DIAGNOSTIC AND PROGNOSTIC VALUE OF GLYCATED HEMOGLOBIN (HbA1c) IN PATIENTS WITH DIABETES MELLITUS AND THALASSEMIA

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Abstract. *Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, which is caused by impaired insulin secretion, insulin action, or both. Four complications of DM have been identified – diabetes microangiopathy including nephropathy, retinopathy and polyneuropathy and diabetes macroangiopathy. DM is diagnosed on the basis of one of the following criteria: 1. Presence of typical symptoms – polyuria, polyphagia, polydipsia, weight loss and accidental plasma glucose value ≥ 11.1 mmol/l. 2. Fasting plasma glucose level ≥ 7.0 mmol/l. 3. Oral Glucose Tolerance Test (OGTT) – 2-hour blood glucose level ≥ 11.1 mmol/l. 4. Test for glycated hemoglobin HbA1c $\geq 6.5\%$. Regular monitoring of blood glucose is crucial for good control of diabetes. Two are the most commonly available methods for glycemic monitoring – 1) home self-assessment; 2) HbA1c test. In this paper we discuss the diagnostic and prognostic value of HbA1c in comorbid patients with DM and diseases, where there are deviations in hemoglobin and erythrocyte values. In the scientific literature, single reports are available that give scarce guidance on how to manage these patients. It is mentioned that anemia should be treated individually, however no data could be found on what should be the standard behavior in such patients. According to the American Diabetes Association, only the blood glucose should be used when the relationship between HbA1c and glycemia is altered. Because of the diverse gene pool of the US population, different variants of hemoglobin may interfere with HbA1c measurements, although tests in the USA are standardized. Discrepancies between HbA1c and plasma glucose should suggest that testing for HbA1c may not be reliable for a particular patient. We present a clinical case of a comorbid patient with DM and thalassemia minor, whose glycemic control with glycated hemoglobin HbA1c is unreliable.*

Key words: *Diabetes mellitus, blood glucose, glycated hemoglobin, hemolytic anemia, thalassemia*

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, which is caused by impaired insulin secretion, insulin action, or both [1]. The increase of blood glucose lev-

els is due to absolute or relative insulin deficiency. Chronic hyperglycemia leads to subsequent complications of various organs, mainly affecting the retina, peripheral nerves, kidneys, small and large blood vessels. There are four classic complications of diabetes mellitus – diabetic retinopathy, diabetic

nephropathy, diabetic polyneuropathy, which together are related to diabetes microangiopathy and diabetes macroangiopathy. The etiological classification of diabetes mellitus is widely accepted [2] – Table 1.

Table 1. Classification of diabetes mellitus

Diabetes mellitus type 1	Presence of β – cellular destruction of the islets of Langerhans in the pancreas leading to absolute insulin deficiency: – Autoimmune – Idiopathic
Diabetes mellitus type 2	Caused by progressive insulin secretory defect in the background of insulin resistance.
Other specific forms – due to other reasons	Other endocrine disorders, pancreatic diseases, effects of drugs and chemical compounds, infections, diabetes during pregnancy, genetic defects.

There are four methods to diagnose diabetes mellitus in clinical practice: 1. presence of clinical symptoms typical of diabetes – polyuria, polyphagia, polydipsia, unexplained weight loss and accidental plasma glucose ≥ 11.1 mmol/l, i.e. sample taken at any time of the day, no matter when the last meal was; 2. Fasting glucose value ≥ 7.0 mmol/l, with interval after the last meal being more than 8 hours; 3. Oral Glucose Tolerance Test (OGTT) at borderline plasma glucose levels – 2-hour blood glucose value ≥ 11.1 mmol/l; 4. HbA1c test – glycated hemoglobin HbA1c $\geq 6.5\%$.

Regular monitoring of blood glucose concentration is crucial for the good glycemic control of the disease. Two methods are available: 1) home self-control of blood glucose (test strips using glucose meters) for fasting and postprandial glycemia assessment and urine glucose and ketone test; 2) Glycated hemoglobin (HbA1c) test.

Glycated hemoglobin is created by non-enzymatic binding of glucose to the N-terminal valine of the he-

moglobin chain. The higher the glycemia for the previous 6-8-week period was, the higher the percentage of glycated hemoglobin will be. It is not possible to assess the quality of the glycemic control without examining the level of glycated hemoglobin, which has to be tested every 3 months to ensure short- and long-term control of the disease. The criteria for optimal metabolic control are listed in table 2.

