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Urinary glycosaminoglycans excretion in diabetic patients

Wydalanie glikozaminoglikanów z moczem u pacjentów z cukrzycą

In patients with diabetes, diabetic nephropathy is a serious complication which significantly lowers the quality of life and increases mortality. Diabetic nephropathy is also a leading cause of end stage renal disease connected with renal replacement therapy. In this group of patients, the possibility of early assessment of the degree of risk of diabetic nephropathy is valuable, which would give a chance for withdrawal or even inhibition of the development of this pathology.

Nowadays, determination of urinary albumin excretion is a recommended laboratory test. It is well known that micralbuminuria does not fully reflect the intrinsic glomerular filtration of proteins due to the fact that final albumin excretion is modified by albumin reabsorption in renal tubules. For this reason, more specific and sensitive markers of filtration barrier disturbances have been searched for many years, which would be helpful not only in diagnosis but also in monitoring diabetic nephropathy [6, 8, 11].

Glycosaminoglycans (GAG) are vital components of glomerular basement membranes and they play an important role in their molecular organization and function. Heparan sulfate (HS) is a major GAG which is a component of perlecan – the most important proteoglycan of the glomerular membrane [4, 10]. It has been proved than changes in the concentration and quantity of sulfates groups of GAG (HS) are connected with the appearance of diabetic nephropathy [9, 13].

HS is metabolized by heparanase (endo-β-D-glucuronidase), which is induced by the presence of glucose in the environment. Lewis in his data showed that incubation of epithelial cells of glomerulus in medium containing high concentration of glucose induces increase in the expression of heparanase-1 gene and decrease in GAG amount in glomerular basement membranes [7]. On the other hand, diminishing of the content of HS in mesangium results in unblocking extracellular synthesis of matrix and proliferation of cells, which leads to hypertrophy of mesangium and progression of nephropathy. Additionally, HS deficiency as an activating factor of lipoprotein lipase favours the progression of atherosclerotic changes and leads to the sclerosis of glomeruli [3]. Lack of synthesis or impaired synthesis of heparane sulfate of a basement membrane leads to the development of microalbuminuria and further to excessive urinary proteins excretion [2, 3].

In search of early indicator of glomerular damages, it was decided to evaluate changes in urinary GAG excretion, as a possible useful marker of the development of diabetic nephropathy. The aim of the study was • searching for differences in urinary GAG excretion in patients with diabetes in comparison to the control group • evaluation of the influence of metabolic control of carbohydrate

balance on urinary GAG excretion • evaluation of possible usefulness of urinary GAG excretion as a laboratory test for diabetic nephropathy

MATERIAL AND METHODS

The research was conducted on 44 patients with diabetes type 1 or 2, hospitalized in the Chair and Department of Endocrinology, Medical University of Lublin. The examined group consisted of 26 women and 18 men, aged from 19 to 87, with the average age of 51.7 years. The average duration of the disease was 11 years, and the average BMI was 29.02kg/m^2. The group of diabetic patients was divided into two subgroups: with HbA_1C <7.5%, which confirms a good metabolic control (13 subjects) and with HbA_1C >7.5%, which indicates poorly controlled diabetes (31 subjects). The control group consisted of 24 healthy subjects: 14 women and 10 men aged from 24 to 73 years. The average age was 50.5 years.

Subjects with excluded coexisting disorders which might have an influence on changes in urinary GAG excretion were qualified to the control group. Laboratory tests were performed in fasting serum and in 24-hour urine collection. Concentrations of glucose, HbA_1C and creatinine were measured with the use of Advia 1650 analyser (Siemens). Quantificational determination of GAG in urine was performed with the use of Blyscan Proteoglycan and Glycosaminoglycan Assay, Bicolor Ltd. (Belfast).

The results obtained underwent statistical analysis and all data were expressed as mean ± standard deviation (SD) and minimum/maximum range. Due to the scatter of results and connected with it elevated SD, standard error of the mean (SE) was used as a parameter better describing the variable. Students'T test for independent variables was used for a comparison between the groups. Statistical significance was considered for *P* values less than 0.05. Statistical analyses were conducted using the Statistica programme (StatSoft, Polska).

