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Changes in the excretion of glycosaminoglycans with urine in patients with kidney stones

Zmiany w wydalaniu glikozoaminoglikanów z moczem
u pacjentów z kamicą nerkową

Glycosaminoglycans (GAG) are long, non-branching and minus-charged polysaccharide chains built with repeated disaccharide units, where one of the components is always an aminosaccharide, i.e. D-glucosamine or D-galactosamine, and the other - uronic acid (9).

GAG can perform mechanico-structural functions and take part in the processes of adhesion, migration, proliferation, differentiation and maturation of cells (8, 9). They also participate in the formation of the urinary system. The selectivity of the glomerular basement membrane is preserved thanks to the presence of polyanionic sulfate radicals in heparan sulfate (HS). The presence of GAG is also detected in renal papillae, medulla and cortex. Moreover, they constitute the main element of the glycocalyx slime layer, which pads the urinary tract. In this way they protect the urotelium from the adhesion of crystals, bacteria and cells and they also guarantee the impenetrability of the urinary tract walls for the substances present in urine (2, 7, 8). So it seems obvious that any changes in the excretion of GAG reflect disturbances of these structures whose components they constitute.

From the aspect of one of the most frequent civilisation illnesses, i.e. kidney stones, the most important and interesting are such factors whose initial disturbances may lead to the development of the illness. As the extensive research shows, GAG have a significant influence on the very process of the formation of crystals, as well as on their aggregation and adhesion to the walls of the urinary tract. It is assumed that the changes in the total content of GAG, both as a substance connected with the slime layer and as a free substance present in urine, may disrupt the homeostasis and remain in the causality relationship with the process of development of kidney stones (2,7,8).

The aim of our research was: 1) looking for differences in GAG excretion in the urine of the patients with kidney stones as compared with the control group, 2) evaluation of the diagnostic usefulness of the method for estimating GAG content in urine with the use of 1,9 dimethylometyleno blue (DMB).

MATERIAL AND METHODS

The material for our research was the urine coming from the patients with diagnosed kidney stones hospitalized at the Urology Department of Cardinal Stefan Wyszyński Specialistic Regional Hospital in Lublin. The research was conducted on 49 patients aged from 22 to 88, their mean age being 49.6. The control group consisted of 25 healthy people, laboratory employees and students, who in their histories had no kidney or bone illnesses. They were aged between 24 and 66, with their mean age of 33.2.

The research material was 12-hour urine collection, conducted from 7 p.m. to 7 a.m. of the next day. The material was collected in winter months, i.e. from November to January. The material collected during the 12-hours urine collection was analysed in such a way that the urine volume was determined and its pH and protein presence was estimated with the use of the routine strip test. The amount of urine necessary for further research was centrifugation at 1500 x g for 10 minutes in order to separate cell elements. Appropriately apportioned material was frozen at -20°C. It was stored in such conditions until its analysis. GAG content was established with the use of the method described by Farndale et al. (5). The basis of this method constitutes specific binding of a cationic dye, 1, 9 dimethylometyleno blue (DMB) with anionic sulfate radicals of GAG. The estimation was conducted with the Blyscan Proteoglycan reagent set and Glycosaminoglycan Assay, produced by Bicolor Ltd. (Belfast). Creatinine concentration in urine was estimated according to Jaffe's reaction, with the use of the colorimetric method.

The results were statistically analysed with STATISTICA for Windows by StatSoft. All the tested hypotheses were verified at the significance level of $p < 0.05$.

RESULTS

Values for GAG excreted with urine in the researched groups did not depend on the age and were not different for women and men groups. Hence in further procedures the researched group and the control one were treated as uniform, with no distinction concerning sex or age. The results of the evaluated parameters are presented in Table 1.

