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EVALUATION OF THE FUNCTIONAL STATE OF THE KIDNEYS IN HEALTHY AND PATIENTS WITH TYPE 1 DIABETES WITH DIFFERENT LEVELS OF ALBUMIN IN THE URINE

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The study analyzed the differences in the functional status of the kidneys in healthy and patients with type 1 diabetes with different levels of albumin in the urine. Significantly, higher values of microalbumin, creatinine, albumin-creatinine coefficient of urine and lower values of glomerular filtration rate according to Cockcroft-Gault, CKD EPI and cystatin C in sick men and women compared with the control group of persons of the same sex. The discrepancies and contradictions e of indicators comparison results in the subjects with different levels of albumin in the urine justified the need to use a full set of criteria for renal dysfunction in the division into comparison groups.

Keywords: type 1 diabetes, urinary albumin levels, assessment of renal function.

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ОЦІНКА ФУНКЦІОНАЛЬНОГО СТАНУ НИРОК У ЗДОРОВИХ ТА ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ 1 ТИПУ З РІЗНИМ РІВНЕМ АЛЬБУМІНУ В СЕЧІ

У ході дослідження проаналізовані відмінності показників функціонального стану нирок у здорових та хворих на цукровий діабет 1 типу з різним рівнем альбуміну в сечі. Встановлено достовірно більші значення мікроальбуміну, креатиніну, альбуміно-креатинінового коефіцієнту сечі та менші значення швидкості клубочкової фільтрації за Кокрофтом-Голтом, CKD EPI і цистатином С у хворих чоловіків і жінок порівняно з контрольною групою осіб аналогічної статі. Встановлено розбіжності і суперечливості результатів порівняння показників у досліджуваних з різним рівнем альбуміну в сечі обгрунтувало необхідність застосування повного комплексу критеріїв ниркової дисфункції при поділі на групи порівняння.

Ключові слова: цукровий діабет 1 типу, рівень альбуміну в сечі, оцінка функціонального стану нирок.

The work is a fragment of the research project "Insulin resistance and hyperinsulinemia as factors in the development of microangiopathies in patients with diabetes mellitus: genesis, diagnosis and medical rehabilitation", state registration No. 0118U003253.

Currently, there is a tendency to a progressive increase in the number of people with a steady decline in renal function. The results of studies focused on identifying the main factors leading to deterioration of renal function suggest that the prevalence of renal failure in the general population is due primarily to nosologies that are not originally renal [2].

Hyperglycemia is a major factor in renal injury and progression of renal failure. Mortality from diabetes-related renal disease increases by 12 % annually [9]. The severity of this problem is provided by the presence of a long period of latent renal dysfunction. This pathological condition can last for years, gradually intensifying and developing into a clear pathology, manifested by clinical markers of chronic diabetic nephropathy and decompensation of renal function. That is why it is especially important to identify the initial period of renal dysfunction [4].

A well-known early marker of kidney damage in diabetes mellitus is microalbuminuria, the appearance of which corresponds to stage III diabetic nephropathy. The increase in albumin in the ultrafiltrate is mainly due to glomerular damage and, accordingly, a decrease in glomerular filtration rate (GFR) [12]. In fact, a decrease in GFR in half of all cases of structural disorders of the glomerular apparatus can occur without microalbuminuria. In general, microalbuminuria does not necessarily predict the development of proteinuria, and the decrease in renal function (according to the decrease in glomerular filtration rate) is not necessarily associated with the development of albuminuria. Moreover, the progression of severe nephropathy can occur without proteinuria. In this regard, the search for early markers of kidney disease in diabetes remains relevant today [15].

It should be recognized that despite the large number of publications on the problems of kidney damage in diabetic nephropathy, the functional state of the tubular apparatus is insufficiently studied, especially in its early stages. Information on the diagnostic significance of some markers of tubular lesions is contradictory, and a comprehensive study of renal status in type 1 diabetes (T1D) is virtually absent [3]. Clarification of these issues may be important for early diagnosis and subsequent correction of diabetic nephropathy.

