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MICROALBUMINURIA AS A POSSIBLE BIOMARKER IN EARLY DETECTION OF KIDNEY LESIONS IN PATIENTS WITH TYPE 2 DIABETES

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Abstract

Today, there is growing evidence supporting the association between renal failure and microalbumin (McA) concentration. The role of McA in the development of microcirculation damage in diabetic nephropathy (DN) has been proven.

In our obsevational study were including 78 patients with type2 diabetes. The baseline level of McA showed to be significantly increased in patients with DN, and it was related to the severity of the renal disease.

We were focusing on patients with a five-year-old diagnosis of diabetes mellitus type 2 (DM-2). After their a two-year follow-up, we found that microalbumin in urine increased afther 6 mounths and one year in some of patients. Microalbumin was determined using of turbidimetric method in the laboratory of the Institute of Medical and Experimental Biochemistry. For serum creatinine, we were using a standardized enzyme method. MDRD4 formula was the best for calculation of glomerular filtration rate. A statistical analysis of the data was performed with the statistical program IBM SPSS 26 for Windows.

The results obtained regarding albumin in urine showed: mean 42.04 mg/L, extremely high CV, and were in negative correlation with GFR. Using a multivariate linear regression model, we proved the fact that the increased McA level in the urine significantly influenced the decline of GFR.

In patients with a high risk of developing diabetic nephropathy, appropriate measures were taken in order to register and prevent the disease on time. The large variability could be due to differences in disease progression among individuals since microalbuminuria is a marker of early kidney damage, besides other promoters of progression.

Keywords: microalbuminuria, glomerular filtration rate, MDRD formula

Introduction

Microalbuminuria (McA) is a term that refers to excretion of small but pathological amounts of albumin in the urine, ranging from 30 to 300 mg/24 h[1]. Standard test strips for qualitative diagnosis of albumin in the urine can detect a daily excretion of albumin greater than 300 mg. More sensitive methods are necessary for the identification of McA.

The presence of permanent microalbuminuria represents the earliest stage of kidney damage in diabetes, which precedes the onset of clinically manifested diabetic nephropathy (DN)[2].

McA is also a marker of elevated cardiovascular (CVD) morbidity and mortality in patients with essential hypertension, and in the general population is associated with insulin resistance, central obesity and atherogenesis with dyslipidemia[3].

Microalbuminuria is also considered an indicator of generalized endothelial lesions that reflect the general vascular permeability disorder. Mechanisms of its formation are complex, and to date the diabetic glomerular lesions have been most frequently tested. This marker is relatively simple and available for diagnosis, and its early detection and monitoring is becoming increasingly important in the treatment of patients with diabetes mellitus. In type 2 diabetes, symptoms may exist in a latent form for several years before their clinical manifestations. Therefore, also appears in diagnosing the

underlying disease in patients McA is used, McA together with hypertension and peripheral obliterative angiopathy[4]. Pathological excretion of small amounts of albumin in the urine can precede the appearance of type 2 diabetes, together with insulin resistance, obesity and hypertension. Therefore, the presence microalbuminuria in this type of diabetes is not very specific for initial diabetic nephropathy[5]. However, due to the significantly higher prevalence of type 2 diabetes patients undergo dialysis treatment[6]. The presence of elevated urinary albumin excretion as well as hyperinsulinemia in people without diabetes can predict the onset of type 2 diabetes[7].

Aims

- To determine the concentration of albumin in the urine patients
- To assess the severity of renal function damage

Material and methods

We analyzed patients with a five-year-old diagnosis of diabetes mellitus type 2 (DM-2) (n=78). Inclusion criteria were McA in diabetic patients. Exclusion criteria were: patients with essential hypertension, insulin resistance, central obesity, atherogenesis, as well as patients who did not have any other disease caused by changes of microalbumin concentration in the urine.

This prospective study were included 78 patients, of both sexes, aged 35-65, with a proven diagnosis of type 2 diabetes at the University Clinic for Endocrinology. For all patients with DM type 2, a questionnaire was filled out with data regarding age, sex, weight, height, family history of diseases in the interest of this study, consumption of alcohol, coffee, smoking. Whole blood/serum was determining creatinine and urine was determining albumin and used, to analysed at the Institute of Medical and Experimental Biochemistry. Serum creatinine was determined using a routine enzymatic method. In the second morning urine, the concentration of microalbumin was determined by the turbidimetric method according to anti-albumin antibodies. The absorbance increased after by the resulting aggregates was measured by a turbidimetric end-point method. GFR was determined mathematically according to the MDRD4 formula.

