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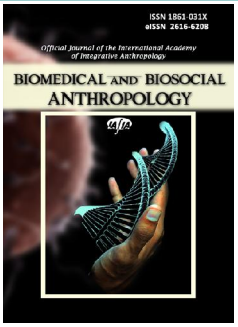

Features of biochemical parameters in healthy and patients with type 1 diabetes mellitus with different levels of albumin in the urine

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The use of multi-label biochemical panels is justified by the fact that different markers reflect the severity of various pathological processes (disorders of carbohydrate and fat metabolism), which in their interaction are indicators of decompensation of a single pathology - diabetes in particular. The aim of the work was to study the differences in biochemical parameters in healthy and patients with type 1 diabetes mellitus (T1D) depending on the level of albumin in the urine. 78 men and 62 women aged 22-26 years, patients with diabetes mellitus type 1, who were hospitalized in the therapeutic department в № 1 and № 2 of Vinnytsia Regional Highly Specialized Endocrinology Center and 8 practically healthy men and 13 practically healthy women of the same age were examined. The level of microalbuminuria was determined in all patients by enzyme-linked immunosorbent assay. Fasting blood glucose and 2 hours after a meal were determined by enzymatic, amperometric analysis on a biochemical analyzer Biosen C_Line, manufactured by EKF Diagnostic (Germany). The average value of blood glucose was calculated mathematically. To determine glycated hemoglobin (HbA1c), we used the method of high performance liquid chromatography on a D 10 analyzer, manufactured by Bio-Rad (USA). The International Normal Ratio (INR) was determined using a set of reagents to determine the prothrombin time, prothrombin ratio and international sensitivity index, which is specified in the passport to the set (manufacturer of PC-K-TEST "Granum"). Total cholesterol and triglycerides were determined by colorimetric photometric method (using enzymes) on a biochemical analyzer using standard kits from Olympus AU480 (USA). Statistical processing of the results was performed in the license package "Statistica 5.5", using non-parametric evaluation methods. When comparing the level of biochemical parameters between patients with T1D and normo-, microalbuminuria and proteinuria with healthy men or women, patients found higher values: fasting glucose (in men - by 29.1 %, 30.9 % and 42.0 %; in women - by 29.7 %, 33.2 % and 46.0 %); glucose 2 hours after exercise (for men - by 27.4 %, 30.8 % and 36.1 %; for women - by 30.4 %, 32.4 % and 40.2 %); the average value of glucose (for men - by 26.2 %, 30.8 % and 39.3 %; for women - by 28.7 %, 34.0 % and 43.1 %); glycated hemoglobin (47.8 %, 48.8 % and 45.8 % for men; 27.2 %, 50.2 % and 54.4 % for women). Also, in patients with T1D men and proteinuria and women with normoalbuminuria and proteinuria compared with healthy men or women found higher cholesterol values (17.8 % in men and 7.6 % and 26.0 % in women); and in patients with T1D men with proteinuria compared to healthy men - 31.3 % higher triglyceride levels. When comparing the level of biochemical parameters between patients with T1D in men or women with proteinuria, higher values were found: fasting glucose compared with men with normoalbuminuria by 22.2 % and with women with normoalbuminuria by 30.3 % and microalbuminuria by 23.7 %; the average value of glucose compared with men with normoalbuminuria by 20.8 % and microalbuminuria by 14.0 %; glycated hemoglobin compared to women with normoalbuminuria by 15.9 %; compared with men with normoalbuminuria and microalbuminuria - by 24.8 % and 20.3 %, or with women with normoalbuminuria and microalbuminuria - by 24.9 % and 28.0 %; triglycerides compared with men with normoalbuminuria by 23.0 %. When comparing

the sex differences in the level of biochemical parameters between healthy or between patients with T1D and normo-, microalbuminuria and proteinuria in men and women, only higher values of glycated hemoglobin by 14.1 % in women with proteinuria and total cholesterol by 8.3 % in women with normoalbuminuria were found. Thus, between healthy and patients with T1D and different levels of albuminuria there are differences in biochemical parameters, and they are greater the higher the level of albumin in the urine.

