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Study on Metabolic and Mineral Balance, Oxidative Stress and Cardiovascular Risk Factors in Type 2 Diabetic Patients on Different Therapy

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***Abstract*—**Intense oxidative stress, increased glycated hemoglobin and mineral imbalance represent risk factors for complications in diabetic patients. Cardiovascular complications are most common in these patients, including nephropathy. This study was conducted in 2015 at the Procardia Laboratory in Tîrgu Mureş, Romania on 40 type 2 diabetic adults. Routine biochemical tests were performed on the Konleab 20XTi analyzer (serum glucose, total cholesterol, LDL and HDL cholesterol, triglyceride, creatinine, urea). We also measured serum uric acid, magnesium and calcium concentration by photometric procedures, potassium, sodium and chloride by ion selective electrode, and chromium by atomic absorption spectrometry in a group of patients. Glycated hemoglobin (HbA1c) dosage was made by reflectometry. Urine analysis was performed using the HandUReader equipment. The level of oxidative stress was measured by serum malondialdehyde dosage using the thiobarbituric acid reactive substances method. MDRD (Modification of Diet in Renal Disease) formula was applied for calculation of creatinine-derived glomerular filtration rate. GraphPad InStat software was used for statistical analysis of the data. The diabetic subject included in the study presented high MDA concentrations, showing intense oxidative stress. Calcium was deficient in 5% of the patients, chromium deficiency was present in 28%. The atherogenic cholesterol fraction was elevated in 13% of the patients. Positive correlation was found between creatinine and MDRD-creatinine values ($p<0.0001$), 68% of the patients presented increased creatinine values. The majority of the diabetic patients had good control of their diabetes, having optimal HbA1c values, 35% of them presented fasting serum glucose over 120 mg/dl and 18% had glucosuria. Intense oxidative stress and mineral deficiencies can increase the risk of cardiovascular complications in diabetic patients in spite of their good metabolic balance. More than two third of the patients present biochemical signs of nephropathy, cystatin C dosage and microalbuminuria could reveal better the kidney disorder, but glomerular filtration rate calculation formulas are also useful for evaluation of renal function.

***Keywords*—**Cardiovascular risk, malondialdehyde, metabolic balance, minerals, type 2 diabetes.

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I. Introduction

DIABETES mellitus, especially type 2, is a common metabolic disease, a disease of civilization, with increasing prevalence all around the world. Oxidative stress, deficiency of some minerals, metabolic imbalance and increased LDL-cholesterol levels represent cardiovascular risk factors for patients suffering from diabetes mellitus. Common complications of this disease are those affecting the microvascular area, such as retinopathy and nephropathy [1].

Carbohydrate metabolic balance can be evaluated based on the serum glucose profile, but the best method is HbA1c measurement [2]. Glucosuria is usually a sign of a serious imbalance.

Obesity and dyslipidemy is also very common in these patients, increased atherogenic LDL-cholesterol and triglyceride concentration represent risk factors for the complications of the diabetes [3].

Deficiency of some minerals, such as magnesium, chromium, calcium can intensify oxidative stress and have a bad influence on the carbohydrate metabolic balance. Calcium supplementation has been proved to enhance the absorption of vitamin B_{12}-intrinsic factor complex at the level of the ileon, thus preventing vitamin B_{12} deficiency, one of the well-known side effects of metformin therapy, which can lead to increased homocysteine concentration, an independent cardiovascular risk factor [4]. Serum potassium and free calcium concentration are influenced by acid-base imbalances [5].

The calculation formulas used for evaluation of glomerular filtration rate (such as MDRD) have a better role in evaluation of kidney function compared to the simple serum creatinine dosage, and their values are highly correlated with calculations made based on cystatin C measurement, an early marker of nephropathy [6].

Several studies revealed that chromium supplementation can lower blood glucose levels in patients suffering from diabetes mellitus. According to the National Institute of

Health: Office of Dietary Supplements, an adequate intake of Cr for men and women is 35 and 25 μg/day, respectively [7], [8]. Chromium is necessary for maintaining normal metabolism and storage of fats, proteins and carbohydrates, this trace element enhances the actions of insulin. Inadequate chromium intake has been linked to the development of glucose intolerance, which can be transformed easily into diabetes mellitus. Chromium can also help to raise the antiatherogenic HDL-cholesterol levels, and may play a role in preventing heart disease [9].

The aim of the study was to evaluate oxidative stress, carbohydrate metabolic balance, lipid profile and deficiency of minerals to define cardiovascular risk factors present, and estimation of glomerular filtration rate to reveal nephropathy in type 2 diabetic subjects.