The purpose of the study was to examine the differences in the functional status of the kidneys in healthy and patients with T1D depending on the level of albumin in the urine.

Materials and methods. A survey of 78 men and 62 women aged 22–26 years with T1D, who underwent inpatient treatment in the therapeutic department No. 1 and No. 2 Vinnytsia Regional Highly Endocrinological Center and 8 healthy men and 13 healthy women of similar age done.

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.

Creatinine was determined spectrophotometrically using picric acid on a biochemical analyzer using standard kits from Pointe Scientific (USA). Regulatory values of this indicator are 0.035-0.124 mmol/l; 35.36–123.76 μmol/l.

Albumin-creatinine ratio was determined using diagnostic strips of MicroalbuFAN for analysis of albumin and creatinine in urine. The ratio of albumin – creatinine in the urine of a healthy person is present in a concentration of less than 30 mg/g. Microalbuminuria is indicated by a ratio of 30 to 300 mg/g, and higher values indicate proteinuria.

The level of cystatin C was determined by enzyme-linked immunosorbent assay (ELISA) using the kit RD191009100 Human Cystatin C ELISA company BioVendor (Czech Republic). The ELISA method (enzyme-linked immunosorbent assay) is based on the ELISA sandwich method. Regulatory values for cystatin C are 0.57-1.12 mg/l for women and 0.6–1.11 mg/l for men.

GFR levels were calculated for creatinine (Cockcroft-Gault formula and CKD EPI) and cystatin C (ml/min/1.73 m^2).

The formula for calculating GFR according to Cockcroft-Gault:

GFR (for men) = [(140 – age) × body weight) / blood creatinine] × 1.23;

GFR (for women) = [(140 – age) × body weight) / blood creatinine] × 1.05;

Where, age – in years; body weight – in kg; blood creatinine – μmol/l.

The calculation of GFR by CKD-EPI was performed using an online calculator. The formula for calculating GFR on cystatin C: GFR = 100 / cystatin C – 14.

Statistical data processing was performed in the license package “Statistica 5.5” using non-parametric methods of evaluation of the obtained results. The mean values for each studied feature and the standard deviation are set. The reliability of the difference between the independent quantitative indicators was evaluated using the Mann-Whitney U-test.

Results of the study and their discussion. There was a lower ($p<0.001$ in all cases) values of microalbumin in healthy men (18.13±6.13) compared with sick men with normo- (88.69±62.23), microalbuminuria (131.1±84.3) and proteinuria (983.3±900.4). In healthy women, lower values of ($p<0.001$ in all cases) microalbumin (17.77±5.60) was found compared with sick women with normoalbuminuria (99.71±55.37), microalbuminuria (100.5±72.7) and proteinuria (2427±2872).

Men with microalbuminuria had higher ($p<0.05$) values of microalbumin (131.1±84.3) compared with men with normoalbuminuria (88.69±62.23). Men with proteinuria had higher ($p<0.001$ in both cases) values of this indicator (983.3±900.4) compared with men with normoalbuminuria (88.69±62.23) and microalbuminuria (131.1±84.3). In women with proteinuria found higher ($p<0.001$ in both cases) values of microalbumin (2427±2872) compared with patients with normoalbuminuria (99.71±55.37) and microalbuminuria (100.5±72.7).

There were lower ($p=0.077$, $p<0.05$ and $p<0.001$) values of creatinine in healthy men (0.068±0.008) compared with sick men with normo- (0.075±0.013), microalbuminuria (0.081±0.016) and proteinuria (0.145±0.171). Healthy women had lower ($p<0.05$) creatinine values (0.071±0.008) compared to sick women with proteinuria (0.187±0.186).

Men with microalbuminuria had higher ($p=0.053$) creatinine values (0.081±0.016) compared to men with normoalbuminuria (0.075±0.013). In men with proteinuria found higher ($p<0.001$ and $p<0.05$) value of this indicator (0.145±0.171) compared with men with normoalbuminuria (0.075±0.013) and microalbuminuria (0.081±0.016). In women with proteinuria, higher ($p<0.01$ and $p<0.05$) creatinine values (0.187±0.186) were found compared with patients with normoalbuminuria (0.068±0.013) and microalbuminuria (0.074±0.017).