Keywords: *type 1 diabetes mellitus, urinary albumin levels, biochemical parameters.*

Introduction

Along with diabetic nephropathy, metabolic disorders are traditionally associated with type 2 diabetes. However, current data suggest that patients with type 1 diabetes mellitus (T1D) may also have a metabolic syndrome, and insulin resistance, as its basis, adversely affects the manifestation and development of micro- and macrovascular complications. The presence of nephropathy and metabolic syndrome in people with T1D requires control not only of glycemia, but also other biochemical parameters associated with them [5, 13]. After all, timely identification of metabolic and cardiovascular risk factors, early drug correction in the subclinical stages of diabetic nephropathy, reduces the possibility of chronic renal failure, retino- and neuropathy [8, 9].

When studying diabetes mellitus and its complications, patients need to study the biochemical parameters of carbohydrate, fat metabolism and blood clotting [3, 15]. In fact, there are many of these indicators. However, based on clinical logic, feasibility and technical availability of use in the outpatient and inpatient stages, fasting glucose and 2 hours after oral glucose loading, as well as glycated hemoglobin are most often determined to assess carbohydrate metabolism. From the indicators of the lipid spectrum, cholesterol and triglycerides are more commonly assessed [10, 12]. Comparative analysis of the differences between these indicators in healthy and patients with T1D and varying degrees of albuminuria will not only be of great scientific and practical importance, but will be extremely important for insurance medicine.

The aim of the study was to examine the differences in biochemical parameters in healthy and patients with T1D depending on the level of albumin in the urine.

Materials and methods

78 men and 62 women aged 22-26 years, patients with T1D, who were hospitalized in the therapeutic department № 1 and № 2 of Vinnytsia Regional Highly Specialized Endocrinology Center and 8 practically healthy men and 13 practically healthy women of the same age were examined.

All patients underwent the procedure of determining the level of microalbuminuria by enzyme-linked immunosorbent assay using spectrophotometry (reagents from ORGenTec, Germany). Regulatory values of microalbumin in the set of reagents used - 0-25 μg/ml.

Venous blood samples were taken from subjects on an empty stomach in the morning (up to 9 am) after 10-12 hours of fasting.

Blood glucose was determined by enzymatic, amperometric method on a biochemical analyzer Biosen C_Line, manufacturer EKF Diagnostic (Germany). Glucose content was determined using special sensor chips. When the sample is applied to the chip sensors, β-D-glucose is converted enzymatically by glucose oxidase into gluconic acid and hydrogen peroxide, which reacts with the electrode. The measurement result was an electric current that is proportional to the glucose concentration. Reference norms of blood glucose 3.3-5.5 mmol/L. Blood glucose levels were determined on an empty stomach and 2 hours after a meal, and the mean was calculated.

Patients underwent glycated hemoglobin (HbA1c) testing. For this analysis, the method of high performance liquid chromatography on the analyzer D 10, manufactured by Bio-Rad (USA) was used. Normative values of this indicator <6 %.

The International Normal Ratio (INR) was determined using a set of reagents to determine the prothrombin time (PT), the prothrombin ratio (PR) and the International Sensitivity Index (ISI), which is specified in the passport to the kit. Manual technique. Initially, the inverter was determined. The sequence of calculation: 1) PR=PT of the patient/PT of control plasma; 2) INR=PRisi. Manufacturer of PC-K-TEST "Granum". The normal INR is approaching 1.0.

Biochemical parameters such as total cholesterol and triglycerides were determined by colorimetric photometric method (using enzymes) on a biochemical analyzer using standard kits from Olympus AU480 (USA). Determination of total cholesterol was performed using phenol and peroxidase. The norm for this indicator is less than 5.2 mmol/L. Serum triglycerides were determined by the same method but with lipase and peroxidase, the norm <1.7 mmol/L.

Statistical data processing was performed in the license package "Statistica 5.5" using non-parametric methods of evaluation of the obtained results.