All respondents were signed and wrouten consent to participate in the study, and ethical approval was obtained from the Ethics Committee of the Faculty of Medicine.

Statistical methods

By using commercial statistical software (SPSS 26 for Windows), a statistical analysis of data was made. The results are presented in tables and figures. Statistically significant difference was defined as a $p<0.05$.

Results

Table 1. Interpretation of descriptive statistics for numeric variables in dataset

	Mean	Standard Deviation	Coefficient of Variation (%)
Creatinine(s) umol/L	84.13	40.98	48.72
Microalbimun mg/L	42.04	84.44	200.85
Age-year	59.17	5.34	9.02
GFR %	84.89	37.49	44.16
A/C mg/g	1.15	0.90	78.02

The CV indicates moderate variability in serum creatinine levels Mean 84 umol/L, SD 41 umol/L. Serum creatinine is an indicator of kidney function, so this variability could reflect different

levels of kidney health across the participants. A lower CV 48.72 % with CV of GFR suggests more consistency in creatinine levels, though still considerable variation.

This extremely high CV 200.85 mg/L of Mca suggests substantial variation in microalbumin levels, which might indicate significant differences in kidney function. The large variability could be due to differences in disease progression among individuals, as microalbuminuria is a marker of early kidney damage.

The low CV of age 9.02 year indicates that age is relatively consistent among participants, with a standard deviation of just over 5 years. This suggests that most participants are within a similar age range, and age variability is minimal.

The CV 44.16% here shows moderate variability in GFR, a critical indicator of kidney function. Participants had varying degrees of kidney function, which could be tied to different stages of disease or health conditions.

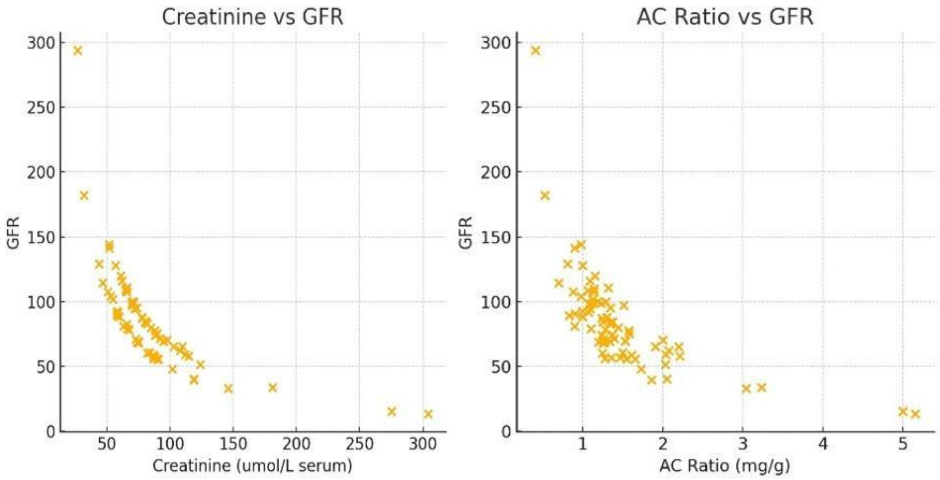
The CV of 78.02% reflected significant variation in the A/C ratio among participants. Since this ratio helps in assessing kidney damage, the high variability suggests that kidney health among participants varies greatly, with some likely experiencing early signs of kidney dysfunction while others are in healthier ranges.

Table 2. Correlation matrix showing the relationships between GFR and other numeric variables

GFR		**Creatinine serum**	**Micro albimun**	**Age**	**A/C ratio**
GFR %	1	-0.66	-0.10	0.12	-0.65
Creatinine (s)µmol/L	-0.67	1	0.21	-0.15	0.98
Microalbimun mg/L	-0.11	0.21	1	-0.07	0.21
Age- year	0.12	-0.15	-0.07	1	-0.29
A/C ratio mg/g	-0.65	0.98	0.21	-0.29	1

- Creatinine (serum): Strong negative correlation (r=-0.67), indicating that as serum creatinine increases, GFR decreases, which is expected since high creatinine levels typically reflect impaired kidney function.
- A/C Ratio: Strong negative correlation (r =-0.65), showing that a higher albumin-to-creatinine ratio tends to accompany lower GFR, indicating worsening kidney function.
- Age: Weak positive correlation (r = 0.12), suggesting small association between age and GFR in this dataset.
- General Insights
- Creatinine and A / C ratio have the strongest correlations with GFR, making them key markers of kidney function in this dataset. As these values increase, GFR decreases, signaling declining kidney health.

