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LABORATORY DIAGNOSTICS OF BETA-THALASSEMIA IN THE REPUBLIC OF UZBEKISTAN.

Davlatova G.N
Juraeva N.T
Boboev K.T
Mirzoeva L.A
Republican Specialized Scientific and Practical Center of Hematology

Abstract: *Thalassemia is a group of genetically determined blood diseases that develop when the synthesis of alpha or beta chains of hemoglobin is impaired, accompanied by hemolysis, hypochromic anemia and microcytosis. In hematology, thalassemia refers to hereditary hemolytic anemias - quantitative hemoglobinopathies. Thalassemia is widespread among the population of the Mediterranean and Black Sea regions; The name of the disease literally translates as "sea coast anemia." Also, cases of thalassemia are not uncommon in the countries of Africa, the Middle East, India and Indonesia, Central Asia and the Caucasus. Every year, 300 thousand people worldwide develop thalassemia syndrome. children. Depending on the course of thalassemia, it can be mild, fatal or mild and asymptomatic.*

Key words: *β-thalassemia, globin chains, molecular defects, molecular genetic research methods.*

INTRODUCTION.

Thalassemia is autosomal recessive, meaning both parents must be affected by the disease or carriers of the disease to pass it on to the next generation [4].

Currently, about 5% of the world's population are carriers of a potentially pathological hemoglobin gene (that is, these are healthy people who received only one mutant gene from their parents) [6]. Unfortunately, at present, the region of Central Asia, including Uzbekistan, remains a blank spot on the thalassemic map of the world, where there are still no basic biochemical and molecular genetic methods for diagnosing this pathology.

Currently, for most countries of the world seriously affected by this disease (Italy, Greece, Iran, Turkey, Azerbaijan, etc.), the prevalence of beta thalassemia has been assessed and the spectra of mutations in the beta globin gene have been determined and optimal prenatal DNA strategies have been developed - diagnostics.

Most of the currently found beta-thalassemic mutations (and about 200 of them are known) are of ancient origin, and each population affected by beta-thalassemia is characterized by its own specific set of mutations, sometimes wide, sometimes narrow, depending on the level of ethnic homogeneity, with prevailing defects or without them [7]. Beta thalassemia results from point mutations in the beta globin gene [1]. The severity of the disease depends on the nature of the mutation [5].

Mutations are characterized as (β0) if they prevent any formation of β-globin chains, mutations are characterized as (β+) if they allow the formation of some β-globin chains to

occur. Three main forms have been described: thalassemia minor, thalassemia intermedia and thalassemia major, which range from asymptomatic or mild symptoms to severe anemia requiring lifelong transfusions [8]. People with beta thalassemia (those who are homozygous for thalassemia mutations or inherit 2 mutations) usually present during the first two years of life with symptomatic severe anemia, poor growth, and skeletal abnormalities. Thalassemia major, if left untreated, eventually leads to death, usually from heart failure; therefore, prenatal screening is very important [3]. Those with beta thalassemia intermedia (those who are compound heterozygotes for the beta thalassemia mutation) typically present later in life with mild to moderate symptoms of transfusion anemia [8]. The disease is divided into several forms:

1. Beta thalassemia minor (mild form) - often asymptomatic, can only manifest as mild anemia.

2. Beta thalassemia interlude (moderate form) - requires periodic treatment, may manifest as moderate anemia and other symptoms.

3. Beta thalassemia major (severe form) - requires regular blood transfusions and intensive treatment, as without treatment it can lead to serious complications and a threat to life.

The current situation in Uzbekistan: Currently in the Republic of Uzbekistan there are 346 children with beta thalassemia and 35 adults over 18 years old in the dispensary, in addition there are also hidden carriers. All registered patients, based on the results of molecular genetic analysis, have been receiving chelation therapy over the past 8 years, as a result of which mortality rates have decreased, quality of life has improved and life expectancy has increased. Before chelation therapy, 99% of children died before reaching adolescence. However, in patients with beta thalassemia, bone marrow transplantation is successful in more than 90% of cases;

Material and methods of research. The study included 120 patients of various age groups (67 men, 53 women, aged from 2 to 45 years, 22.4 ± 1.2), sent for examination and treatment at the Republican Specialized Medical Center of Hematology of the Ministry of Health of the Republic of Uzbekistan. with beta thalassemia (MCV ‹ 80 fL) hypochromic (MCH ‹ 27 pg) anemia with Hb › 70 g/l.

The following parameters were used: 1) erythrocyte (Sysmex XT-4000i, Japan) and Mentzer index (Rbc/Hb); 2) serum iron and ferritin (Cobas 6000, Roche, Switzerland); 3) hemoglobin fractions (isoelectric focusing (IEF) on agarose, system for electrophoresis sas1 and sas2 helenabiosciences, UK).