Results

There was a lower ($p<0.01$-0.001) value of fasting blood glucose in healthy men (4.525±0.599) compared with sick men with normo- (6.385±1.539), microalbuminuria (6.548±2.032) and proteinuria (7.800±2.627). In healthy women, lower ($p<0.001$ in all cases) values of fasting blood glucose (4.662±0.479) compared with sick women with

normoalbuminuria (6.632±1.573), microalbuminuria (6.974±1.695) and proteinuria (8.638±2.520) were found (Fig. 1).

Men with proteinuria had higher ($p<0.05$) fasting blood glucose levels (7.800±2.627) compared with men with normoalbuminuria (6.385±1.539). In women with proteinuria, higher ($p<0.05$ and $p=0.064$) values of fasting blood glucose (8.638±2.520) were found compared with patients with normoalbuminuria (6.632±1.573) and microalbuminuria (6.974±1.695) (see Fig. 1).

There were lower ($p<0.05$-0.001) PPBGL in healthy men (5.175±0.417) compared with sick men with normo- (7.128±1.533), microalbuminuria (7.476±2.321) and proteinuria (8.100±3.409). Healthy women had lower ($p<0.01$-0.001) PPBGL (5.169±0.471) compared with sick women with normoalbuminuria (7.423±1.624), microalbuminuria (7.674±1.889) and proteinuria (8.638±3.802). (Fig. 2).

There was a lower ($p<0.001$ in all cases) average value of blood glucose levels in healthy men (4.850±0.307) compared with sick men with normo- (6.609±1.339), microalbuminuria (7.006±1.860) and proteinuria (7.985±2.379). Healthy women had a lower ($p<0.001$ in all cases) average blood glucose level (4.915±0.410) compared with sick women with normoalbuminuria (6.892±1.200), microalbuminuria (7.441±1.451) and proteinuria (8.638±3.080) (Fig. 3).

In men with proteinuria, a higher ($p<0.05$ and $p=0.074$) ABGL (7.985±2.379) was found compared with men with normoalbuminuria (6.609±1.339) and microalbuminuria (7.006±1.860) (see Fig. 3).

There was a lower ($p<0.001$ in all cases) values of glycated hemoglobin in healthy men (4.925±0.537) compared with sick men with normo- (9.433±2.036), microalbuminuria (9.620±1.861) and proteinuria (9.092±1.870). In healthy women, less ($p<0.001$ in all cases) values of glycated hemoglobin (4.823±0.446) compared with sick women with normoalbuminuria (9.129±1.795), microalbuminuria (9.683±1.902) and proteinuria (10.58±1.40) were found (Fig. 4).

Women with proteinuria had higher ($p=0.063$) values of glycated hemoglobin (10.58±1.40) compared with women with normoalbuminuria (9.129±1.795) (see Fig. 4).

Women with proteinuria had higher ($p=0.060$) values of glycated hemoglobin (10.58±1.40) compared to men with proteinuria (9.092±1.870) (see Fig. 4).

There was a lower ($p<0.05$) value of total cholesterol in healthy men (4.813±0.383) compared with sick men with proteinuria (5.852±1.171). Healthy women had lower ($p<0.05$ in both cases) values of total cholesterol (4.722±0.304) compared with sick women with normoalbuminuria (5.111±0.674) and proteinuria (6.381±1.721) (Fig. 5).

Men with proteinuria had higher ($p<0.01$ and $p<0.05$) values of total cholesterol (5.852±1.171) compared with men with normoalbuminuria (4.688±0.901) and microalbuminuria (4.863±0.831). Women with proteinuria had higher ($p<0.05$ in both cases) values of total cholesterol (6.381±1.721) compared with patients with normoalbuminuria