Figure 1. Scatter plots illustrating significant correlations



It seems that the logistic regression is designed for binary outcomes (0 or 1), but our dependent variable "Groups" had more than two categories. We had to perform a multinomial logistic regression instead, since we were dealing with multiple categories.

Here are the key results from the multinomial logistic regression analysis, where "Groups" was a dependent variable, and the other numeric variables (creatinine, microalbumin, age, A/C ratio, and GFR) were independent variables.

Significant predictors:

GFR:

- Coefficient: 0.05
- p-value: 0.02 (significant)
- Interpretation: As GFR increases, the likelihood of being in a higher-risk group increases, suggesting a protective effect of higher kidney filtration rates.
- Non-significant predictors:
- Creatinine (serum), microalbumin, age, and AC ratio: These variables did not show significant contributions to predicting group membership in this model.

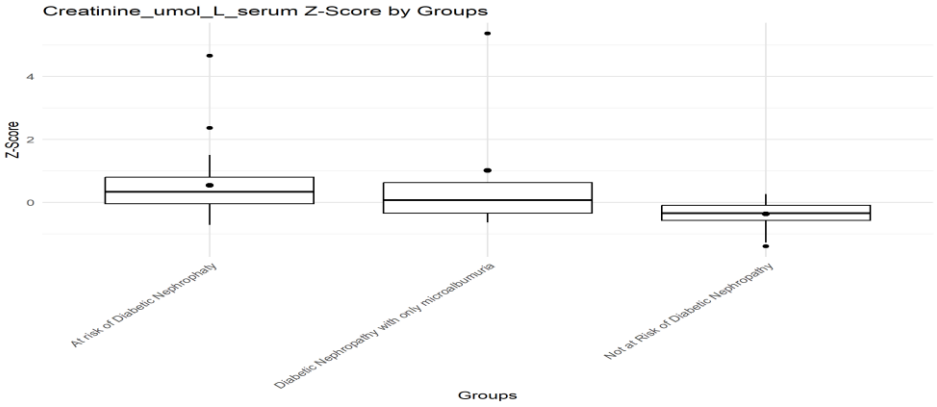
1. Creatinine *vs.* GFR: Showed a strong negative relationship where GFR decreased as creatinine levels increased.
2. A/C Ratio *vs* GFR: Showed a strong negative correlation where higher A/C ratios were associated with lower GFR, indicating worsening kidney function.

