

LABORATORY DIAGNOSIS OF COMPLEX PHENOTYPES IN HEMOGLOBINOPATHIES

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ABSTRACT

β -Thalassaemia is one of the most extensively studied genetic diseases with extremely diverse genotype-phenotype correlation. Extensive investigations carried out in the last two decades helped define and understand the molecular basis many types of β -thalassaemia syndromes as well as shedding light into elucidating their clinical phenotypic heterogeneity. Many laboratories were able to use state-of-the-art technology to decipher some of the complex forms of gene rearrangements, DNA sequence variations, gene deletions and insertions and many more. In this presentation, I will review some of the laboratory approaches, which helped us decode numerous complex genotypes.

Thalassemys are a heterogeneous group of inherited blood disorders characterized by a decreased or absent globin chain synthesis. A genetic defect that impairs the α -globin chain production results in α -thalassaemia (thal), the most common single gene disorder in the world. A relative decrease or absence of β -globin synthesis causes β -thal. It is estimated that approximately 400 million people throughout the world are heterozygous for the hemoglobinopathies. In many developing countries, the disease poses a major public health problem with considerable burden on the medical and financial resources.

Thalassemys are prevalent in high frequencies in many parts of the world. Thus, prevention of thalassemys presents a major challenge in many developing and under-developed countries with limited resources. By applying appropriate hematological and DNA methods, much can be achieved in relatively short time in obtaining reliable database to establish preventive programs including premarital, carrier, newborn screening as well as setting up comprehensive genetic counseling and prenatal diagnosis facilities.

The study of hemoglobinopathies has contributed enormously to the understanding of basic principles of genetics and has provided an excellent example of how new methods can be applied to analyze genetic diseases. Many of the advances made in molecular medicine can be illustrated by reference to the thalassemys. The recent advances in molecular biology have enabled researchers to define the basic molecular defects responsible for diverse phenotypes. Further understanding the underlying molecular mechanisms in different types of globin gene defects has paved way to investigate the molecular basis of many other genetic diseases.

The application of molecular biology techniques to the study of genetic diseases has provided an enormous wealth of information during the past two decades. This knowledge has been vital in understanding the fundamental molecular basis of genetic diseases and was used extensively in their treatment and management. Without reliable, accurate and rapid laboratory techniques, it would have been impossible to establish comprehensive screening programs for carrier detection, mutation analysis, and prenatal diagnosis (PND).

SOME EXAMPLES OF COMPLEX HEMOGLOBINOPATHIES:a. β -THALASSEMIA CARRIERS WITH A CONCOMITANT GLOBIN DEFECT

A typical β -thalassemia carrier state, clinically silent and characterized by reduced MCV (<79 fl), MCH (<27 pg), increased HbA₂ (>3.5 %) and imbalanced α/β globin chain (α/β ratio = 1.3-1.8), may be modified by several genetic factors, such as concomitant α and δ globin gene lesions. Moreover, the primary defect in β globin synthesis can have a considerable effect on the phenotype and result in an outcome that may be silent hematologically or be relatively severe as thalassemia intermedia.

b. HETEROZYGOUS β -THALASSEMIA AND α -GENE DEFECTS

Coinheritance of α -thalassemia, in particular $-\alpha/-\alpha$ and $--/\alpha\alpha$ genotypes, results in increased MCV and MCH, sometimes up to normal values. The practical consequence of this knowledge is that in populations where both α and β -thalassemia are common, as in Dubai, screening by MCV determination may inadvertently miss some of double heterozygotes. By contrast, coinheritance of HbH disease genotype ($--/-\alpha$) with heterozygous β -thalassemia markedly reduces MCV and MCH, which are even lower than in simple β -thalassemia carrier state. The presence of triplicated α -globin gene in association with β -thalassemia trait can worsen the imbalance of α/β ratio, resulting in the clinical phenotype of thalassemia intermedia (see below).

In all above conditions, HbA₂ level is always in the carrier range (3.5-6.5 %), and hence useful for diagnosis. The α/β globin chain synthesis ratio is reduced, sometimes lower than 1, while it is increased (α/β ratio >2.0) when the triplicated α -globin gene complex is associated with heterozygous β -thalassemia.

c. HETEROZYGOUS β -THALASSEMIA WITH NORMAL HbA₂ LEVELS

- i. Elevation of HbA₂ is the most relevant indicator of the β -thalassemia carrier state. However, β -thalassemia carriers may have normal or borderline (HbA₂ = 3.0 - 3.5 %) HbA₂ levels.
- ii. Normal HbA₂ δ and β -thalassemia: Normal HbA₂ is a feature of the double heterozygotes for δ and β -thalassemia (both *in cis* and *in trans*); nevertheless these subjects have low MCV, MCH and the α/β ratio, which is in the range of β -thalassemia carriers. This is used for their differentiation from the α -thalassemia carrier state.
- iii. Normal HbA₂ / β -thalassemia: Another form of normal HbA₂/ β -thalassemia, relatively common in Greek population, is that associated with the β -globin chain variant Hb Knossos ($\beta27$ G \rightarrow T). The normal HbA₂ level is due to the presence of a δ° mutation (δ° 59 -A) *in cis* to the β -globin gene.