RESULTS

The clinical and laboratory characteristics of examined groups are shown in Table 1. Fasting glucose in most patients differed significantly from normal values and mean glucose concentrations was 157.7mg/dl. 24-hour urine collection varied from 700 ml to 5700 ml. The average 24-hour urine collection was 1757 ml. Mean HbA_1C was 8.87% in the examined group. The excretion of GAG with urine in patients with diabetes in comparison to the control group is presented in Table 2.

The amount of GAG in urine was expressed as: 1) GAG concentration in the examined urine sample (µg/ml), 2) The total amount of GAG excreted in 24-hour urine collection (µg/24h), 3) GAG concentration calculated per urine creatinine (µg/mg creatinine). While comparing results in the group of diabetic patients with the results obtained in the control group, a statistically significant decrease in urinary GAG excretion was found when GAG excretion was expressed as concentration in the examined urine sample and was 2.87 µg/ml for the group of diabetic subjects and 4.77 µg/ml for the control group (p=0.0023).

No significant differences were found between both groups when GAG excretion was expressed as the total amount of GAG in 24-hour urine collection as well as calculated per urine creatinine. During the analysis of urinary GAG excretion with regard to the degree of metabolic control of carbohydrates balance on the basis of HbA_1C level, no significant relationships were found between results of patients with good metabolic control (HbA_1C < 7.5%) and patients with poorly controlled diabetes (HbA_1C >7.5%). These results were 3.39 µg/ml and 2.66 µg/ml for patients with HbA_1C < 7.5% and HbA_{1C} > 7.5%, respectively. The results obtained are presented in Table 3.

Table 1. Characteristics of control group and patients with diabetes

Parameters	n	mean (x)	minimum	maximum	Standard deviation (SD)
Control group					
Age (years)	24	50.5	24.0	73.0	15.3
Diuresis (ml)	24	1160.4	560.0	2000	465.2
Urinary creatinine (mg/l)	24	0.872	0.340	1.54	0.339
BMI	24	25.128	18.8	32.8	3.866
Diabetes					
Age (years)	44	51.7	19.0	87.0	16.6
Diuresis (ml)	44	1757.2	700	5700	833.3
Urinary creatinine (mg/l)	44	0.525	0.169	1.53	0.289
BMI	44	29.02	15.4	42.2	6.670
Duration of the disease (years)	44	11.12	0	46.0	10.09
Glycemia (mg/dl)	44	157.7	79.0	364.0	55.68
HbA_{1C} (%)	44	8.87	5.48	13.67	1.98

Table 2. Results of 24-hour GAG urine excretion in control group and patients with diabetes. SD – Standard deviation, SE – Standard error of the mean

Parameters	Control group				Patients group				(p<0.05)
	N	Mean (x)	SD	SE	N	Mean (x)	SD	SE	
GAG concentration (µg/ml)	24	4.77	2.691	0.54	44	2.87	2.154	0.32	p=0.0023
GAG total excretion in 24-hour (µg/24 h)	24	4979.1	2727.8	56.8	44	4686.7	3823.2	576.3	NS
GAG concentration (µg/mg creatinine)	24	5.66	2.839	0.57	44	5.70	3.957	0.59	NS

Table 3. Results of 24-hour GAG urine excretion in patients with diabetes, depending on HbA_{1C} concentration

Parameters	HbA_{1C} <7.5%	HbA_{1C} >7.5%
GAG concentration (µg/ml)	3.39	2.66
GAG total excretion in 24-hour (µg/24 h)	4975.2	4581.3
GAG concentration (µg/mg creatinine)	7.01	5.13

DISCUSSION

Data from the literature differ significantly regarding urinary GAG excretion in the course of diabetes. In the 1980's Deckert at al. introduced the hypothesis that increased metabolism of HS plays an important role in the pathogenesis of diabetic nephropathy. However, scientific studies conducted in recent years do not provide complete and unequivocal results upon changes in urinary GAG excretions in the course of diabetes [3, 9].

Results of the conducted studies revealed that in patients with diabetes urinary GAG excretion in 24-hour urine collection is lower in comparison to the control group. Only results of urinary GAG

excretion expressed as GAG concentration in urine demonstrate statistical significance: 2.87 µg/ml in the group of diabetic patients, 4.77 µg/ml in the control group.

This very general outline of a declining tendency for urinary GAG excretion in patients with diabetes suggests that the possible cause of these changes may be a decrease in production or an increase in degradation of GAG in structures of glomerular basement membranes observed during hyperglycemia states.