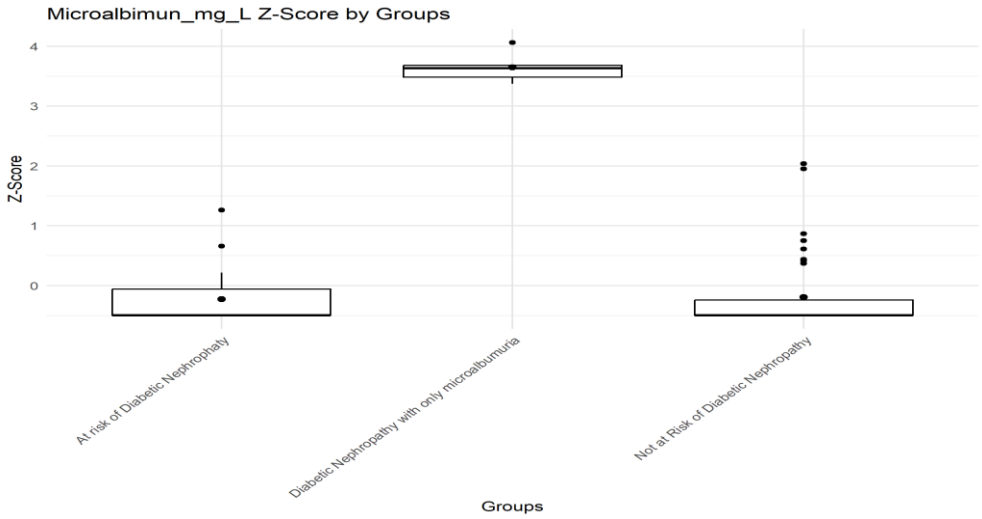
General Comments

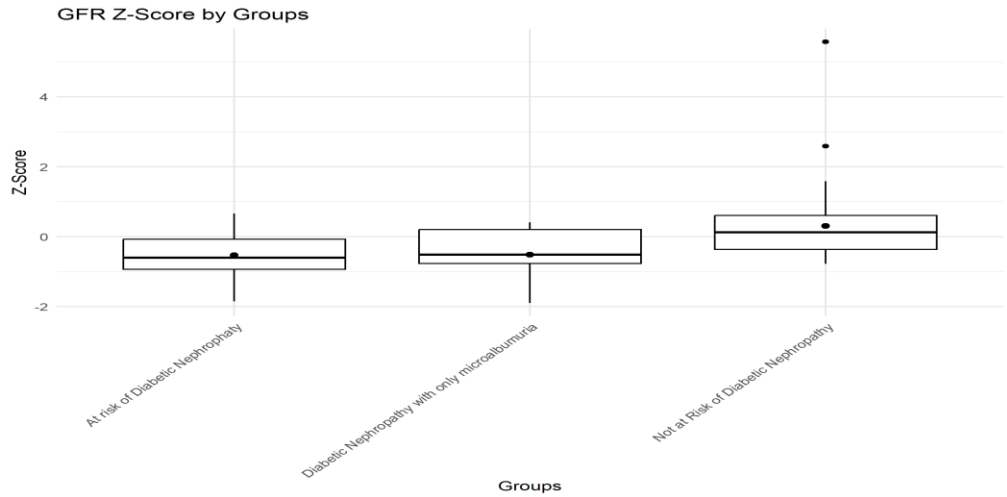
- Creatinine and microalbumin are commonly used markers but appeared less significant in this model, possibly due to overlap with GFR or the specific characteristics of the dataset.
- A/C ratio and age did not contribute significantly to differentiating between risk groups in this dataset, although they are known to be important in broader clinical contexts.

In summary, the logistic regression model suggests that GFR is the most important markers for predicting diabetic nephropathy risk, while other variables like creatinine and microalbumin may play a less direct role in this particular dataset.

Figure 2. Detailed interpretation of logistic regression analysis where dependent variable was group classification (risk of diabetic nephropathy) and independent variables were creatinine, microalbumin, age, A/C ratio, and GFR







The analysis highlighted the strong influence of A/C ratio in defining nephropathy risk stages, while other markers like creatinine and GFR may not provide as much differentiation in this context. These findings suggest that focusing on these specific biomarkers could help in better identifying individuals at different stages of diabetic nephropathy progression.

Here are the key results from the multinomial logistic regression analysis:

A/C Ratio (converted):

- Coefficient: -0.02
- p-value: 0.01 (significant)
- Interpretation: Higher A/C ratios are associated with a lower probability of being in more advanced nephropathy groups. This suggests the A/C ratio is important in distinguishing between groups.

Overall insights:

- The A/C ratio is a strong differentiator between those with diabetic nephropathy and the other groups.
- It is less useful in distinguishing those at risk from those not at risk, which could imply that the A/C ratio is more indicative of established kidney damage rather than early-stage risk factors.

This suggests that monitoring A/C ratio is particularly valuable for detecting the transition from at risk to established nephropathy.

Table 3. Established categories according to literature

Category	A/C index (µg/mg)	Daily portion (mg/24 h)	Time portion (µg/min)
Normal albuminuria	<30	<30	<20
Microalbuminuria	30-299	30-299	20-199
Macroalbuminuria	≥300	≥300	≥200

Discussion

Microalbuminuria (McA) is a term that refers to excretion of small but pathological amounts of albumin in the urine, ranging from 30 to 300 mg/24 h. After two years of follow-up, increased albumin levels were correlated with greater renal failure, characterized by a faster decline in the percentage of glomerular filtration rate[8]. Standard test strips for qualitative diagnosis of albumin in urine can detect a daily albumin excretion of over 300 mg/24h. Now on the market are more sensitive methods which are necessary for the identification of McA between 30-300mg/24h, such as turbidimetric methods[9]. As recommended by the American Diabetes Association (ADA) and the National Kidney Foundation, diagnosing persistent McA requires a positive finding in two out of

three urine samples, three to six months apart. For monitoring of therapeutic procedures, an average value of urinary albumin excretion rate in two to three 24-hour urine samples should be measured, collected in an interval of two to three days, so that the influence of individual and daily variations reduces to the smallest measure. In this sense, a one-time measurement urinary excretion of albumin has no particular significance in diagnostics, specific nor in the therapeutic monitoring of a patient[10].

