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Estimation of the glycosaminoglycans excretion with urine in different collections

Glycosaminoglycans (GAG) are long, non-branching and minus-charged polysaccharide chains composed of repeated disaccharides units, where one of the components is always an aminosaccharide, i.e. D-glucosamine or D-galactosamine, and the others – uronic acid. GAG are degradation products of high molecular weight proteoglycans. GAG can perform mechanical-structural function and take part in the processes of adhesion, migration, proliferation, differentiation and maturation of cells. In the kidney they are part of the mucous layer lining the urothelium of the urinary tract and are also distributed along the membranes of glomeruli, Bowmans' capsule, tubular and peritubular capilaries and arterioles (2). The urinary bladder is covered with a layer of glycocalyx where GAG are fundamental components. GAG are assumed to maintain the electronegativity of surface for the high anionic charge of sulfated groups and they have a role in the aggregation process in the renal cells (8). A diminished urinary level of GAG could reflect their decrease in the glycocalyx, with decreased electronegativity and reducted function as a barrier against the infections and prevention of caliculi formation (1).

The aim of this study was to investigate the relation between GAG excretion in human urine in the first morning sample, 12-h and 24-h specimens, expressed as total GAG amounts excreted in 12 and 24 hours (μg/12 h, μg/24 h); GAG concentration (μg/ml of urine); GAG concentration calculated per urine creatinine (μg/mg creatinine), and osmolality (μg/mOsmol).

MATERIAL AND METHODS

The material for our research was the urine coming from 42 healthy volunteers, who in their histories had no kidney or bone illnesses. They were aged between 18 and 80, on average 37.7. The research material was 12-h urine collection, conducted from 7 p. m. to 7 a.m. of the next day. The first morning urine sample and 24-h sample was collected from 14 of them. The urine collection was analysed in such a way that the urine volume was determined and its pH, osmolalities and protein presence was estimated with the use of the routine strip test. The amount of urine necessary for further research was centrifugated at 1500 x g for 10 min. 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 Farendal et al. (4). The basis of this method constitutes specific binding of a cationic dye 1.9-dimethylomethyleno blue (DMB) with anionic sulfate radicals of GAG. Urinary GAG were measured spectrophotometrically using the Blyscan Sulfated Glycosaminoglycan Assay, produced by Biocolor Ltd. (Belfast). GAG excretion was epressed as: total GAG excretion in 12-h and 24-h speciments (μg/12 h, μg/24 h), concentration (μg/ml of urine) and concentration calculated per urine creatinine (μg/mg creatinine), and osmolality (μg/mOsmol).

Creatinine concentration in urine was estimated according to Jaffe's reaction, with the use of the colorimetric method, and BIOFARM (Poland) kits. Urine osmolality was measured with Trident Osmometer by freezing-point depression. The results were statistically analysed with STATISTICA by StatSoft. The data are presented as the mean and standard deviation. Statistical analysis was preformed with Student's test and Pearson correlation test test for comparison between two groups. All hypotheses were verified at the significance level of $p<0.05$.

RESULTS AND DISCUSSION

The results of GAG excretion in the first morning sample,12-h and 24-h specimens estimation as concentration and 12-h and 24-h excretion and creatinine or osmolality calculated per mg are shown in Table 1.

Table 1. Urine GAG in first morning, 12-hour, 24-hour samples estimation as concentration and 12-hour and 24-hour total excretion

Urine sample	n	X	SD	MIN	MAX
GAG (μg/ml) concentration					
First morning sample	14	4,71	2,22	0,36	7,26
12-hour sample	42	4,48	1,84	0,6	6,52
24-hour sample	14	3,24	1,55	1,59	6,4
GAG total excretion					
12-hour sample (μg/12h)	42	2461	1292	392	5542
24-hour sample (μg/24h)	14	6280	4720	3039	18868

No statistically significant differences were found between medium levels of GAG urine concentration in different time collections. Medium values of GAG concentration, calculated per urine creatinine did not differ significantly between the studied collections, either.

Table 2. Urine GAG concentration in first morning, 12-hour, 24-hour samples, calculated per mg creatinine and osmolality

	n	X	SD	MIN	MAX
GAG (μg/mg creatinine)					
First morning sample	14	4.34	1.78	0.68	6.95
12-hour sample	42	3.65	1.56	0.89	7.54
24-hour sample	14	4.49	2.89	1.77	12.08
GAG (μg/mOsm)					
First morning sample	14	7.89	3.95	1.03	13.92
12-hour sample	42	6.86	2.82	1.58	11.92
24-hour sample	14	5.87	3.47	2.23	12.52

Table 2 showed the correlation between GAG excretion in different samples, expressed as concentration, total excretion and calculated per creatinine and osmolality.

Table 3. Correlation between GAG excretion in different samples expressed as concentration, total exccretion and calculated per creatinine and osmolality