According to Vilar, intensive degradation of HS in glomerular basement membranes, or directly in urine, may have an influence on the decrease in urinary GAG excretion in patients with diabetes [12]. Under his hypothesis, nitric acid (III), which is one of the products of metabolic processes of nitric oxide (NO), contributes to the degradation of HS. This acid detaches N-sulfates groups from HS chain and heparin chain [12]. Tissue damage due to inflammatory process intensifies the synthesis of NO by endothelium and leads to HS breakdown in glomerular basement membranes. The synthesis of enzymes that metabolize HS is increased in diabetes, which may also contribute to degradation of HS in glomerular basement membranes [7].

A similar direction of changes in urinary GAG excretion in diabetes was found by Yokoyama et al. [13]. Japanese scientists examined patients with type 2 diabetes and showed that HS concentration in urine of individuals with diabetic nephropathy was decreased in comparison to the control group [13]. Similar results were also obtained by Cadaval et al. [1]. In their study, decreased urinary GAG excretion in rats with streptozocin-induced diabetes was found. After 12 weeks of the experiment, all animals developed hyperglycemia and hypertension and maintained their weight. In the examined urine sample, decreased concentrations of HS, chondroitin sulfate and dermatan sulfate were observed in comparison to the control group. Changes in the concentration of urinary GAG excretion were observed in the second week of the experiment [1].

Contrary to these findingsk, there are data in which an increase in urinary GAG excretion in patients with diabetes was revealed. In Juretic et al. [5], data on urinary GAG concentration in adults patients with type 2 diabetes was significantly increased in comparison to the control group, similarly to urinary GAG concentration in children with type 1 diabetes. In the latter group, urinary GAG concentrations were significantly lower than in adult patients with type 2 diabetes.

Microalbuminuria was observed only in 10 adults diabetic type 2 patients and the authors drew the conclusion that increased urinary GAG concentration in diabetic patients might precede overt clinical albuminuria [5].

De Muro et al. were working on possible correlations between urinary GAG excretions and the level of HbA_1C [2]. They proved in their research that the level of HbA_1C has an influence on urinary GAG concentration. However, there were no statistical significant differences between the group of patients with $HbA_1C < 8\%$ and the control group. Significantly increased urinary GAG concentrations were found in patients with an elevated level of $HbA_1C > 8\%$ [2].

It was found in our own studies that urinary GAG excretion in patients with poorly controlled diabetes ($HbA_1C > 7.5\%$) was lower in comparison to the group of diabetic patients with good metabolic control of the disease (4581.3 µg/24h and 4975.2 µg/24h, respectively) although there were no statistical significant differences. And what is important, urinary GAG concentration in patients with good metabolic control of diabetes was almost identical to that in healthy subjects (4979.1 µg/24h). It can be assumed that good metabolic control of carbohydrate balance has an influence on correct metabolism of GAG.

Albuminuria has been a laboratory marker of diabetic nephropathy so far. Proven diagnostic usability of urinary GAG assessment would allow for noninvasive and rapid method for diagnosis of early stages of diabetic nephropathy. Despite so much research conducted in recent years, we are still not able to draw a final conclusion whether the assessment of urinary GAG excretion may

be a valuable diagnostic method for early stages of diabetic nephropathy. It results from the fact that urinary GAG excretion depends on many factors including age, sex, comorbidities, pregnancy, seasons of the year, concomitant therapy, climatic conditions. Differences in methodology and the preanalytic phase of testing also influence the results.

The data from the literature as well as the results obtained indicate that GAG can be a valuable marker in diagnostics of diabetic nephropathy. However, research results require confirmation on a greater number of cases considering many factors influencing the progression of diabetic nephropathy, such as: type of diabetes, duration of disease, administered treatment and comorbidities.

CONCLUSIONS

The conducted studies suggest that in subjects with diabetes changes in urinary GAG excretion are observed, which confirms changes in GAG metabolism in these patients. For complete determining of the existing statistical correlations between urinary GAG excretion in patients with diabetes, study results require confirmation on a greater number of cases considering the duration of the disease and the type of diabetes.