The logical question arises: “What is the diagnostic and prognostic value of HbA1c in comorbid patients with diabetes mellitus and diseases with hemoglobin and erythrocyte abnormalities?” We searched the literature but seemingly only single case reports and case series were found to address this question. Anecdotal reports mention the need of individual approach; however, even in the guidelines of the leading scientific organizations, which study diabetes worldwide, we did not find data on what the standard behavior should be.

According to the American Diabetes Association (ADA) [4], when finding a discrepancy between HbA1c and plasma glucose levels due to hemoglobin variants (i.e. hemoglobinopathies), the use of laboratory assays that do not interfere with or alter the diabetes diagnostic criteria should be considered [5-8]. In conditions associated with an altered relationship between HbA1c and glycemia, such as sickle cell anemia, pregnancy (second and third trimester and postpartum period), glucose-6-phosphate dehydrogenase deficiency, HIV, hemodialysis, recent blood loss, or transfusion, or erythropoietin therapy, etc., only blood glucose criteria should be used to diagnose diabetes [9]. Due to the big genetic diversity of the American population (African American, Hispanic/Latino, non-Hispanic white, Native American, Asian American, Pacific Islander), the different hemoglobin variants may interfere with HbA1c measurements, although most tests used in the US are standardized and should not be affected by differences [10-11]. Big discrepancies between HbA1c and plasma glucose levels should suggest that testing for HbA1c may not be reliable for a particular patient.

Table 2. Criteria for metabolic control of most non-pregnant adults and risk of complications (American Diabetes Association 6. Glycemic Targets: Standards of Medical Care in Diabetes – 2020; Diabetes Care Jan 2020, 43 (Supplement 1) S66-S76; DOI: 10.2337/dc20-S006) (3).

Blood glucose / HbA1	Optimal control Low risk	Insufficient control High risk
1. Preprandial capillary plasma glucose	4.4-7.2 mmol/L	> 7.2 mmol/l
2. Postprandial plasma glucose (2 hours after meals)	< 10 mmol/l	> 10 mmol/l
3. HbA1c	< 7.0%	> 7.0%

Among the hemolytic anemias due to a disorder of hemoglobin synthesis, the anemias with quantitative disorders called thalassemias or thalassemia syndromes are the most important ones. Thalassemias are a heterogeneous group of genetic disorders of hemoglobin synthesis characterized by the lack or reduced synthesis of one of the types of the globin chains, which leads to a chronic hemolytic condition or anemia. Anemic syndrome is caused by reduced hemoglobin content and erythrocyte count per unit of blood volume [2].

Hemoglobin accounts for 34% of the total erythrocyte mass, or 95% of erythrocyte dry weight, and has a molecular weight of 64,000. It contains of globin, which is about 97% of the whole molecule and of pigment called heme, which accounts for the remaining 3%. The globin molecule is composed of 4 polypeptide chains, each of which is linked to one heme. There are 141 amino acids in the alpha chains, and 146 in the rest of the chains, arranged in strictly sequential order. The arrangement of the polypeptide chains is regulated by specific regulatory genes. The heme has a protoporphyrin ring structure with a centrally located divalent iron atom. Normally, erythrocytes of an adult human contain 90-95% hemoglobin A1 (α_2, β_2), 2-4% hemoglobin A2 (α_2, δ_2) and 0.5-1% hemoglobin F (α_2, γ_2).

Thalassemia is inherited as an autosomal codominant disease. Some of the heterozygotes may be healthy, but they transmit the anomaly to their generation. The disorders in the regulatory genes reduce the synthesis of the α -polypeptide chains (α -thalassemia) or β -chains (β -thalassemia). Homozygous β -thalassemia is thalassemia major, and its heterozygous form is thalassemia minor or thalassemia minima. The reduction of hemoglobin A1 in erythrocytes is partially compensated by an increase in fetal hemoglobin F and hemoglobin A2. In homozygotes, hemoglobin F can reach 90% of the total hemoglobin, whereas in heterozygotes, hemoglobin A2 increases above 5% and, to a lesser extent, hemoglobin F.

