ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 2, 17 SECTIO DDD 2010

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Urinary heparan sulfate excretion depending on microalbuminuria in patients with diabetes

Wydalanie siarczanu heparanu z moczem zależnie od mikroalbuminurii u pacjentów z cukrzycą

INTRODUCTION

One of the first signs of diabetic nephropathy is a discrete increase in urinary excretion of albumin called microalbuminuria. Microalbuminuria is predictive of renal lesions and its determination is useful in making decisions on the initiation of drug treatment for incipient nephropathy. When microalbuminuria appears, glomeruli have already demonstrated histological advanced glomerulopathy [3]. It is important to establish predictive markers, earlier than microalbuminuraia, to stop the process, or even to reverse it.

The heparan sulfate (HS) chains are composed of alternating glucosamine and D-glucuronic/L-iduronic acid residues, which are negatively charged as a result of the presence of multiple carboxylic groups and sulfate groups. The HS is a major glycosaminoglycan (GAG) which is a component of perlecan and agrin – the most important proteoglycans of the glomerular membrane (GBM), which may maintain the GBM charge barrier. Therefore, HS in the GBM is assumed to repel negatively charged proteins, including albumin, and prevent their filtration – HS is essential for the maintenance of proper glomerular filtration [1, 4].

It has been proved than changes in the concentration and quantity of sulfates groups of HS are connected with the appearance of renal pathologies, including diabetic nephropathy [5, 11]. A decreased GBM HS expression correlates with an increase in the level of proteinuria. The HS is metabolized by heparanase (endo-β-D-glucuronidase), which is induced by the presence of glucose in the environment. It has been shown that removal of HS from the GBM *in vivo* in rats by digestion with heparitinase leads to increased urinary secretion of both ferritin and albumin [7,8]. The lack of synthesis or impaired synthesis of HS of a GBM leads to the development of microalbuminuria and further to excessive urinary proteins excretion [10].

In search of an early indicator of glomerular damages it was decided to evaluate changes in urinary HS excretion, as a possible useful marker of the development of diabetic nephropathy. The

aim of the study was ● searching differences in urinary HS excretion in patients with diabetes in comparison to the control group ● evaluation of possible relationships between the microalbuminuria and urinary HS excretion ● evaluation of possible usefulness of urinary HS excretion as a laboratory test for diabetic nephropathy.

MATERIAL AND METHODS

The research was conducted on 42 patients with type 2 diabetes mellitus, 22 women and 20 men, aged 19 to 82 years, hospitalized at the Chair and Department of Endocrinology, Medical University of Lublin. Control group consisted of 25 healthy individuals, 14 women and 11 men, aged 24 to 73 years, with no kidney or bone illnesses history.

Laboratory tests were performed in fasting serum and in 24-hour urine collection. Quantificational determination of HS in urine was performed with the use of Heparan Sulfate ELISA kit, (Seikagaku Corporation). HS excretion was expressed as: total HS excretion in 24h speciments (μg/24h), concentration (μg/ml of urine) and concentration calculated per urine creatinine (μg/mg creatinine). Concentrations of creatinine and microalbuminuria were measured with the use of Advia 1650 analyser (Siemens). Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula, such as:

eGFR= 186 x serum creatinine $^{-1,154}$ x Age$^{-0,203}$ x [0,742 if Female]

Results of the conducted studies were statistical analysed using basic parameters of descriptive statistics (mean, SD, median, quartile). Partial Spearman correlation coefficient was used to establish the association between urinary HS excretion and microalbuminuria, eGRF. Statistical analyses were conducted using the Statistica programme (StatSoft, Polska).

All hypotheses were verified at the significance level of $p<0.05$

RESULTS

Characteristics of selected clinical and laboratory parametres of the study and control group are presented in Table 1. The mean eGFR volume was 94.1 (ml/min/1.73m^2), and mean albuminuria was 17.7mg/24h in the diabetic group. Urinary HS excretion in 24-hour urine collection in the examined group of patients with diabetes was significantly lower in comparison with the control group. Significant differences between study groups were observed in regards to urinary HS excretion expressed as concentration ($p=0.00001$), as well as total HS excretion in 24-hour specimen ($p=0.0007$) and as HS concentration calculated per urine creatinine ($p=0.0088$). Urinary HS excretion in patients with diabetes and in the control group is shown in Table 2.