Table 1. The results of diuresis, creatinine and glycosaminoglycans excretions in the control group and stone formers

Parameters	Control group			Stone formers			($p < 0.05$)
	N	mean	SD	N	mean	SD	
Diuresis 12 h (ml)	25	493.4	102.0	49	522.8	272.7	0.604
GAG (mg)	25	1.55	0.82	49	0.76	0.53	0.001
Creatinine (g)	25	0.46	0.20	49	0.38	0.17	0.672
GAG/creatinine (mg/g)	25	3.41	1.42	49	2.09	1.10	0.001

No significant differences were noticed between the amount of diuresis and creatinine excretion in the evaluated groups: the control one and the one with kidney stones patients. However, statistically significant differences were noticed with respect to GAG excretion. The 12–hour GAG excretion in the control group was by nearly 50% greater than in the group of kidney stone patients and the levels were, respectively, 1.55 mg in the control group and 0.76 mg in the group of patients (Fig. 1). Similar differences were observed when GAG excretion was expressed with respect to 1 gram of creatinine, the figures being respectively: 3.41 mg/g of creatinine for the control group and 2.09 mg/g of creatinine for the group of patients (Fig. 2).

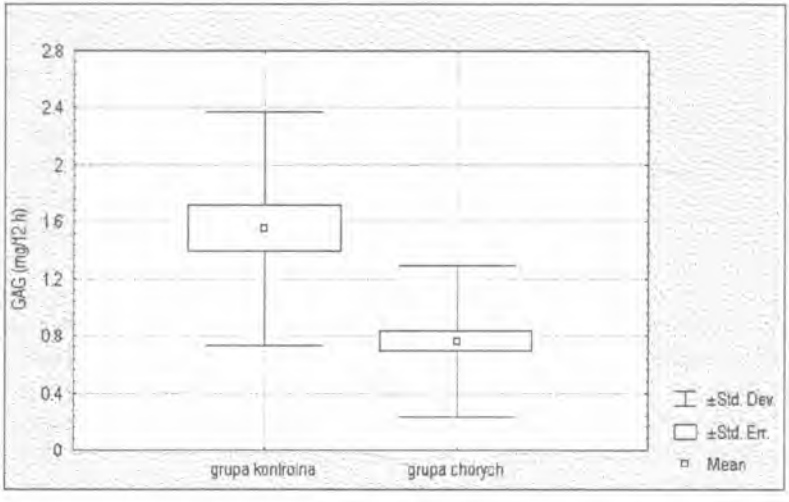

Fig. 1. The comparison of the 12–h glycosaminoglycans excretion in the control group and patients with renal stone disease

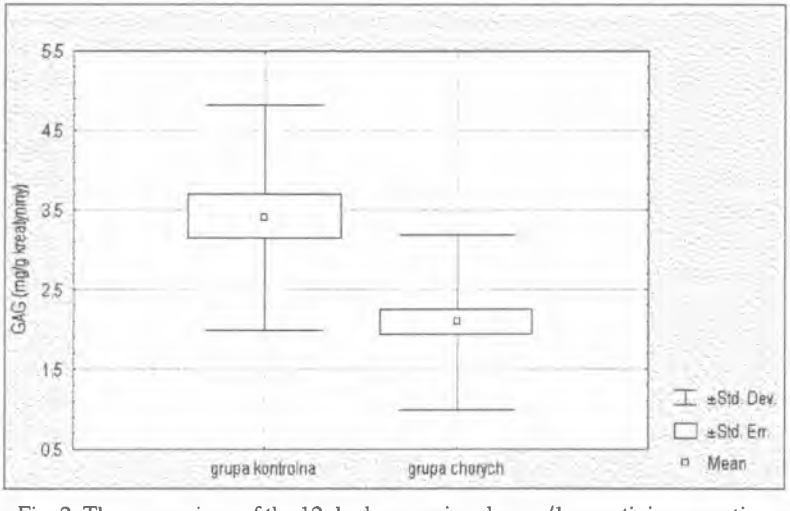

Fig. 2. The comparison of the 12–h glycosaminoglycans/1g creatinine excretion in control group and patients with renal stone disease

DISCUSSION

GAG are constant components of urine. Their excretion reflects the turnover of cells, especially in connective tissue and that is why, physiologically, it remains at the constant level. The final amount of GAG excreted with urine also depends on the penetration of these substances into urine throughout the urinary tract, as it was established that GAG are present in the mucus layer (glycocalyx) covering renal tubules, walls of the bladder and urine outflow routes (8).