EPIDEMIOLOGY

β -thalassemia is one of the most common monogenic diseases in the world. Thalassemia is found in the populations of the Mediterranean, Africa, Asia, and in our country it is found mainly in southern Bulgaria. In particular, β -thalassemia is the most common genetic disease in the Mediterranean countries. In some Mediterranean islands, the frequency of heterozygotes varies between 5% and 20%. The prevalence of thalassemia minor is predominant in Cypriots (14.7%), Greeks (8.0%), Albanians (7.1%),

and Italians (3.7%). The incidence of the disease in Bulgaria is between 2% and 4% of the total population, which means that at least 200,000 Bulgarians are carriers and 18-20 babies are born with thalassemia minor [12].

CLINICAL PICTURE

Heterozygous individuals with thalassemia minor usually show minor complaints or are fully asymptomatic. Splenomegaly is not mandatory and if it occurs, it is low-graded and accidentally detected. Anemia may occur during pregnancy or other concomitant conditions with increased requirements to hematopoiesis. Iron overload (IOL) is one of the most significant complications in thalassemia and, if not properly prevented and treated, leads to fatal outcomes in patients. The four major target organs of IOL are the liver, heart, bones and endocrine glands. The accumulation of toxic amounts of iron causes tissue damage and leads to cardiomyopathy and other heart diseases, liver fibrosis and cirrhosis, diabetes, hypo- or hyperthyroidism, etc. Cardiac complications are the most common cause of the fatal outcome.

We present a **clinical case** of a comorbid female patient with diabetes mellitus and thalassemia minor. The patient was a 69-years-old female. The medical history was collected and described according to the data provided by the patient and the medical documentation from the regional hospital, discharge summaries from the University hospitals in Sofia and laboratory tests conducted by medical-diagnostic laboratories.

In 2002, the patient was diagnosed with diabetes mellitus type 2 through oral glucose tolerance test (OGTT) carried out due to manifestation of typical clinical symptoms – polyphagia, polyuria and polydipsia and repeatedly high blood glucose levels above 7.8 mmol/l in the fasted state and above 11.0 mmol/l after meals, while the HbA1c levels were within the reference range: 4.0-5.6%. The initial treatment was diet and physical activity. Due to the failure of these measures, drug treatment with sulphonylurea drugs was initiated in 2003 (Glibenclamide). In the background of sulfonylureas treatment, excellent carbohydrate metabolism was achieved, which was evident by the three- and six-point glucose profiles over 24 hours and lasting normal glycated HbA1c.

In addition, arterial hypertension with values up to 180/120 mmHg was diagnosed. The patient was treated with calcium antagonists, ARBs, ACE-inhibitors, beta-blockers, diuretics. The control over the hypertension was unstable with periods of exacerbation. A nodose euthyroid goiter II-III grade was found.

Occasional control laboratory tests showed microcytic anemia and hyperbilirubinemia, at the expense of the indirect fraction and permanently elevated serum iron levels. For this reason, the patient was tested for pathological hemoglobins and thalassemia minor was diagnosed.

In 2010-2015, the patient had episodes of atrial fibrillation with absolute arrhythmia, due to which she was regularly hospitalized and had drug regularization with beta-blockers and amiodarone with partial success. Throughout 2015, she received maintenance antiarrhythmic treatment with amiodarone. During this period, the patient was normoglycemic, with normal glycated hemoglobin values and tendency to hypoglycemia, so her treatment for diabetes mellitus with Glibenclamide was discontinued and she remained on diet only.

Misdiagnosed diabetes mellitus was suspected and OGTT was carried out again. The result clearly showed that diabetes mellitus was correctly diagnosed, although blood glucose values corresponded rather to impaired carbohydrate tolerance, and glycated hemoglobin was again within the reference range of up to 5.6%.

The patient gradually developed a clinical picture of thyrotoxicosis and the diagnosis was confirmed at the end of 2016. Thyrotoxicosis was proven by hormonal tests. Thyroid nodules enlarged up to 1.0-1.5 cm were also found on ultrasound examination. Thyrostatic treatment with maximum doses of Thiamazole was initiated. Thyrotoxicosis was believed to be caused by the amiodarone therapy. However, toxic thyroid adenoma was also suspected, but no further tests were performed due to the damaged general condition of the patient. The patient quickly became euthyroid and developed hypothyroidism. By the end of 2019, all attempts to discontinue the thyroid therapy lead to a return to the clinical picture of thyrotoxicosis, with even one-month low-dose Thiamazole (5-10 mg) treatment leading to hypothyroidism.

