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PRIMARY DIAGNOSIS OF THREE POLYMORPHISMS IN HETEROSYGOTIC CONDITION OF BETA-THALASSEMIA IN THE REPUBLIC OF UZBEKISTAN, CLINICAL CASE.

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Annotation: *Thalassemias are a heterogeneous group of blood diseases related to quantitative hemoglobinopathies, in which there is a decrease or complete absence of the synthesis of globin chains that make up the human hemoglobin molecule (HbA, Hbf and Hba2). Beta thalassemia is based on hereditary inhibition of the synthesis of chains that make up HbA. Currently, in the Republic of Uzbekistan, 346 children with beta thalassemia and 35 adults over 18 years of age are registered at 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.*

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]. Clinical manifestations of this form of thalassemia become noticeable from the 1st year of a child's life and initially do not have specific features: lag in weight, pallor of the skin, moderate anemia, attacks of fever. However, by the end of the 2nd year of life, signs of

severe hemolytic anemia, ineffective erythropoiesis and splenomegaly clearly appear. Severe hyperplasia of the red line of the bone marrow is the cause of skeletal disorders. The intermediate form of beta thalassemia is similar in symptoms to beta thalassemia major, but all symptoms are much less pronounced. Such patients live to adulthood and can have offspring. Clinical manifestations of hemosiderosis are much less pronounced and appear 10–20 years later than with thalassemia major. The main cause of death in these patients is heart failure.

Clinical manifestations of beta thalassemia minor are very minor [3, 6, 7]. Patients complain of rapid fatigue, weakness after physical activity, and a decrease in hemoglobin content during intercurrent illnesses. A slight enlargement of the spleen and slight bilirubinemia (due to the indirect fraction) are often observed. In women, the above symptoms sometimes appear only during pregnancy. The clinical significance of this form of thalassemia is that it aggravates the course of acute and chronic diseases, pregnancy, and is often accompanied by folic acid deficiency [4,8]. The minimal form of beta thalassemia is an asymptomatic carrier of the thalassemia gene, diagnosed only by laboratory methods (the most reliable is a DNA test) [6]. The diagnosis of thalassemia should be assumed if the patient has microcytic hypochromic anemia with normal or elevated serum iron levels [1].

All forms of thalassemia are characterized by a decrease in quantitative erythrocyte indices: average erythrocyte volume (MCV), average hemoglobin content in one erythrocyte (MCH), average hemoglobin concentration in one erythrocyte (MCHC).

A normal or slightly increased level of erythrocyte volume distribution (rdw) is usually observed in heterozygotes for the beta-thalassemia gene; in homozygotes this indicator is significantly higher than normal [3, 5].

The hemoglobin content in thalassemia major does not reach 40–50 g/l, in intermediate thalassemia it ranges from 60–80 g/l, in small and minimal forms it ranges from 90–120 g/l.

The osmotic resistance of erythrocytes is increased, 100% hemolysis occurs in a 0.25–0.15% NaCl solution, in rare cases - only in distilled water. Biochemical examination reveals indirect hyperbilirubinemia, normal or elevated serum iron levels in combination with a decrease in total serum iron-binding capacity (TIBC) and a relatively high level of ferritin.

The main diagnostic sign of beta thalassemia is a quantitative change in small fractions of hemoglobin. The HbA2 level in homozygotes can be low, normal or elevated, but the HbA2:HbA ratio is always greater than 1:40, the level of fetal hemoglobin usually exceeds 60%, in some cases it is 90–95%. In heterozygotes, HbA2 accounts for 4–8% of total hemoglobin.

The most reliable method for diagnosing thalassemia today is the DNA study of hemoglobin globin genes, which makes it possible to accurately determine the genetic nature of the disease [6]. It is well known that heterozygous beta thalassemia manifests itself already at 6 months of age, and by 2–3 years the clinical manifestations are pronounced.

Heterozogous beta thalassemia is a fatal disease of childhood, and sufferers rarely survive beyond 16 years of age [7]. The pathogenesis of thalassemia is a disruption of the

synthesis of normal hemoglobin due to an excess amount of free α- or β-chains of hemoglobin, which leads to the development of microcytic hypochromic anemia.

Excess α-chains in homozygous beta-thalassemia rapidly precipitate and form insoluble inclusions in bone marrow normoblasts, destroying them, which leads to the development of ineffective erythropoiesis and bone marrow hyperplasia.

Precipitates in reticulocytes and mature erythrocytes are removed in the spleen, which damages the erythrocyte membrane and increases its permeability to cations, resulting in a reduction in the lifespan of erythrocytes and the development of hemolysis. Severe erythroid hyperplasia is the main cause of increased hematopoiesis in the bones, which causes their deformation. Often, foci of red hematopoiesis are found in the liver and spleen. Ineffective erythropoiesis induces increased iron absorption, which leads to pathological iron overload in the body.

The main clinical signs of the disease: microcytic hypochromic anemia, delayed physical and sexual development, hepatosplenomegaly, changes in the skull bones, iron overload [8]. Beta thalassemia major, or Cooley's anemia, is a severe form of progressive hemolytic anemia that requires constant blood transfusions.

Clinical manifestations of this form of thalassemia include hyperbilirubinemia, normal or elevated serum iron levels in combination with a decrease in total serum iron binding capacity (TIBC) and a relatively high ferritin level. The main diagnostic sign of beta thalassemia is a quantitative change in small fractions of hemoglobin. The HbA2 level in homozygotes can be low, normal or elevated, but the HbA2:HbA ratio is always greater than 1:40, the level of fetal hemoglobin usually exceeds 60%, in some cases it is 90–95%. In heterozygotes, HbA2 accounts for 4–8% of total hemoglobin.