We used “cutoff” values recommended by the manufacturer: Hb A2 › 3.7%, HbF › 2%; 4) mutations of the beta-globin gene (method of reverse hybridization of multiplex PCR products with oligonucleotide probes fixed on strips, β-globin stripassay kit test system, viennalab diagnostics, Austria).

The material for the study was venous blood. For hematological studies, electrophoresis and molecular genetic analysis, blood was collected into tubes with K3EDTA as an anticoagulant, and to obtain serum - into tubes with a coagulation activator (vacuette, greinerbio-one, Austria).

Results. Based on the results of electrophoresis, iron metabolism and Mentzer index, patients were divided into two groups. The main group included 70 patients (37 men, 33 women, age from 2 to 58 years, 14.5 ± 1.5, median 9 years) with beta thalassemia minor. The comparison group consisted of 50 patients with IDA (28 men, 23 women, age from 2 to 54 years, 16.3±2.15). In the main group, the number of erythrocytes was significantly higher than in the comparison group ($p < 0.05$), while the values of the MCV and MCH parameters were lower ($p < 0.05$).

The differences in MCHC and RDW indicators were not significant ($p > 0.05$). A comparison of the analytical characteristics of the main erythrocyte parameters and the Mentzer index (M) for the differential diagnosis of beta thalassemia minor and IDA showed that the MCV parameter has the greatest sensitivity, and the Mentzer index has the greatest specificity. The lr(+) value – the ability of positive results of these tests to recognize the presence of the disease – was regarded as "excellent" for the Mentzer index = 34. The likelihood ratio lr(-) made it possible to rank the tests under study according to the ability of a negative result to recognize the absence of disease and was regarded as excellent for MCV = 0.03.

As part of these programs, the laboratory was entrusted with performing a set of methods of varying complexity, which included hematological, biochemical, and molecular genetic. There were no significant differences in the structure of the programs; individual details were determined by the population and ethnic composition of a particular region and the frequency of carriage of the beta-globin gene. In regions with a low prevalence of the beta-globin gene, screening begins, as a rule, with identifying a group of putative carriers by collecting a family history and assessing the clinical picture, although minor forms of thalassemia do not always have pronounced clinical manifestations [5].

To confirm thalassemia in patients at risk, various methods for separating hemoglobin fractions are used: electrophoresis on carriers (cellulose acetate, agarose), isoelectric focusing (IEF), capillary electrophoresis (CE), high-performance liquid chromatography (HPLC) [4]. Serum iron concentration was significantly lower in patients with IDA (7.9±3.7 μmol/l, $p < 0.01$) and remained within the reference values in patients with beta thalassemia minor (17.9±5.7 μmol /l).

At the same time, in 11.5% of patients in the main group, this indicator was also reduced. The concentration of ferritin in the blood serum in the comparison group was also significantly lower (7.1 ± 0.6 ng/ml, $p < 0.01$), which indicated in favor of IDA, while in the patients of the main group it was within the range normal (42.2±23 ng/ml), and only 2.7% had a decrease. In patients of the main group, the diagnosis of thalassemia was confirmed using isoelectric focusing (Hb A2 > 3.7% and/or Hb f > 2%): the Hb A2 content ranged from 4 to 7.9%, median – 5.1%; Hb F – from 7 to 12%, median – 9.9%).

In the comparison group, when separating hemoglobin fractions, the following data were obtained: the content of Hb A ranged from 97 to 98%, Hb A2 - from 1.7 to 3.5% (median 2.4%).

Thus, the data we obtained allowed us to propose a set of laboratory markers suitable for use, including in screening programs, and to develop a diagnostic algorithm that includes several stages.

The first stage is to identify carriers of beta thalassemia in a group of patients with microcytic anemia based on the erythrocyte mcv parameter, the Mentzer index (M) and iron metabolism indicators.

The second stage is confirmation of the diagnosis of thalassemia in patients identified at the first stage using isoelectric focusing. Its results will allow us to confirm the presence of beta-thalassemia in patients in whom it was previously identified.

The third stage is the identification of beta-globin gene mutations for final confirmation of thalassemia carriage.

Conclusion. Based on clinical, laboratory and geneological studies, a molecular genetic study of patients with beta-thalassemia in Uzbekistan is being carried out by identifying the spectrum of thalassemic mutations, which will allow for a targeted search for new mutations in the beta-globin gene characteristic of our region.

In addition, studies are being conducted to identify heterozygous carriage, and prenatal DNA diagnosis of β-thalassemia is planned in early pregnancy. These studies will significantly reduce beta thalassemia in Uzbekistan.

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