II. Material and Methods

The clinical study was performed in 2015 on 40 type 2 diabetic subjects, patients of the Procardia Laboratory in Tîrgu Mureș, Romania, 40% of them being male patients. We included in the research patients who signed the participation form, subject who were not admitted to the hospital in the period of the study, but were previously diagnosed with type 2 diabetes mellitus. We excluded from the study pregnant patients and subjects having alarm values at the laboratory parameters regarding carbohydrate metabolism (HbA1c over 12%, serum glucose over 400 mg/dl or under 40 mg/dl).

The Konleab 20XTi analyzer was used for the performed biochemical tests (glycaemia, serum total cholesterol, atherogenic LDL-cholesterol, antiatherogenic HDL-cholesterol, serum triglyceride, creatinine, urea, uric acid, magnesium, calcium concentration), the equipment uses photometric procedures for these parameters. The same analyzer was used for the dosage of serum potassium, sodium and chloride by ion selective electrode. Chromium dosage was made by atomic absorption spectrometry in a certain group of patients.

The dosage of HbA1c was made by reflectometry. HandUReader equipment was used for urine analysis, besides the test results based on the strip the sediment was also analyzed with the photomicroscope ML313.

Oxidative stress was measured by thiobarbituric acid reactive substances method, evaluating serum malondialdehyde concentration, which is a stable molecule, a good marker of lipid peroxidation. The serum values of the patients were compared with a scale of standard MDA solutions using a concentrated 1,1,3,3-tetramethoxypropane stock solution.

Creatinine-derived glomerular filtration rate was calculated using the MDRD (Modification of Diet in Renal Disease) formula.

The research data were statistically analyzed using Microsoft Excel and GraphPad InStat software (Pearson test for correlations and Kolmogorov-Smirnov test for data distribution). The level of significance was set at p below 0.05.

III. Results

The average creatinine value of the studied diabetic patients was 1.27 mg/dL +/- 0.36 (SD), 68% presented increased values. It's important to mention that the normal range is different in male and female patients, in male patients, creatinine concentrations exceeding 1.2 mg/dL are considered pathological values, while in case of female subjects, normal values are considered up to 1.00 mg/dL.

Serum creatinine values were highly correlated to urea concentration, r=0.5455, p=0.0003, as represented in Fig. 1.

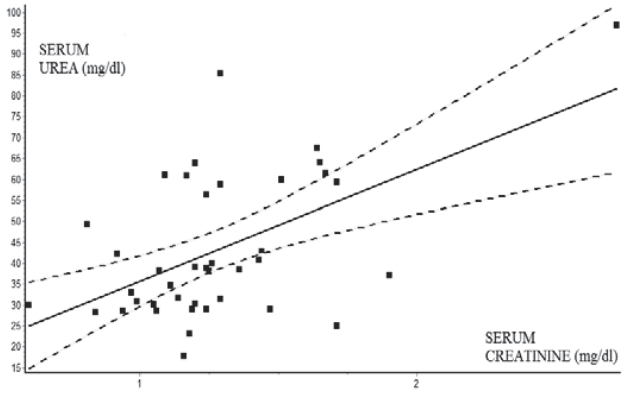

Fig. 1 Positive correlation between serum creatinine and urea concentration

Positive correlation was found between serum creatinine concentration and glomerular filtration rate based on the MDRD-creatinine calculation formula ($p<0.0001$).

In the study group, 5% of the patients presented mild calcium deficiency, the same percentage had increased potassium concentration, and serum magnesium was lower than normal in 3% of the subjects. Sodium was increased in 3% of the patients, and the same percentage showed lower serum sodium values. Serum chloride concentration was increased in 8% of the subjects. Chromium deficiency was present in 28% of the patients.

The majority of the diabetic patients had good control of their diabetes (HbA1c under 7%), the distribution of the values can be observed in Fig. 2. It's worth to be mentioned that 10% of the diabetic patients presented normal HbA1c values, in the 4-6% range.

35% of the diabetic patients included in the study had fasting serum glucose exceeding 120 mg/dl on the day of sampling and 18% of them presented glucosuria.

Knowing the fact that glucose in the urine causes osmotic diuresis, we focused also on the values of urine specific gravity. The average obtained was 1.022 g/ml +/- 0.006 (SD), 15% of the patients presented values at the limit of hypersthenuria (1.030 g/ml). The average pH of the urine was 5.24 +/- 0.55 SD.