Microalbuminuria is therefore considered an element of the metabolic syndrome, which predisposes to the manifestation of this type of diabetes. It is also important that type 2 diabetes patients have a higher degree of insulin resistance compared to those with normal albuminuria (physiological excretion of albumin). Apart from the basally poor metabolic control expressed as elevated glycosylated hemoglobin (HbA1c), McA is extremely strong, independent risk factor for the occurrence of all type of diabetes, too. Results of a study conducted in patients with newly discovered type 2 diabetes showed that at the time of diagnosis, 10.6% of patients already had diabetes McA. This group of patients also had a higher degree of insulin resistance determined by measuring HOMA model[11].

In the EURODIAB prospective study that monitored factors progression of McA in patients with type 1 diabetes, it was observed that patients with a lower volume index waist/hip, showed a greater tendency to return to normoalbuminuric level. In addition, patients with a higher central obesity, had an increased risk of McA and DN from those with lower abdominal mass and *vice versa*. Pathological excretion of small amounts of albumin in the urine can precede the appearance of type 2 diabetes, together with insulin resistance, obesity and hypertension. The presence of microalbuminuria in this of type 2 diabetes is not highly specific of initial diabetic nephropathy. But, monitoring of McA is inportant because, without therapeutic ones procedure of patients with continuous McA will progress to manifest DN, and after 5 years from the appearance of manifest, 20% will develop terminal insufficiency.Due to the significantly higher prevalence of type 2 diabetes, this population of patients makes more than half of people with diabetes who start with dialysis treatment[12].

The mechanism of association between insulin resistance and microalbuminuria is unclear. Insulin resistance can be the expression of diffuse endothelial arterial dysfunction when it contributes to the onset of atherosclerosis or directly affects the arterial damage by the toxic effect of hyperinsulinemia. Although, to some extent insulin resistance is involved in the pathogenesis of McA, it remains less important than microalbuminuria which represents a higher stage of DN[13].

Microalbuminuria is also associated with increased transvascular albumin (TPA), and hence, it is considered a surrogate marker of endothelial dysfunction. Diabetics with McA have increased capillary permeability at the level of vascular circulation, and TPA is one of the best parameter for this vascular disease ,beacuse the rate of transcapillary loss is increased[14]. Similar results were observed for fibrinogen, which is accompanied by an increase of von Willebrand factor (vWf), an indicator of increased extravascular coagulation and generalized vascular lesions. In patients with normoalbuminuria, the vWf level is not elevated, and a similar correlation was observed for other markers of endothelial lesions, such as tissue plasminogen activator (tPA) and angiotensin converting enzyme. Therefore, McA indicates a widespread vascular dysfunction [15].

Ultrastructural changes in glomerul are the result, above all, of non-enzymatic tissue glycosylation protein, absolute reduction of heparin sulfate, magnesium expansion and other complex processes characteristic of microalbuminuria and advanced glomerulopathy[16].

Finally, indicators of central and visceral fat, such as waist circumference or waist-to-hip ratio, were not available in this study[16].In the Asia-Pacific Study, a cohort study of 454 adult participants, elevations in these factors were more strongly associated with prevalent diabetes. In the future, variables that we determined (McA, A/C and GFR) should be used together with BMI to create an obesity index that more clearly indicates the risk of developing cardiovascular disease (CVD) in men and women without diabetes[17].

Conclusions:

Microalbuminuria in patients with type 2 DM is a potential marker which identifies patients in the earliest stage of renal lesions. It also identifies patients with increased risk, and they require adequate and timely therapy in order to slow progression of chronic complications. McA monitoring

is also a simple method for the assessment of therapy efficacy, so it should be introduced into the routine procedure of treatment and monitoring of patients with diabetes.

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