predicted prenatally[5] (Fig. 2). Both parents are heterozygous for β-thalassaemia mutation at codon 41-42. To date we have performed prenatal diagnosis on 5 cases of β-thalassaemia and 4 α-thalassaemia and in 3 cases parents elected to terminate the pregnancy[6] (Fig. 1). DNA analysis of foetal tissue obtained at curretage confirmed the diagnosis.

Malaysian Association of Thalassaemias, Kuala Lumpur

This was set up in June 1988. Public education programmes and community projects were organised and genetic counselling was provided. Affiliated associations were subsequently formed in two other states.

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