REFERENCES

1. Cadaval R., Kohlman O., Michelacci Y.: Urinary excretion of glycosaminoglycans and albumin in experimental diabetes mellitus. Glycobiology, 10(2), 185, 2000.
2. De Muro et al.: A longitudinal evaluation of urinary glycosaminoglycans excretion in normoalbuminuric type 1 diabetics patients. Clin. Chem. Lab. Med., 44, 561, 2006.
3. Gambaro G., van Der Woude J.: Glycosaminoglycans: Use in treatment of Diabetic Nephropathy. J. Am. Soc. Nephrol., 11, 359, 2000.
4. Hurst R. E. : Structure, function, and pathology of proteoglycans and glycosaminoglycans in the urinary tract. World Journal of Urology, 3, 12, 1994.
5. Juretic D., Krajnovic V., Bajalo L.: Altered distribution of urinary glycosaminoglycans in diabetic subjects. Acta Diabetol., 39, 123, 2002.
6. Kaznowska-Bystryk I. et al.: Changes in the excretion of glycosaminoglycans with urine in patients with glomerular disease. Annales UMCS, Sectio DDD, vol. XX, 35, 2007.
7. Lewis J. E., Xu X.: Abnormal Glomerular Permeability Characteristics in Diabetic Nephropathy. Diabetes Care, 31, 202, 2008.
8. McAuliffe A. V. et al.: Urinary Glycosaminoglycan Excretion in NIDDM Subjects: Its Relationship to Albuminura. Diabetic Medicine, 13 (8), 758, 1996.
9. Perez-Blanco F. et al.: Urinary Excretion of Glycosaminoglycans in Patients with early Diabetic Nephropathy. Nephron, 73, 344, 1996.
10. Prydz K., Dalen K. T: Synthesis and sorting of proteoglycans. Journal of Cell Science, 113, 193, 2000.
11. Tuomi T.: Type 1 and type 2 Diabetes-What do they have in common? Diabetes, Vol. 54, Supplement 2, 40, 2005.
12. Vilar R. E. et al.: Nitric oxide degradation of heparin and heparan sulfate. Biochem. J., 324, 473, 1997.
13. Yokoyama H. et al.: Serum and urinary concentrations of heparan sulfate in patients with diabetic nephropathy. Kidney International, 56, 650, 1999.

SUMMARY

GAG are vital components of glomerular basement membranes and they play an important role in their molecular organization and function. In search of an early indicator of glomerular damages in patients with diabetes, it was decided to evaluate changes in urinary GAG excretion as a possible useful marker of the development of diabetic nephropathy. The aim of the conducted study was to find differences in urinary GAG excretion in patients with diabetes in comparison to the control group, to assess the influence of the degree of metabolic control on urinary GAG excretion, and to evaluate the possible usefulness of urinary GAG excretion as a laboratory test for diabetic nephropathy. The conducted studies suggest that in subjects with diabetes, changes in urinary GAG excretion are observed, which confirms changes in GAG metabolism in these patients. For complete determining of existing statistical correlations between urinary GAG excretion in patients with diabetes, study results require confirmation on a greater number of cases considering the duration of the disease and type of diabetes.

STRESZCZENIE

GAG są istotnym składnikiem budującym błonę podstawną kłębuszków nerkowych oraz pełnią znaczącą rolę w jej organizacji i funkcji. W poszukiwaniu wczesnego wykładnika uszkodzeń kłębuszka u pacjentów z cukrzycą postanowiono ocenić zmiany w wydalaniu GAG z moczem jako ewentualnie przydatnego markera rozwoju nefropatii cukrzycowej. Celem pracy było poszukiwanie różnic w wydalaniu GAG w moczu pacjentów z cukrzycą w porównaniu z grupą kontrolną, ocena wpływu stopnia wyrównania gospodarki węglowodanowej na wydalanie GAG z moczem oraz ocena ewentualnej użyteczności diagnostycznej oznaczania wydalania GAG w moczu jako wskaźnika nefropatii cukrzycowej. Przeprowadzone badania sugerują, iż u chorych na cukrzycę dochodzi do zmian wydalaniu GAG z moczem, co potwierdza zmiany w metabolizmie glikozaminoglikanów u tych chorych. Dla pełnego określenia zachodzących zależności statystycznych w wydalaniu GAG z moczem u chorych na cukrzycę wyniki badań wymagają potwierdzenia na szerszej grupie pacjentów z uwzględnieniem czasu trwania choroby oraz typu cukrzycy.