- iv. $[\epsilon\gamma\delta\beta]^0$ -Thalassemia is a rare form of β -thalassemia with normal HbA₂ level, caused by a series of extended deletion which remove the β globin gene cluster or the β LCR.
- v. Corfu $\delta\beta$ -thalassemia: This is another rare form of β -thalassemia with normal HbA₂, in which a partial deletion the δ gene is linked *in cis* to a G→A mutation in the β -IVS-1-5 position. The $\delta\beta$ -thalassemia carriers also have normal HbA₂ levels, but they are easily identified by the presence of a marked increase of HbF and imbalance in the α/β ratio.
- vi. α , δ and β -thalassemia: A rare complex thalassemia genotype which may be missed during thalassemia carrier screening is the coexistence of α , δ and β -thalassemia. These carriers may have normal MCV and α/β ratio (as a consequence of α and β -thalassemia interaction) and normal HbA₂ (as a consequence of δ and β -thalassemia interaction).

d. MILD AND SILENT β -THALASSEMIA ALLELES

- i. Heterozygotes for some mild (IVS-I-6; T→C) and silent β -thalassemia alleles (-101 C→T; -92 C→T; IVS-II-844; C→G) have borderline or even normal levels of HbA₂. Carriers of these silent alleles may have also normal MCV, MCH and normal α/β ratio. Their identification is usually retrospective, in parents of patients with thalassemia intermedia.
- ii. The α -globin gene triplication can be considered as a very mild β -thalassemia allele. There is an excess production of α -globin chain, and this is usually associated with a silent hematological phenotype.

e. SEVERE β -THALASSEMIA CARRIERS

In some rare cases, a simple β -thalassemia heterozygote allele may result in a severe β -thalassemia major phenotype. This is known as 'dominantly inherited β -thalassemia' and is mostly due to the presence of β globin Exon 3 mutations that lead to the formation of hyperunstable β chains.

DIFFERENTIATION BETWEEN THALASSEMIA MAJOR AND INTERMEDIA- IMPORTANCE OF LABORATORY DIAGNOSIS

The differentiation between thalassemia major and intermedia is critical for optimal and appropriate treatment. In fact, an accurate prediction of a mild phenotype may avoid unnecessary packed red blood cell transfusions and the interruption of pregnancy in case of prenatal diagnosis. Since the degree of globin chain imbalance is the main determinant of the severity of thalassemia syndromes, any factor able to reduce this imbalance may produce the mild phenotype of no transfusion dependent thalassemia intermedia. Three main factors have been so far been identified:

- a) *mild or silent β -thalassemia alleles*
- b) *co-inheritance of α -thalassemia*
- c) *co-inheritance of determinants that increase γ -chain production*

The relative importance of these determinants is different in varying conditions. Apart from some well-defined factors, such as the presence of the silent -101 C→T β gene promoter mutation, most of these conditions will always be associated with moderate thalassemia intermedia even in combination with severe β^0 -thalassemia mutations. Thus rendering it harder to predict the severity of the phenotype consistently.

β -THALASSEMIA INTERMEDIA IN THE UAE

In the UAE, β -thalassemia intermedia in several cases deserve particular attention. A total of 53% of the β -thalassemia homozygote patients in the UAE are homozygous for the severe IVS-I-5 (G→C) mutation. Through haplotype studies, it was determined that some of these alleles are on a chromosome positive for the G γ -158 C→T polymorphism (also known as the Xmn I site). This genetic marker increases the upregulation of the G γ chains and leads to the manifestation of a mild disease. Only in some cases molecular mechanisms responsible for the increased γ chain production, and hence for the amelioration of the phenotype, have been identified. Two siblings homozygous for this mutation were free of regular blood transfusions until they were late teenage years. Both were found to be +/+ for the Xmn I polymorphism.

Furthermore, it was established a decade ago that the incidence of α -thalassemia in the UAE was 50%. This makes the concomitant presence of this genetic determinant with another hemoglobinopathy highly likely. The co-inheritance of a β gene defect together with α -thalassemia and possibly with Xmn-I polymorphism in many patients render them virtually free of blood transfusions for life. This is of paramount significance in certain areas where hemoglobinopathies are prevalent.

Understanding the complex phenotypes and the relationship between genotype and phenotype is useful in clinical practice for planning appropriate treatment, in genetic counselling for predicting the phenotype and in prenatal diagnosis for the identification of atypical carriers during β -thalassemia screening. Special consideration must be made for the implications of such complex phenotypes in prenatal diagnosis of thalassemias, management of thalassemic patients and requirement of life long blood transfusions.