In men with normoalbuminuria found higher ($p<0.05$) creatinine values (0.075±0.013) compared with sick women with normoalbuminuria (0.068±0.013).

The value of cystatin C in healthy men (0.681±0.117) was lower ($p<0.01$ in both cases) in comparison with sick men with normo- (1.041±0.360) and microalbuminuria (1.122±0.373). In healthy women, lower values of ($p<0.001$ in all cases) cystatin C (0.586±0.072) was found compared with sick women with normo- (0.957±0.428), microalbuminuria (0.977±0.431) and proteinuria (1.331±0.645).

Men with proteinuria had lower ($p<0.05$ and $p<0.01$) values of cystatin C (0.842±0.527) compared with men with normoalbuminuria (1.041±0.360) and microalbuminuria (1.122±0.373). In women with proteinuria found higher ($p<0.05$) values of cystatin C (1.331±0.645) compared with patients with normoalbuminuria (0.957±0.428).

In men with proteinuria, a lower ($p<0.01$) value of cystatin C (0.842±0.527) was found compared with sick women with proteinuria (1.331±0.645).

The value of albumin-creatinine coefficient of urine in healthy men (16.25±5.18) was lower ($p<0.001$ in all cases) compared with sick men with normo- (105.0±19.6), microalbuminuria (117.8±87.7) and proteinuria (742.3±750.4). In healthy women lower ($p<0.001$ in all cases) values of albumin-creatinine coefficient of urine (16.92±4.80) were found in comparison with sick women with normoalbuminuria (111.7±24.1), microalbuminuria (105.0±59.3) and proteinuria (1883±2082).

Men with proteinuria had higher ($p<0.001$ in all cases) values of urinary albumin-creatinine ratio (742.3±750.4) compared with men with normoalbuminuria (105.0±19.6) and microalbuminuria (117.8±87.7). In women with proteinuria found higher ($p<0.05$ and $p<0.01$) values of albumin-creatinine coefficient of urine (1883±2082) compared with women with normoalbuminuria (111.7±24.1) and microalbuminuria (105.0±59.3).

There was a higher ($p<0.001$ in all cases) values of GFR according to Cockcroft-Gault in healthy men (152.4±22.3) compared with sick men with normo- (124.7±35.2), microalbuminuria (100.1±30.2) and proteinuria (70.50±28.91). Healthy women had higher ($p<0.01$) GFR values according to Cockcroft-Golt (123.6±13.4) compared with sick women with proteinuria (72.13±45.52).

Men with microalbuminuria had a lower ($p<0.01$) GFR according to Cockcroft-Gault (100.1±30.2) compared with men with normoalbuminuria (124.7±35.2). Men with proteinuria had lower ($p<0.01$ and $p<0.001$) GFR according to Cockcroft-Gault (70.50±28.91) compared with men with normoalbuminuria (124.7±35.2) and microalbuminuria (100.1±30.2). Women with proteinuria had lower ($p<0.01$ and $p<0.05$) GFR according to Cockcroft-Gault (72.13±45.52) compared with patients with normoalbuminuria (134.4±41.1) and microalbuminuria (126.4±46.6).

Healthy men had higher ($p=0.067$) GFR according to Cockcroft-Gault (152.4±22.3) compared to healthy women (123.6±13.4). In men with microalbuminuria found lower ($p<0.05$) values of this indicator (100.1±30.2) compared with sick women with microalbuminuria (126.4±46.6).

Established higher ($p<0.05$, $p<0.01$ and $p<0.001$) GFR values for CKD EPI in healthy men (128.6±6.3) compared with sick men with normo- (117.9±12.6), microalbuminuria (109.2±16.2) and proteinuria (83.69±34.38). In healthy women, greater ($p<0.05$) GFR values for CKD EPI (96.77±26.70) compared with sick women with proteinuria (60.75±43.35).