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The purpose of the work is to develop laboratory criteria for identifying carriers of thalassemia in a group of patients with microcytic anemia.

Materials and methods. We present an unusual clinical case of heterozygous beta thalassemia with severe course. Patient Bakhtiyorov Kh., born June 27, 2021, ethnic Uzbek, was sent to the Republican Specialized Scientific and Practical Center of Hematology for further examination with a preliminary diagnosis: Hereditary microspherocytic anemia?, splenomegaly.

From the anamnesis it is known that a decrease in hemoglobin levels was first detected in the first days of birth and iron deficiency anemia was diagnosed. Iron therapy was periodically administered without monitoring the dynamics of hemoglobin content. Ultrasound of the abdominal organs revealed splenomegaly. At the time of examination, she complained of fatigue, shortness of breath with little physical exertion, and periodic jaundice of the sclera.

Objectively: the skin and visible mucous membranes are pale, clean, the sclera is subicteric. Peripheral lymph nodes are not enlarged. In the lungs there is vesicular breathing.

The heart sounds are sonorous, the rhythm is correct, and a systolic murmur of a functional nature is heard at the apex. The abdomen is soft and painless on palpation, the liver is at the edge of the costal arch, the spleen is enlarged 7 cm below the costal arch.

The patient underwent the following studies: – a general blood test (on a hematological analyzer micros 18 OT) with the determination of quantitative erythrocyte parameters and the study of a histogram of erythrocytes; – study of the morphology of red blood cells in a stained blood smear; – determination of the number of reticulocytes with supravital staining with brilliant cresyl blue; – determination of osmotic resistance of erythrocytes (ORE) by a screening method in 0.35% NaCl solution (normal 90–100% hemolysis) and 0.5% NaCl solution (normal 0–5% hemolysis); – Hb electrophoresis on cellulose acetate films at pH 8.6; – quantitative determination of HbF according to Betka (norm up to 2%); – quantitative determination of HbA2 by elution method (standard 3.5%); – determination of blood bilirubin (total and indirect) and study of fe metabolism (serum fe, TBL, ferritin); – DNA determination by direct sequencing of PCR fragments of the beta-globin gene.

Results and discussion. In the general blood test, the following changes were observed: leukocytes -5.8 · 10*9/l, lymphocytes -28.6%, medium cells -6.9%, granulocytes - 64.5%); erythrocytes -3.46 · 10*12/l; hemoglobin – 77 g/l; hematocrit – 26.6%; MCV – 77 fl; MSN -25.2 pg; MCHC -328 g/l; platelets - 267 · 10*9/l; thrombocrit 234%; average platelet volume - 9.3 fL; platelet distribution width by volume -20.4%.

The morphology of erythrocytes was characterized by hypochromia, microcytosis, and “target appearance.” Anisocytosis, poikilocytosis, normocytosis and reticulocytosis were observed (2.2%). A biochemical blood test revealed hyperbilirubinemia due to the indirect group (total bilirubin 38.8 μmol/l, indirect bilirubin 34.1 μmol/l), serum iron 28.1 μmol/l, PVSS 43.7 μmol/l, ferritin serum 75 μg/l; HbF 97.0%, HbA2 2.96%.

При анализе эритроцитарных показателей было выявлено незначительное снижение MCV и MCH, нормальный уровень MCHC и высокий показатель RDW. However, as is known, microspherocytosis is characterized by a significant increase in MSHC with normal or slightly reduced MCV. The patient's serum iron level was normal. In accordance with this, a study of small fractions of hemoglobin was carried out to exclude hemoglobinopathy. The results of the analysis showed a complete absence of HbA, hemoglobin was represented by Hbf (97.0%) and slightly HbA2 (2.96%).

Result of Molecular Genetic Testing

Name according to HGVS	Mutation name	Genotype polymorphisms	Mutation location	Link SNP	Disease type
HBB:c.9T>C	HBB:c.9T>C	beta 2(NA2) His>His	exon hg38:chr 11 5,227,013	rs7130 40	polymorp hism
HBB:c.315+16G>C	IVS-II-16G>C	beta nt 511G>C	intron hg38:chr 11 5,226,561	rs1076 8683	neutral polymorphism

HBB:c.316-185C>T	IVS-II-666C>T	beta nt 1161C>T	intron hg38:chr 11 5,225,911	rs1609812	neutral polymorphism
HBB:c.316-45G >C	These changes were not detected in the NBB database (globin.bx.psu.edu).				
HBB:c.445+96T >C					

Since the Republican Specialized Scientific and Practical Center of Hematology has a genetic laboratory where diagnostics for mutation and carriage are carried out, including premarital diagnosis of beta thalassemia on the basis of the grant project "Development of a new diagnostic panel for identifying carriage and pre- and post-implantation diagnosis of beta thalassemia". In connection with the increase in the life expectancy of patients, the issue of prenatal diagnosis to prevent the birth of a sick child is relevant.

Conclusion. Sequencing results indicate the presence of three polymorphisms in the heterozygous state: c.9T>C, IVS-II-16G>C, IVS-II-666C>T and two polymorphisms in the heterozygous state HBB: c.316-45G>C and HBB:c.455+96T>C in the HBB gene. These two changes were not found in the NBB database (globin.bx.psu.edu).

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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