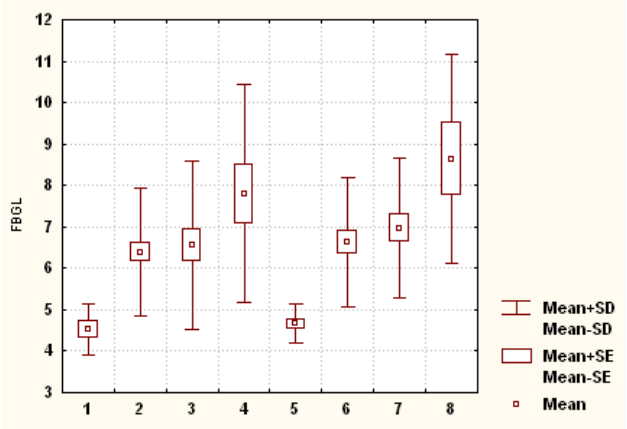

Fig. 1. Fasting blood glucose levels (FBGL) in patients with T1D men and women depending on the level of albumin in the urine (mmol/L). Here and in the following figures, 1 - healthy men; 2 - sick men with normoalbuminuria; 3 - sick men with microalbuminuria; 4 - sick men with proteinuria; 5 - healthy women; 6 - sick women with normoalbuminuria; 7 - sick women with microalbuminuria; 8 - sick women with proteinuria; Mean - average value; Mean±SE - average value ± mean error; Mean±SD - mean ± standard deviation.

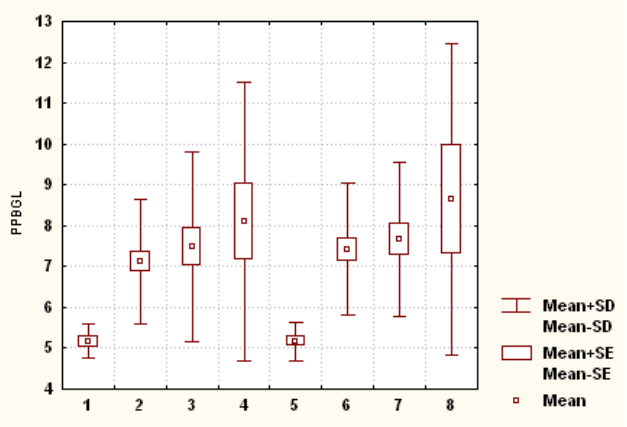

Fig. 2. Postprandial blood glucose level (PPBGL) in patients with T1D men and women depending on the level of albumin in the urine (mmol/L).

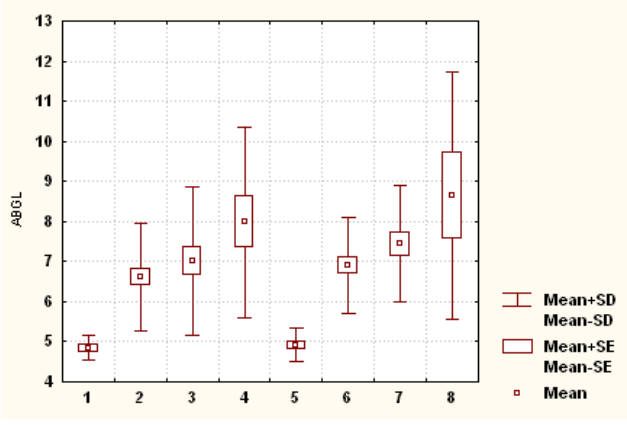

Fig. 3. The average blood glucose level (ABGL) in patients with T1D men and women depending on the level of albumin in the urine (mmol/L).

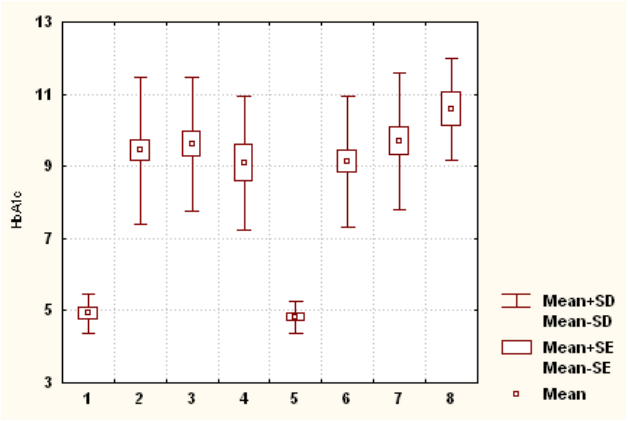

Fig. 4. The level of glycated hemoglobin (HbA1c) in patients with T1D men and women depending on the level of albumin in the urine (%).

