

## DIAGNOSTIC METHODS FOR $\alpha$ - AND $\beta$ -THALASSEMIA

**Davlatova G.N., Juraeva N.T., Boboev K.T., Mirzoeva L.A.**

*Republican Specialized Scientific and Practical Center of Hematology*

**Key words:**  $\alpha$ - or  $\beta$ -thalassemia, globin chains, molecular defects, molecular genetic research methods.

**Annotation:** *Thalasseмии are a group of congenital microcytic hemolytic anemias that are characterized by a defect in hemoglobin synthesis. Thalasseмии can be defined as  $\alpha$ - or  $\beta$ -thalassemias depending on the defective globin chain and the underlying molecular defects. Alpha thalasseμία is especially common among people of African, Mediterranean, or South Asian descent. Beta thalasseμία is more common in people of Mediterranean, Middle Eastern, South Asian and Indian origin. Symptoms and signs are caused by anemia, hemolysis, splenomegaly, bone marrow hyperplasia, and iron overload may occur with repeated blood transfusions.*

### INTRODUCTION

Carriers of  $\alpha$ - and  $\beta$ -thalassemia (heterozygotes) exhibit microcytic hypochromic parameters with mild or no anemia. Although the main research methods in routine clinical practice used in the diagnosis of thalasseμία are still considered hematological research methods and Southern blotting, and molecular analysis was not mandatory until recently to confirm the diagnosis of  $\beta$ -carrier. However, molecular genetic testing is necessary to confirm  $\alpha$ -thalassemia carrier status. Molecular diagnostics are needed to predict severe transfusion-dependent cases and intermediate and mild non-transfusion-dependent cases. Chorionic villus DNA testing is a prenatal diagnostic method, and the methods are the same as for detecting mutations, depending on laboratory equipment and experience.

Thalasseμία syndromes are a heterogeneous group of hemoglobin disorders caused by decreased or absent production of normal globin chains. They are the most common recessive diseases worldwide, with an estimated 1–5% of the world's population carrying the thalasseμία genetic mutation [1]. Thalasseμία is most common in Southeast and South Asia, the Middle East, Mediterranean countries, and North and Central Africa. However, due to ongoing population migration, thalasseμία is now becoming increasingly common in Northern Europe and North America. These changes have challenged health professionals and policymakers throughout the region to ensure equitable access to quality services for the prevention, diagnosis and adequate treatment of thalasseμία. Despite the many animal models described to date, the study of natural deletions and point mutations in carriers and patients remains important for understanding expression regulation and disease mechanisms. In the present review, we will highlight aspects of the molecular basis of  $\alpha$ -thalassemia that will provide insight

into how we can understand the mechanisms underlying  $\alpha$ -thalassemia [3]. Human hemoglobin is a tetrameric protein consisting of two alpha-like and two beta-like globin chains, each forming a pocket containing a heme group for oxygen binding. The globin genes are located in two distinct gene clusters at different chromosomal loci in the order of their expression during development. Expression is regulated by complex interactions of transcription factors and regulatory elements (promoters and enhancers) to turn genes on and off in a stage-specific and tissue-specific manner. Globin gene disorders (hemoglobinopathies) are characterized by either abnormal variants of globin chains, such as sickle cell disease, or decreased synthesis of globin chains in erythroid cells (thalassemia) during hematopoiesis [1]. Hemoglobinopathies are inherited predominantly in an autosomal recessive manner. Reduction or absence of  $\alpha$ -globin chains leads to an excess of unpaired beta-( $\beta$ )-like globin chains that form insoluble homotetramers, leading to intracellular precipitation, ineffective erythropoiesis and acute hemolytic anemia typical of severe forms of  $\alpha$ -thalassemia [4,5]. Alpha thalassemia is an inherited autosomal recessive disease characterized by microcytic hypochromic anemia. This is one of the most common monogenic gene disorders among the world's population. Clinical severity ranges from almost asymptomatic to mild microcytic hypochromia and a fatal hemolytic condition called Barth fetal hydrops syndrome.

Materials and methods of research. If thalassemia is suspected, the following will be checked:

- Peripheral blood smear
- Hemoglobin electrophoresis
- Study of DNA structure (prenatal diagnosis)

Diagnosis is based on genetic research and quantitative analysis of the structure of hemoglobin. Studies of thalassemia patients have been instrumental in identifying numerous causative mutations in globin genes, upstream and downstream untranslated regions, and regulatory elements controlling the expression of  $\alpha$ - and  $\beta$ -globin gene families and hence hemoglobin switching. Cell lines and mouse models have allowed the identification of a diverse collection of interacting transcription factors and other protein complexes involved in the regulation of the expression of these genes [2,5]. There are more than 200 known  $\beta$ -thalassemia mutations, most of which are single nucleotide substitutions, insertions, or short deletions. Several large  $\beta$  gene deletions have been identified, and most of them can be diagnosed using Gap-PCR. Fortunately, a limited number of point mutations are common among different ethnic groups; Therefore, for any given ethnic region, a PCR method is initially used, designed to simultaneously detect a general specific mutation. With this approach, more than 80% of cases can be identified for most ethnic groups.