Table 1. Characteristics of control group and patients with diabetes

Parameter	Diabetic group			Control group		
	n	mean	SD	n	mean	SD
Age (years)	42	50.4	15.8	25	50.5	15.3
BMI (km/m^2)	42	28.9	7.1	25	25.1	12.2
Diuresis (ml)	42	1823.8	959.3	25	1160.4	465.2
Urine creatinine concentration (mg/ml)	42	0.53	0.23	25	0.87	0.33
Duration of diabetes (years)	42	9.2	7.1	-	-	-

Table 2. Results of 24-hour HS urine excretion in control group and patients with diabetes

Parameter	Control group			Diabetic Group			p=
	n	median	quartile	n	median	quartile	
HS concentration (µg/ml of urine)	25	5.50	3.87/7.03	42	2.29	1.28/3.05	0.00001
HS concentration per urine creatinine (µg/mg creatinine)	25	5.87	5.04/6.75	42	4.79	2.78/6.55	0.0088
HS total excretion (µg/24 h)	25	4978	3684/7733	42	3216	1870/4925	0.0007

A significant negative correlation between urinary HS excretion and microalbuminuria was observed. HS excretion was positively associated with eGFR values. Data are presented in Table 3.

Table 3. Correlation between HS excretion, microalbuminuria and eGFR in diabetic patients

	n	p	r Spearmana
HS cocentration & mikroalbuminuria	42	0.021	-0.35
HS 24-h & mikroalbuminuria	42	0.049	-0.30
HS / creatinie & mikroalbuminuria	42	0.008	-0.40
HS concentration & eGFR	38	0.017	0.38
HS 24-h & eGFR	38	0.0028	0.47
HS / creatinine & eGFR	38	0.000001	0.69

DISCUSSION

In patients with diabetes, diabetic nephropathy is a serious complication which significantly deteriorates quality of life and is one of the main causes of renal insufficiency. For that reason the opportunity of early assessment of the degree of development of diabetic nephropathy, which may give a chance for regression or inhibition of the pathology, is very important. Nowadays determination of urinary albumin excretion is a recommended laboratory test for diagnosis of diabetic nephropathy. However, it is well known that microalbuminuria does not fully reflect the effective protein filtration in glomeruli. For many years, the researchers have been looking for much more specific and sensitive markers of disturbances in glomerular filtration barrier, which may be helpful in diagnosis and monitoring of nephropathy. The HS is a component of the glomerular filtration barrier, mainly in the form of perlecan – the most important proteoglican [4, 9]. It has been reported that changes in the amount and concentration of sulfated units of HS are associated with the development of diabetic nephropathy [6, 11]. Lewis observed that HS is degradated by heparanase, en enzyme induced by the presence of increased glucose concentrations in the environment, which leads as a consequence to the diminishing HS content in GBM [9].

According to STENO hypothesis increased HS proteoglycan metabolism within the glomerulus may play an important role in the pathogenesis of diabetic nephropathy [2]. Defects in HS synthesis can be a marker of development of pathological changes in glomeruli [2, 9].

For determining of the early stages of diabetic complications we used the quantitative assessment of urinary HS excretion. Results of the conducted research revealed that in patients with diabetes urinary HS excretion in 24-hour urine collection is lower in comparison with the control group. Significant differences were observed in regards to all methods of expression of urinary HS excretion: as a concentration, 24-hour excretion and also as calculation per urine creatinine. Decreased urinary HS excretion in patients with diabetes may result from impaired production or increased degradation of GAG in glomeruli in the course of hyperglycemia.

Yokoyama and co-workers observed a similar direction of changes in HS excretion in diabetes. They examined patients with type 2 diabetes and revealed that in urine of patients with diabetic nephropathy HS concentration was significantly decreased as compared to the control group.

What is important, similarly as in our data, HS concentration was determined by ELISA method with the use of antibodies mAb 10E4 and mAb 3G10. The decrease in binding of mAb 10E4 in the examined sample is connected with the diminished sulfated units in HS proteoglycan, which is followed by decreased urinary HS excretion [11].

Also Cadaval and co-workers, who determined urinary total GAG excretion in rats with streptozocin-induced diabetes, obtained similar results. They observed decreased urinary GAG excretion in diabetes in comparison with the control group. In the examined urine sample HS concentration as well as chondroitin sulfate and dermatan sulfate were diminished. Disturbances in urinary GAG concentrations were observed already in the second week of the experiment [1].

So far microalbuminuria was defined as a laboratory marker of diabetic nephropathy. Proven diagnostic usability of urinary GAG assessment would allow for early, noninvasive and rapid method for diagnosis of initial stages of diabetic nephropathy, before the microalbuminuria was

present. In our research we observed a significant negative correlation between urinary HS excretion and microalbuminuria and positive association with GFR values. It can by explained by the increase in albuminuria as a consequence of alteration of the permeability of filtration barrier, which is accompanied by the decrease in urinary HS excretion, as was shown in our data. The observed decrease in GFR exclude the effect of diminishing urine HS concentration due to the increased filtration.