GAG excretion, more extensive in children and teenagers, with adults does not show any significant dependency concerning sex or age (11). Also our research presented in this paper supports the claim that there is no relevant correlation between GAG excretion and the age of the people both in the control group and the one with kidney stones. It turns out, however, that GAG correlates significantly with creatinine excretion and to some extent it seems to have a value depending on a particular individual. This fact undermines the conclusion that GAG is excreted into urine only through washing out or peeling from the surface of the slime layer covering the urotelium. The positive correlation of GAG and creatinine excretion with urine allows us to support the claims of those researchers who maintain that GAG get into urine not only through being washed out from the slime layer, but also through glomerular filtration (2, 7).

Because of the multianionic charge connected with the presence of sulfate groups, GAG protect the slime layer from the adhesion of other molecules, bacteria and cells. In this way they disrupt their adhesion to the walls of the urinary tract as well as their aggregation, which allows us to treat them as inhibitors of crystallization (2, 8). The role of GAG in the process of stone formation is connected with the appearance of these substances in the glycocalyx, as well as with their presence directly in urine. The mechanisms through which GAG influence crystallization and formation of stones in urine can be presented as a direct interaction of polysaccharides with crystals, blocking of their growth, or as a change of the characteristics of crystalline surfaces through the reduction of the zeta potential (2, 3, 7). The characteristics preventing the nucleus formation depend on the quantity of the polysaccharide connected with the surface, on its molecular weight, degree of sulfatization and the appearance of other polymers in the solution. These qualities are represented to the greatest extent – apart from semisynthetic polysaccharides – by heparin and chondroitin sulfate (CS) (12).

The data in the literature on the subject concerning GAG excretion with urine show significant discrepancies resulting from varied urine collection conditions and uncomparable estimation methods. The method of GAG determination with the use of 1,9 dimethylometyleno blue (DMB), as compared with other methods applied so far, stands out as a simple procedure and there is a possibility of adapting it to diagnostic procedures (5, 6).

The results of the conducted research show that in patients with kidney stones the excretion of GAG with urine is significantly lower than in the group of healthy individuals with a similar sex and age structure. Low content of GAG in urine reflects their lowered synthesis, whereas a decrease in the content of GAG in the environment of the urinary tract and in the slime layer lining the urinary tract brings about an insufficient protection of the cells. Such a state promotes greater adhesion of solid particles which make easier the formation of the crystalline nucleus and then – stone formation. These results agree with the results of the majority of the authors (1, 4, 6, 10). It seems necessary to assume that individual reports concerning an increase in GAG excretion with urine in patients with kidney stones (11) concern the situations when the increase in GAG excretion is caused by other factors, and they do not possess the inhibiting characteristics, e.g. like HS or hyaluronic acid (2, 7,

12). It was established that not all the types of GAG inhibit crystallization and aggregation to the same extent and the lowering of the level of a strong inhibitor of crystallization does not always equal a decrease in the excretion of total GAG. In this context it seems significant to establish the composition of the excreted GAG, as well as the degree of their sulfatization.

CONCLUSIONS

1. GAG urinary excretion was independent of age and sex in control grup, as well as of urinary stone formers.

2. GAG urinary excretion was significantly lower in stone formers than in controls.

3. The method of 1,9 dimethylometyleno blue (DMB) is simple and can quickly test for presence of GAG in urine and can be adapted for diagnostic procedure.

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STRESZCZENIE

Celem pracy było wykazanie różnic w wydalaniu glikozoaminoglikanów (GAG) z moczem u 49 pacjentów z rozpoznaną kamicą nerkową. GAG oznaczano w 12–godzinnej zbiórce moczu i wyrażano w przeliczeniu na 1 gram kreatyniny. Do oznaczania wykorzystano metodę z błękitem 1,9 dwumetylometylenowym (DMB). Badania wykazały, że:

1. W badanej grupie kontrolnej oraz chorych z kamicą nerkową poziom glikozoaminoglikanów (GAG) nie wykazywał istotnych statystycznie różnic zależnych od płci i wieku.

2. Wydalanie GAG z moczem u chorych na kamicę nerkową wykazało istotne statystycznie obniżenie w porównaniu z grupą kontrolną.

3. Zastosowana w pracy metoda oznaczania GAG z użyciem błękitu 1,9–dwu metylo–metylenowego jest metodą nieskomplikowaną i stosunkowo szybką, co pozwala dostosować ją do procedur diagnostycznych.