For  $\beta$ -thalassemia mutations, the reverse dot blot technique, in which amplified DNA is hybridized to a panel of mutation-specific probes attached to a nylon strip, is

widely used. Some laboratories also use the amplification-resistant mutation system: it allows you to quickly, economically and conveniently test several mutations at the same time. Denaturing gradient gel electrophoresis is an alternative to the above methods and is useful in countries where a very wide range of  $\beta$ -mutations occurs. Almost 90% of  $\beta$ -mutations can be detected by a shift in the band structure towards normal. However, improvements and decreasing costs of DNA sequencing have made the  $\beta$  gene direct sequence the most widely used in many laboratories, allowing analysis of the exact nucleotide sequence of a gene or the gene region being assessed. The original method described by Sanger et al. was modified in 1977, and several types of automatic sequencers are available today. Direct sequencing also allows the identification of unknown mutations [2].

Diagnosis of alpha thalassemia. The molecular basis is usually deletions and, less commonly, point mutations affecting the expression of one or more duplicated  $\alpha$  genes. Clinical variations and increasing disease severity are directly associated with decreased expression of one, two, three, or four copies of the  $\alpha$ -globin genes. Deletions and point mutations in  $\alpha$ -globin genes and their regulatory elements have been studied extensively in carriers and patients, and these studies have provided insight into whether  $\alpha$ -globin genes are regulated. By looking at natural deletions and point mutations, our knowledge of globin gene regulation and expression will continue to expand and lead to new therapeutic targets. [1, 2]. Alpha thalassemias arise mainly from deletions of varying lengths and can be detected predominantly by reverse dot blot and Gap-PCR. Primer sequences have been published for the diagnosis of several types of  $\alpha^+$  or  $\alpha$  thalassemias [8, 13, 15]. Amplification of sequences in the  $\alpha$ -globin gene cluster is more difficult than in the  $\beta$ -globin gene cluster due to significant sequence homology within the  $\alpha$ -globin gene cluster. For unknown  $\alpha$ -deletions, Southern blotting is still used in some laboratories, but more recently multiplex ligation-dependent probe amplification has been introduced into research practice. This method is now used instead of Southern blotting because of its sensitivity and reliability, without the need for the former radioactive detection method.

After the era of Sanger sequencing, next generation sequencing (NGS) represents a new principle of sequencing technology, as this technology increases sequencing throughput from a few hundred base pairs to several thousand in a single analysis. NGS, based on massively parallel sequencing of clonally amplified DNA molecules, coupled with sufficient computing power and appropriate software for efficient data analysis, has allowed genetic diagnostics to enter clinical practice at an affordable cost. It can be applied to the entire genome, the entire exon, and to specific target regions of the genome. Currently, there are no data in the literature on sequencing globin genes using this approach. There is only one commercial sequencing panel available (TruSight Inherited Disease, Illumina Inc.) that reports that the HBB and HBA1 genes are included, but sequencing only two genes does not justify the cost of the entire procedure

for one patient. The most important step of NGS is the experimental development of a set of probes that will be used to capture DNA: the high level of homology between genes in the alpha and beta clusters makes the NGS approach to thalassemia very complex, time-consuming and expensive. Another important point about NGS is that it is useful for detecting single nucleotide substitutions and insertions or small deletions (depending on the type of study), but is less accurate for other types of genomic variations.

Although bioinformatics research is now also working on tools to test for the presence of complex rearrangements, large deletions or triplications are currently missed, and especially in the case of alpha thalassemia, this renders the entire procedure completely useless.

In conclusion, it is important to emphasize that DNA testing for globin gene mutations is not required to diagnose most carrier status. It is advisable to confirm the diagnosis of TDT by knowing the mutations of the parents, and in cases of NTDT, where co-inheritance of different globin gene defects may be responsible for the different expression of the phenotype. Some cases are hematologically and clinically difficult to diagnose even with family hematological workup due to the presence of mild mutations or  $\alpha$ - and  $\beta$ -interactions; Therefore, in such cases, it is convenient to carry out molecular analysis before starting treatment.

Chorionic villus DNA testing is a prenatal diagnostic method and the methods used are the same as for detecting mutations, depending on laboratory equipment and experience. An important step in prenatal diagnosis is the removal of any maternal tissue, since the presence of residual maternal tissue may lead to diagnostic error. To exclude maternal contamination, the presence of short tandem repeats or variable numbers of tandem repeats in fetal and parental DNA samples by PCR is important. Details of the methods commonly used for molecular diagnosis of thalassemia are described in the International Thalassemia Federation publications Prevention of Thalassemia and Other Hemoglobin Disorders.

#### LIST OF USED LITERATURE:

1. Brancaleoni et al. Laboratory diagnosis of thalassemia //International journal of laboratory hematology, 2016; 38: 32-40.
2. Cardiero G. et al., Alpha-Thalassemia in Southern Italy: Characterization of Five New Deletions Removing the Alpha-Globin Gene Cluster, International Journal of Molecular Sciences, 2023; 3(24): 2577,
3. Farashi S., Harteveld C. L. Molecular basis of  $\alpha$ -thalassemia //Blood Cells, Molecules, and Diseases, 2018; 70: 43-53.
4. Ferih Kh., et al. Applications of Artificial Intelligence in Thalassemia: A Comprehensive Review, Diagnostics, 10.3390/diagnostics13091551, 2023; 9 (13):1551.



5. Saad H. K. M., et al. , HBB Gene Mutations and Their Pathological Impacts on HbE/ $\beta$ -Thalassaemia in Kuala Terengganu, Malaysia, *Diagnostics*, 2023; 7(13): 1247.
6. Krishnevskaya E. et al. New-Generation Ektacytometry Study of Red Blood Cells in Different Hemoglobinopathies and Thalassemia, *Thalassemia Reports*, 10.3390/thalassrep13010007, 2023; 1(13): 70-76.