Serum MDA concentrations was high in the tested diabetic patients, 78% of them had values exceeding 5 ng/mL, which shows intensified oxidative stress.

The average serum cholesterol value of the studied patients was 186.7 mg/dl +/- 49.33 (SD), 38% of them showing values exceeding 200 mg/dl, which represents the highest acceptable value for subjects presenting cardiovascular risk factors. The minimum cholesterol value measured in the studied patients was 111 mg/dl, the highest was 367 mg/dl.

In case of the protective, so called „good" HDL-cholesterol, the average value was 48.01 mg/dl +/- 12.32 (SD), the lowest value being 25 mg/dl, the highest 85 mg/dl. 15% of the patients presented low HDL-cholesterol values, which represents an important cardiovascular risk factor. It has to be mentioned that in female subject normal values of HDL-cholesterol are considered over 42 mg/dl, and in male patients over 35 mg/dl.

Increased LDL-cholesterol occurred in 8% of the patients. Especially the chemically modified particles are very dangerous, because they can trigger atheroschlerosis.

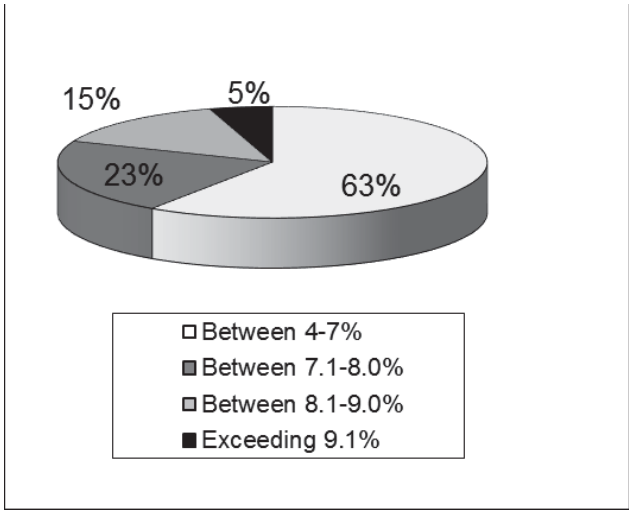

Fig. 2 Distribution of glycated hemoglobin levels in diabetic patients

IV. Discussion

In case of diabetic patients, cardiovascular complications are often under-diagnosed, this is true especially regarding nephropathy and rethinopathy. Both of these diseases affecting the microvascular area can develop silently. Glomerular filtration rate calculation formulas, such as MDRD or eGFR, based on serum creatinine or cystatin C measurements and determination of microalbuminuria could improve the percentage of early diagnosis [10], [11].

The majority of the diabetic patients included in the study presented good metabolic balance, but oxidative stress was intensified in a high percentage of the patients, revealing that cellular damage due to reactive oxygen species is an important mechanism enhancing the occurrence of complications, besides molecular changes due to glycosylation processes [12].

There is a low percentage of patients having deficiencies regarding serum calcium and magnesium concentration (which can be considered routine laboratory tests), but the percentage of chromium deficiency is very high, showing that the dosage of trace elements would be appropriate to complete the list of laboratory tests regularly performed in diabetic patients. Serum selenium, zinc, copper measurement and the dosage of hydro-and lipo-soluble vitamins could reveal additional deficiencies, which can be related to poor antioxidant status [13], [14].

In a group of the studied diabetic patients HDL-cholesterol level is decreased, which together with other risk factors can increase the likelihood of cardiovascular complications. The percentage of this protective cholesterol can be elevated by certain medication and by special diet, which also can help in maintaining a proper body weight [15].

There is a relationship between intense oxidative stress and the probability of having increased number of chemically modified (oxidized) lipids in the cardiovascular system. Another mechanism is related to poor carbohydrate metabolic balance, persistent hyperglycaemia can trigger the accumulation of glycosylation end products, changing the surface of certain biomolecules [16].

V. Conclusion

Our research data revealed that oxidative stress is intensified in the studied type 2 diabetic patients and several other cardiovascular risk factors are present in case of several participating subjects.

The list of current laboratory analysis and paraclinical investigations should be completed with parameters for the early diagnosis of microvascular diseases, especially nephropathy and rethinopathy, which are often under-diagnosed.

In addition to regular treatment, dietary supplements, diabetic teas and other phytotherapeutical products rich in trace elements, vitamins and bioflavonoids could be benefic in the management of type 2 diabetic patients. Some of these products contain compounds having insulin-like effect (such as myrtilline), thus could improve the patients' antioxidant status and carbohydrate metabolic balance, helping the prevention of cardiovascular complications.

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