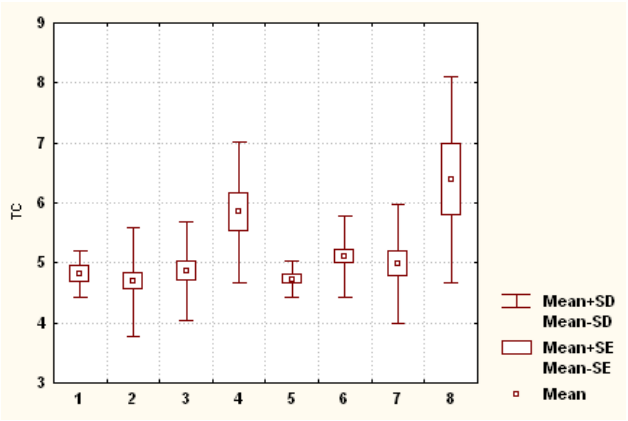

Fig. 5. The level of total cholesterol (TC) in patients with T1D men and women depending on the level of albumin in the urine (mmol/L).

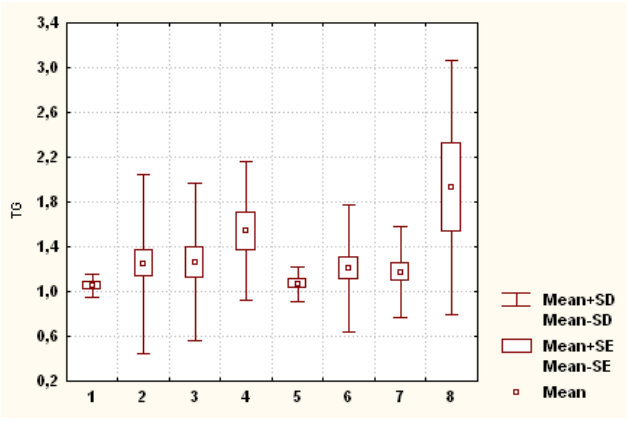

Fig. 6. The level of triglycerides (TG) in patients with T1D men and women depending on the level of albumin in the urine (mmol/L).

(5.111±0.674) and microalbuminuria (4.985±0.994) (see Fig. 5).

Men with microalbuminuria had lower ($p<0.01$) values of total cholesterol compared with women with normoalbuminuria (4.688±0.901 and 5.111±0.674, respectively) (see Fig. 5).

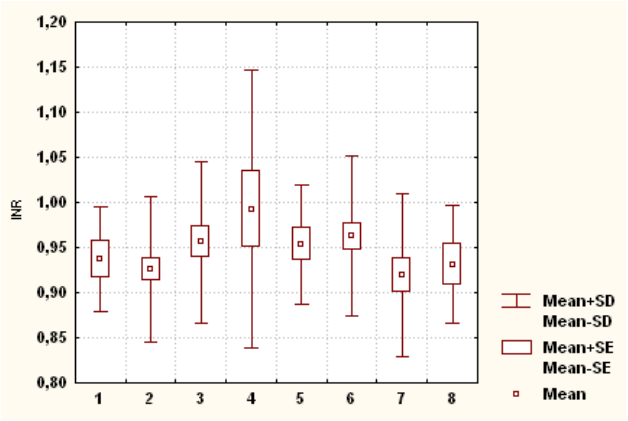

Fig. 7. Indicator of the international normal ratio (INR) in patients with T1D men and women depending on the level of albumin in the urine (abs.u.).

In men with proteinuria, higher ($p=0.061$ and $p=0.065$) values of triglyceride levels (1.537±0.620) were found in comparison with men with normoalbuminuria (1.250±0.802) and healthy men (1.056±0.105) (Fig. 6).

There are significant differences in international normal ratios (INR) between healthy men or women with T1D patients of the same sex with normo-, microalbuminuria or proteinuria, or between patients of the same sex with normo-, microalbuminuria or proteinuria, as well as between healthy or sick men and women of the respective comparison groups (Fig. 7).