Men with microalbuminuria had lower ($p<0.01$) GFR values for CKD EPI (109.2±16.2) compared with men with normoalbuminuria (117.9±12.6). Men with proteinuria had lower ($p<0.05$, $p<0.001$ and $p<0.05$) GFR values for CKD EPI (83.69±34.38) compared to men with normoalbuminuria (117.9±12.6) and microalbuminuria (109.2±16.2). In women with proteinuria found lower ($p<0.01$ and $p<0.05$) GFR values for CKD EPI (60.75±43.35) compared to patients with normoalbuminuria (104.3±18.6) and microalbuminuria (97.65±24.17).

In healthy men, men with normo- and microalbuminuria found higher ($p<0.001$, $p<0.01$ and $p=0.087$) GFR values for CKD EPI (respectively 128.6±6.3; 117.9±12.6 and 109.2±16.2) compared to women of similar comparison groups (respectively 96.77±26.70; 104.3±18.6 and 97.65±24.17).

GFR values for cystatin C were found to be higher ($p<0.01$ in both cases) in healthy men (137.0±27.9) compared to sick men with normo- (95.97±44.93) and microalbuminuria (87.10±40.06). In healthy women, higher ($p<0.001$ in all cases) values of GFR on cystatin C (158.9±20.6) compared to sick women with normoalbuminuria (110.8±53.1), microalbuminuria (105.3±44.6) and proteinuria (72.50±29.14).

Higher values of ($p<0.05$ and $p<0.01$) GFR according to cystatin C was found in sick men with proteinuria (132.9±66.5) compared to sick men with normo- (95.97±44.93) and microalbuminuria (87.10±40.06). Women with proteinuria had lower values of ($p<0.05$) GFR according to cystatin C (72.50±29.14) compared to women with normoalbuminuria (110.8±53.1).

In men with proteinuria, a higher ($p<0.01$) GFR value according to cystatin C (132.9±66.5) was found compared with patients with proteinuria (72.50±29.14).

The high prevalence of persistent decline and impairment of renal function in T1D and the unfavorable overall prognosis inherent in this category of individuals determine the need for early detection and, if possible, prevention of diabetic nephropathy. The relevance of attempts at a population-based approach to the comparative assessment of the functional status of the kidneys in healthy and T1D patients is obvious [13].

In our study, in patients with T1D compared with the control group found significantly higher values: microalbumin in patients with normo-, microalbuminuria and proteinuria (in men – by 80.0 %, 86.2 % and 98.2 %; in women – by 82.2 %, 82.3 % and 99.03 %); creatinine in sick men with normo-, microalbuminuria and proteinuria (by 9.3 %, 16.1 % and 53.1 %); in women with proteinuria – by 62.0 %; cystatin C in sick men with normo- and microalbuminuria (by 34.6 % and 39.0 %); in sick women with normo-, microalbuminuria and proteinuria (by 38.8 %, 40.0 % and 56.0 %); albumin-creatinine ratio of urine in patients with normo-, microalbuminuria and proteinuria (in men – by 84.5 %, 86.2 % and 97.8 %; in women – by 84.9 %, 83.9 % and 99.1 %). Found significantly lower values: GFR according to Cockcroft-Gault in sick men with normo-, microalbuminuria and proteinuria (by 22.2 %, 52.3 % and 116.2 %); in women with proteinuria – by 71.4 %; GFR according to CKD EPI in sick men with normo-, microalbuminuria and proteinuria (by 9.1 %, 17.8 % and 53.7 %); in women with proteinuria – by 59.3 %; GFR according to cystatin C in sick men with normo- and microalbuminuria (by 42.8 % and 57.3 %); in sick women with normo-, microalbuminuria and proteinuria (by 43.4 %, 50.9 % and 119.2 %).

To date, most clinicians around the world prescribe albumin and creatinine analyses for the diagnosis of renal function. Until now, it was and is believed that an increase in these biochemical parameters is a predictor of the development of severe stages of diabetic nephropathy [6]. Indeed, by dividing patients into groups depending on the level of albumin in the urine, we obtained quite expected (at first glance) results: with increasing levels of albumin in the urine of men or women with proteinuria there were higher values of microalbumin, creatinine, albumin-creatinine compared with those studied with normoalbuminuria and microalbuminuria. Men with microalbuminuria had higher levels of microalbumin and creatinine than in men with normoalbuminuria.