Throughout 2017, the arterial hypertension of the patient was difficult to control, signs of heart failure with shortness of breath at rest and physical exertion developed, and swelling of the lower extremities, cyanosis, cough, rhythmic and conduction disorders persisted even though the thyroid function was in the euthyroid phase. The fasting blood glucose at that time ranged from 6.0 mmol/L to 7.0 mmol/L, postprandially not exceeding 7.8 mmol/L. Glycated hemoglobin HbA1c remained within the reference range, and the blood-glucose profiles showed blood glucose values not exceeding 7.8 mmol/L. The leading clinical symptoms showed severe total heart failure accompanied by respiratory failure.

In this background, hematological parameters worsened – anemia deepened, the hemoglobin dropped to and below 100 g/l, at normal erythrocyte count, the hematocrit remained at the lower limit of the reference range (0.35-0.49). MCV, MCH and MCHC indicated microcytosis, iron was above the reference range (9-22.2 $\mu\text{mol/l}$), iron-binding capacity was also above the reference range (45-72 $\mu\text{mol/l}$).

At the same time, the patient developed a clinical picture of acute cholecystitis. At the beginning of 2018, she was hospitalized to a gastroenterology clinic and as a result of laboratory and instrumental investigations, a clinical and laboratory constellation of chronic acute calculous cholecystitis was established. An ultrasound examination revealed an enlarged gall bladder with a length of 12 cm, and the lumen filled with concrements. A further abdominal examination showed that there were no X-ray positive shadows visible, which lead to the conclusion that calculus was of bilirubin nature, most probably due to the chronic hemolytic thalassemia syndrome. The condition of the patient was put under control and she was referred to a cardiology clinic for more precise diagnosis of her cardiovascular and pulmonary status.

During her stay at the cardiology clinic in May 2018, the patient was diagnosed with congestive heart failure NYHA III-IV functional class, severe left ventricular dysfunction, combined heart disease – mitral stenosis and grade III-IV insufficiency, high-grade tricuspid regurgitation, persistent atrial fibrillation, pulmonary hypertension, pleural effusions, grade III arterial hypertension, type 2 diabetes mellitus, hyperlipidemia, diffuse parenchymal process of the liver, chronic calculous cholecystitis, iodine-induced thyrotoxicosis (Jodbasedow), chronic pyelonephritis.

Under conditions of increased cardiovascular and surgical risk, surgical treatment was started – mitral valve prosthesis with biological valve prosthesis was performed. The postoperative period went smoothly. The patient quickly restored her cardiac function, the pump function and blood pressure were stabilized, pulmonary hypertension disappeared, respiratory failure was completely overcome, pleural effusions dissolved.

However, the question whether diabetes mellitus was present remained unanswered. The tests again found that the glycated hemoglobin was in the reference values and blood glucose was within the borderline values more characteristic of impaired carbohydrate tolerance. We could not objectively measure the effect of the thyroid dysfunction on carbohydrate metabolism. The patient was left untreated, only on a strict diet, under strict laboratory control.

DISCUSSION

We present a typical case of the course of heterozygous form of β -thalassemia minor. Unfortunately, in our case the disease was diagnosed too late – when the patient was 50 years old. In the described period, hemolysis with hyperbilirubinemia, anemia and high serum iron occurred with worsening of the general condition of the patient. Deposition of iron in the target organs in combination with relative tissue and organ ischemia and the advanced age of our patient evolved into all the typical complications of the disease – cardiomyopathy and other cardiac diseases, liver fibrosis, diabetes mellitus, hypo- or hyperthyroidism, cholecystitis with calculosis, etc. [13-14]. In addition, our case manifested that OGTT alone is indicative of the diagnosis of diabetes, and that routine testing of blood glucose and glycated HbA1c in patients with β -thalassemia may be completely unreliable. This is important to know because there are at least 200,000 people with β -thalassemia minor in Bulgaria, who are predisposed to develop diabetes mellitus. The use of standardized tests for glycated hemoglobin is a mandatory prerequisite, and OGTT remains the primary method of diagnosis of diabetes mellitus in this specific patient population.

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