Discussion

It is well known that hyperglycemia has its detrimental effect through numerous mechanisms, given that glucose and its metabolites are utilized in cells by various metabolic processes [13]. Elevated fasting glucose, 2 hours after glucose loading and glycated hemoglobin in patients with T1D are predictors of the likelihood of diabetic nephropathy, which proves the need to identify and compare them with the control group [2, 10]. The lipid spectrum, as one of the markers of dysmetabolic disorders, in patients with diabetes is characterized by increased triglycerides, decreased cholesterol levels of high-density lipoproteins and increased cholesterol levels of low-density lipoproteins [6, 11].

In our study, in patients with T1D compared with the control group found significantly higher values: fasting blood glucose in patients with normo-, microalbuminuria and proteinuria (in men - by 29.1 %, 30.9 % and 42.0 %; in women - by 29.7 %, 33.2 % and 46.0 %); blood glucose 2 h after exercise in patients with normo-, microalbuminuria and proteinuria (in men - by 27.4 %, 30.8 % and 36.1 %; in women - by 30.4 %, 32.4 % and 40.2 %); the average value of blood glucose in patients with normo-, microalbuminuria and proteinuria (in men - by 26.2 %, 30.8 % and 39.3 %; in women - by 28.7 %, 34.0 % and 43.1 %); glycated hemoglobin in patients with normo-, microalbuminuria and proteinuria (in men - by 47.8 %, 48.8 % and 45.8 %; in

women - by 27.2 %, 50.2 % and 54.4 %); total cholesterol in sick men with proteinuria by 17.8 %; in women with normo- and proteinuria - by 7.6 % and 26.0 %, respectively); triglycerides in sick men with proteinuria by 31.3 %.

The mechanism of damage to the renal parenchyma in diabetes mellitus includes progression of hyperglycemia, glomerular hyperfiltration, decreased number of structural and functional units of the kidney (nephrons), systemic hypertension, proteinuria, accumulation of end products of glycation, dysfunction of renin-angiotensin system and the development of disorders of lipid metabolism [4, 7, 18].

Leonova N. V. et al. [14] in the distribution of patients depending on the severity of diabetic nephropathy, evaluation of biochemical analysis of blood revealed significantly higher values of total cholesterol, low-density lipoproteins and glucose in patients with T1D with higher blood creatinine and urinary albumin. High-density lipoproteins, in contrast, were higher in patients with normoalbumineria and lower creatinine levels.

Korolev V. O. et al. [12] found that in patients with T1D there were direct weak or medium correlations of proteinuria with fasting and postprandial glycemia, as well as with the content of glycated hemoglobin.

The results obtained in the clinical study groups coincide with the literature data described above. Thus, with an increase in the level of albumin in the urine in men or women with proteinuria, greater values were observed: fasting blood glucose compared to men with normoalbuminuria by 22.2 % and women with normoalbuminuria and microalbuminuria - by 30.3 % and 23.7 %; the average value of blood glucose compared to men with normoalbuminuria and microalbuminuria - by 20.8 % and 14.0 %; glycated hemoglobin compared to women with normoalbuminuria - by 15.9 %; total cholesterol compared with men with normoalbuminuria and microalbuminuria - by 24.8 % and 20.3 %; compared with women with normoalbuminuria and microalbuminuria - by 24.9 % and 28.0 %; triglycerides in comparison with men with normoalbuminuria - by 23.0 %.

We did not notice any significant sex differences when comparing the level of biochemical parameters between both healthy and patients with T1D and normo-, microalbuminuria and proteinuria in men and women. Only 14.1 % higher values of glycated hemoglobin were found in women with proteinuria and 8.3 % higher values of total cholesterol in women with normoalbuminuria compared to the corresponding groups of men.

The presence of protein in the urine of patients with T1D, usually indicates a process that has already developed. Diagnosis of early and preclinical stages of diabetic nephropathy is not available for most hospitals [1, 16, 17]. Therefore, in our opinion, to screen the initial stages and verify the severity of kidney damage in the future it is possible to use the results of monitoring the biochemical parameters described by us.

Conclusions

Differences in biochemical parameters found in patients with T1D and different degrees of albuminuria compared to healthy subjects indicate a more unfavorable metabolic profile and indicate the potential usefulness of their use as a screening tool to identify risk groups for this pathology and its complications.

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