Our data could be used as evidence that the predictor of severe stages of diabetic nephropathy is microalbuminuria and groups of subjects on the severity of diabetic nephropathy should be formed by the level of albumin in the urine. Confirmation of this conclusion could be the work carried out in the early eighties. However, in these studies, patients with microalbuminuria were observed for 7-14 years, and the late stages of nephropathy did develop in most patients [5]. As it turned out later, microalbuminuria can occur simultaneously with a decrease in glomerular filtration rate and is ambiguously a mandatory stage of progression to late stages of nephropathy. In patients with early stages of T1D, it was found that suppression of microalbuminuria in the early stages of the disease does not prevent further falls in GFR. Microalbuminuria is a dynamic process that is more likely to return to normoalbuminuria than to lead to proteinuria [1]. Therefore, the conclusion, unfortunately, turned out to be hasty and was based on a common scientific error – "unreasonably broad generalization" for all stages of diabetic nephropathy.

It has been proven that with the development of diabetic nephropathy, the initial hemodynamic changes cause glomerular hyperfiltration, and hyperfiltration, as such, leads to a chain of pathological events – a sharp and progressive decline in renal function [10, 11]. That is, the earliest diagnostic sign of the development of chronic renal failure is not a decrease in GFR, but, conversely – its increase.

With increasing urinary albumin levels in men or women with proteinuria, there were lower GFR according to Cockcroft-Gault and CKD EPI compared with those studied with normoalbuminuria and microalbuminuria. In men with microalbuminuria compared to men with normoalbuminuria, lower values were observed for GFR according to Cockcroft-Gault and GFR by CKD EPI.

The logical question is whether to take into account the analysis of data to detect initial renal impairment, obtained by the formulas of Cockcroft-Gault and CKD-EPI. The first formula was developed to assess the clearance of creatinine, which systematically overestimates GFR due to tubular creatinine secretion, especially at low GFR values. The CKD-EPI formula, in contrast to the first, allows a more accurate assessment of GFR if it exceeds 60 ml/min/1.73 m^2 [7].

Detection of renal dysfunction in people with prediabetes and early stages of diabetes may be crucial for the appointment of preventive measures. Indicators such as microalbumin, creatinine, urinary albumin-creatinine, GFR according to Cockcroft-Golt and CKD EPI are not able to correctly reflect the function of the kidneys in its entire range, especially in the earliest and potentially reversible stage – hyperfiltration. It is believed that the most accurate algorithm for the diagnosis of renal dysfunction is the regular determination of not two, as before, the markers "micro/macroalbumin and creatinine", and three "micro/macroalbumin and creatinine/cystatin C" [8].

The most accurate endogenous marker of hyperfiltration is cystatin C. It is a marker of both glomerular and tubular dysfunction [14]. Among patients, depending on the level of albumin in the urine, the following differences were found in cystatin C and GFR on cystatin C.

Cystatin C was found to be lower in men with proteinuria than in men with normoalbuminuria and microalbuminuria – by 19.1 % and 25.0 %. In women with proteinuria, on the contrary, this indicator was higher than in women with normoalbuminuria – by 39.1 %.

Men with proteinuria had higher GFR values for cystatin C compared to men with normoalbuminuria and microalbuminuria – by 38.5 % and 52.6 %. In women with proteinuria, on the contrary, GFR according to cystatin C was lower than in women with normoalbuminuria – by 34.6 %.

The opposite pattern in men and women cannot be attributed to sexual dimorphism. The inconsistency of the obtained data indicates that the division of the groups studied only by the level of albumin without taking into account the level of cystatin C is impractical.

Conclusions

1. Establishing differences in the level of functional status (cystatin C in particular) in healthy individuals and patients with diabetes has given a new understanding of what is “normal kidney function”.

2. For correct and timely diagnosis of renal function, GFR, microalbumin, cystatin C and proteinuria should be regularly measured as a set of independent diagnostic markers.

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