

Diabetic Type II related with Anemia - Luljeta Hetemi - Institute of Biochemistry Skopje

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Introduction:

The purpose of this study was to: follow-up patients with diabetes type II through testing of: glycated hemoglobin (HbA1c) derivatives, but the importance of the HPLC method during HbA1c testing in: diagnosis, monitoring, follow-up, access to diabetes type II therapy also obtained results from: diabetes, pre diabetes, patient insulin insertion, monotherapy, but also findings of abnormal Hb forms in diabetic patients, such as hemoglobinopathies, and during the research we found that the diagnostic orientation through a HbA1c test with HPLC is in some pathologies, which was both preventive and diagnostic in other pathologies. The research lasted over 1 year at the Olive medical & laboratory diagnostic center.

Method:

HPLC (high performance liquid chromatography) G8 Tosoh, an exclusive distributor: Keis group pharmaceutical Kosovo. For the first time in our country we performed HbA1c with HPLC. The G8 Analyzer is the next generation of Tosoh's leading HPLC testing systems for fast and accurate HbA1c results. The analyzer dilutes the whole blood specimen with Hemolysis & Wash Solution and then injects a small volume of the treated specimen onto the TSKgel Glyco HSi Variant Column. Separation is achieved by utilizing differences in ionic interactions between the cation exchange group on the column resin surface and the hemoglobin components in a step gradient elution. The hemoglobin fractions (designated as A1a, A1b, F, LA1c, SA1c, A0, and H-V0, H-V1, H-V2) are subsequently removed from the column material by performing a step-wise elution using Elution Buffers HSi Variant 1, 2, and 3 that have specific salt and pH concentrations. The separated hemoglobin components pass through the LED photometer flow cell where changes in absorbance are measured at 415nm. The G8 software integrates and reduces the raw data, and then calculates the relative percentages of each hemoglobin fraction. The print-out consists of the numerical results and the chromatogram. This represents the changes in absorbance versus retention time for each peak fraction. An analysis requires only 1.6 minutes. The study was conducted on 150 patients, of which 80% were diabetes type II, age 40-65 years, with HbA1c ranging from 6.9-11%, HbA from 6-8, HbF of over 1% -8% (in 48% of cases was HbF positive), the control group were non-diabetic, suspect, dyslipidemia, patients with no signs of type II diabetes, the reason for testing being because they had high insulin resistance, or moderate, some of these patients were with polycystic ovary syndrome. In addition to the absolute accuracy of the HPLC method in testing HbA1c, in this study we found HbF positive of patients with type II diabetes,

which is leading us to anemia, and the cause of this anemia is thought to be impairment of renal function, such as diabetic nephropathy - complication of type II diabetes, but a still unclear mechanism between abnormal HbF congenital and type II diabetes will remain uninvolved as we were not able to test all the patients for Hb electrophoresis. From these laboratory findings, HbF results in increased pathology: in addition to being an innate congenital form, its elevated level was also found in cases of pernicious anemia, hemolytic anemia, thalassemia, thyrotoxicosis, deep venous thrombosis, etc. LA1c as the labile form of glycated Hb, could be increased in case of presence of carbamylated Hb (urea, patients with kidney diseases) and acetylated Hb (alcohol abuses, liver diseases, etc), of course increase of blood sugar glucose.

When blood glucose level is high, glucose molecules attached to RBC, the longer hyperglycemia occurs, more the glucose binds to Hb, and higher the glycated Hb. So from this study we found approximately 35% of cases with LA1c positive, where diabetic nephropathy had already begun, which was confirmed by positive microalbuminuria, and with nephrologist doctor report. Abnormal forms of HbF values in our country, and in these 150 cases there were no other abnormal forms of Hb, so on the other hand we found a correlation between HbF positive had easily or moderately increased serum Homocystein or hyperhomocysteinemia and 10% of them were with MTHFR mutant positive (about 15% of these patients that had opportunity to be tested, their laboratory findings were serum Homocystein values were from 15-17, reference range for adults 5-15 $\mu\text{mol/L}$, method Immunoassay-Imulite-2000 analyzers for the quantitative determination L-Homocystein in human serum (Siemens), and other anamnestic data of these cases were partly with clinical signs such as leg edema, and leg pain, so as we know Hyperhomocystein is an indicator for cardiovascular disease, coronary disease, early arteriosclerotic disease, arterial thromboembolism, and this risk is present by Hypercholesterolemia, also by Homocystinuria as genetic defects.

Results:

Total Homocystein (tHcy) has emerged as an important risk factor in the assessment of cardiovascular disease. tHcy a thiol containing amino acid is produced by the intracellular demethylation of methionine, Hcy thereby serves as a pool that can be later scavenged for use in the remanufacture of either methionine through the action of the folate dependent enzyme methionine synthase or cysteine using the B6 dependent transsulphuration pathway, folate and B6 dependent is another reason for anemia connection with Diabetes type II, because is

present positive HbF form that told us for more and more tests, so these made us to follow up consultation with vascular surgeon, he requires MTHFR with 2 mutations and other Hemostasis tests. MTHFR mutations results were from Synlab laboratory in Germany. MTHFR mutation c677T on 11% of these cases was mutant positive.

Conclusion:

We concluded with the facts that HPLC can freely be called the gold standard diagnostic method, as it has proven to be the best method. Risk for pre-diabetic, were 20% of our patients, with HbA1c values between 6-6.4%, about 10% of our patients were with HbA1c values more than:6.5%, tested for the first time, that consider diabetic, about 25% were with adequate control: with 6.6-7% values, and inadequate control according ADA (American Diabetic association criteria's) with 7-8% values of HbA1c, about 45% of them. In mechanism from HbF findings and HbA1c findings, with HPLC the same diabetic patient may receive an early diagnosis for: complications of diabetes such as kidney damage, liver damage, anemia, early arteriosclerosis, thromboembolism, risk for ICV etc.