The presented data indicate that determining HS can be a valuable marker in a diagnosis of diabetic nephropathy; however, details of the preanalytic and analytic phase need to be processed. In the clinical aspect further investigations are required on a greater number of cases considering many factors such as the type of diabetes, administered treatment and the duration of the disease.

CONCLUSIONS

The results suggest that a decreased HS in the urine, in patients with diabetes, may reflect a structural change or an altered processing of HS within the GBM. Determination of urine HS excretion can be regarded as a test for diabetic nephropathy and a potentially useful diagnostic tool following more precise and extended observations involving a larger number of clinical cases.

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SUMMARY

The HS is a component of the glomerular filtration barrier, mainly in the form of perlecan and agrin. It has been reported that changes in the amount and concentration of sulfated units of HS are associated with the development of diabetic nephropathy. The lack of or impaired synthesis of HS in GBM leads to the development of microalbuminuria and further excessive urinary protein excretion. To determine the early stages of diabetic complications we used the quantitative assessment of urinary HS excretion. The aim of the study was searching for differences in urinary HS excretion in patients with diabetes in comparison to the control group, evaluation of the influence of microalbuminuria on urinary HS excretion and evaluation of possible usefulness of urinary HS excretion as a laboratory test for diabetic nephropathy. Results of the conducted research revealed that in patients with diabetes urinary HS excretion in 24-hour urine collection is lower in comparison with the control group. Significant differences were observed in regards to all methods of expression of urinary HS excretion: as a concentration, 24-hour excretion and also as calculation per urine creatinine. Decreased urinary HS excretion in patients with diabetes may result from impaired production or increased degradation of GAG in glomeruli in the course of hyperglycemia. In our research we observed a significant negative correlation between urinary HS excretion and microalbuminuria and positive association with eGFR values. The presented data indicate that determining HS can be a valuable marker in the diagnosis of diabetic nephropathy; however, details of the preanalytic and analytic phase need to be processed. In the clinical aspect further investigations are required on a greater number of cases considering many factors such as the type of diabetes, administered treatment and the duration of the disease.

Key words: diabetes, heparan sulphate, diabetic nephropathy

STRESZCZENIE

Siarczan heparanu (HS) stanowi składnik bariery filtracyjnej kłębuszków nerkowych, głównie w postaci perlekanu i agryny. Dowiedziono, że zmiany w koncentracji i ilości grup siarczanowych HS są powiązane z wystąpieniem nefropatii cukrzycowej. Brak lub upośledzona synteza HS błony podstawnej prowadzi do rozwoju mikroalbuminurii i dalej do nadmiernego wydalania białek z moczem. Do określenia powikłań cukrzycy posłużono się więc oceną ilościową wydalania HS z moczem. Celem pracy było: poszukiwanie różnic w wydalaniu HS z moczem u pacjentów z cukrzycą w porównaniu z grupą kontrolną, ocena zależności pomiędzy mikroalbuminurią a wydalaniem HS z moczem oraz ocena ewentualnej przydatności oznaczania wydalania HS z moczem jako testu laboratoryjnego w nefropatii cukrzycowej. Wyniki przeprowadzonych badań wykazały, że u osób cierpiących na cukrzycę wydalanie HS z moczem w zbiórce 24-godzinnej jest niższe niż w podobnej pod względem płci i wieku grupie osób zdrowych. Istotność statystyczną uzyskano dla wszystkich sposobów wyrażania wydalania HS z moczem tj. jako stężenia, wydalania 24-h i po przeliczeniu na kreatyninę. Obniżone wydalanie HS z moczem u pacjentów z cukrzycą sugeruje, że prawdopodobnym powodem tych zmian może być spadek produkcji lub przyspieszona degradacja GAG w strukturach błony kłębuszka, obserwowana podczas hiperglikemii. W naszych badaniach

uzyskaliśmy istotną statystycznie ujemną korelację między wydalaniem HS a mikroalbuminurią, widoczną w zakresie wszystkich sposobów wyrażania wydalania HS z moczem, oraz dodatnią korelację z wartościami eGFR. Przedstawione dane wskazują, że oznaczanie HS może być przydatnym markerem w diagnostyce nefropatii cukrzycowej, wymaga jednak opracowania szczegółów fazy przedanalitycznej i analitycznej. W aspekcie klinicznym konieczne są obserwacje na większej liczbie pacjentów z uwzględnieniem szeregu czynników, takich jak: typ cukrzycy, stosowane leczenie oraz czas trwania choroby.

Słowa kluczowe: cukrzyca, siarczan heparanu, nefropatia cukrzycowa