Correlation between GAG excretion		r	p
GAG R (s)	: GAG 12h (s)	0.008	NS
	: GAG 12h (w)	0.31	NS
	: GAG 24h (s)	0.45	NS
	: GAG 24h (w)	0.62	NS
GAG R/Osm	: GAG 12h (s)	0.11	NS
	: GAG 12h (w)	0.38	NS
	: GAG 12h/Osm	0.38	NS
	: GAG 24h (s)	0.29	NS
	: GAG 12h (w)	0.53	NS
	: GAG 24h/Osm	0.39	NS
GAG R/Kr	: GAG 12h (s)	0.08	NS
	: GAG 12h (w)	0.15	NS
	: GAG 12h/Osm	0.24	NS
	: GAG 12h/Kr	-0.03	NS
	: GAG 24h (s)	0.77	<0.01
	: GAG 24h (w)	0.82	<0.001
	: GAG 24h/Kr	0.78	<0.01
	: GAG 24h/Osm	0.85	<0.001
GAG 12h (s)	: GAG 24h (s)	0.16	NS
	: GAG 24h (w)	0.35	NS
GAG 12h (w)	: GAG 24h (s)	0.017	NS
	: GAG 24h (w)	0.4	NS
GAG 12h/Osm	: GAG2 4h (s)	0.12	NS
	: GAG 24h (w)	0.49	NS
	: GAG 24h/Osm	0.24	NS
GAG 12h/Kr	: GAG 24h (s)	-0.1	NS
	: GAG 24h (w)	0.22	NS
	: GAG 24h/Kr	-0.21	NS
GAG R/Kr	: GAG R/Osm	0.77	< 0.01
GAG 12h/Kr	: GAG 12h/Osm	0.91	< 0.001
GAG 24h/Kr	: GAG 24h/Osm	0.94	< 0.001

GAG R/(s) – GAG concentration in first morning sample (μg/ml)
GAG R/Osm – GAG concentration in first morning sample calculated per osmolality
GAG R/ Kr - GAG concentration in first morning sample calculated per creatinine
GAG 12h(w) - GAG total excretion in 12-hour sample (μg/12h)
GAG 12h(s) - GAG concentration in 12-hour sample (μg/ml)
GAG 12h/Osm - GAG concentration in 12-hour sample calculated per osmolality
GAG 12h/Kr - GAG concentration in 12-hour sample calculated per creatinine
GAG 24h(w) - GAG total excretion in 24-hour sample (μg/24h)
GAG 24h(s) - GAG concentration in 24-hour sample (μg/ml)
GAG 24h/Osm - GAG concentration in 24-hour sample calculated per osmolality
GAG 24h/Kr - GAG concentration in 24-hour sample calculated per creatinine

Comparing the tabulated results of the experiments, in all the urine sample collections we have observed a high correlation between the results of GAG excretion into urine as calculated per creatinine and those calculated per osmolality. Moreover, we have found a high index of correlation between GAG excretion in the morning urine calculated per creatinine and 24-h GAG excretion expressed both as a concentration and as calculated per creatinine and osmolality.

The values reflecting GAG excretion as examined and explained by various authors show significant discrepancies. According to Stone (9), that is a result of incompatible circumstances in which the material is collected, different methods of sample preparation as well as the application of varying analytical procedures. It is difficult or even impossible to compare own results with literature data and at the same time it requires a precise elaboration of the control. On the other hand, the incongruity or incompatibility of the results makes it hard to accept this study as a clinical diagnostic tool.

The crucial advantage of the method using DMB is a simple procedure that does not require prior GAG isolation from the examined material. This significantly decreases the time of determination and does not cause GAG depletion or urinary protein interference. It also means smaller volume of urine samples sufficient to determine GAG contents (3, 4, 6).

GAG excretion, increased during childhood and puberty, does not exhibit significant sex or age correlations (9). According to other authors, the contents of GAG are higher in men's urine than in that of women. Hence the suggestions to calculate the obtained values of GAG excretion to urine-secreted creatinine (7, 9). Such a conversion has also been done in this work, and enriched with an additional method of calculating urinary GAG excretion as calculated per osmolality. The symptomatic positive correlation between the results obtained from conversion both ways for all urinary sample collections allows us to recognize them as being diagnostically equivalent and expands the spectrum of possibilities of harnessing the results for disorders leading to changes in glomerular filtration.

Another issue related to urinary GAG excretion determination is the question of the optimum choice of the method and time of urine collection. Available literature reports differences in GAG excretion throughout the 24-hour period. It has been pointed out that morning physiological excretion is 20–30% higher compared with a day-and-night collection (9). Hence many authors suggest the analysis of a 24-hour urine collection, which greatly complicates the availability of this diagnostic material.

In our own work, we have compared the values of GAG excretion from morning collection, a 12- and 24-hour one. No statistically significant differences were noticed indicating a clear 24-hour pattern of GAG excretion. This has been confirmed by the results of the comparison of urinary GAG excretion from a morning collection and a 24-hour one. The reason might be a limited number of cases in the compared groups. What is interesting, however, is the positive symptomatic correlation that has been observed between the results obtained in the determination of GAG from the morning collection calculated per creatinine values and the values obtained in the 24-hour urine collection expressed as excretion, concentration and calculated per creatinine and osmolality values. The obtained results indicate a similar diagnostic value of both urine samples, which has practical consequences of good accessibility of morning urine samples as compared with troublesome 24-hour collection routine.

CONCLUSIONS

1. GAG excretion in a 12-hour urine sample was 2461 μg/12h with the concentration of 4.48 μg/ml.

2. GAG excretion calculated per creatinine shows positive correlation with GAG excretion in the urine calculated per urine osmolality.

3. GAG excretion determined in the urine sample coming from the morning collection and calculated per mg creatinine shows positive correlation with GAG

excretion in a urine sample from the 24-hour collection, which indicates a comparable diagnostic value of the two urine samples.

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SUMMARY

Various morbidity processes are accompanied by metabolic changes in GAG and, consequently, the changes in the excretion of these compounds in the urine. The urine GAG were an object of interest to many authors. Since the results of the studies often vary, an attempt to estimate GAG excretion in the urine was suggested using a currently recommended method of determining with 1.9-dimethylomethylene blue, where it would be possible to compare the methods of calculating GAG excretion in the urine. The objective of the work was: to determine the scope of GAG urinary excretion in a sample of a 12-hour urine collection from healthy patients; to compare the method of calculating the results of GAG excretion in the urine as a concentration, excretion and as calculated per creatinine and osmolality values; to determine the correlation between urinary GAG excretion in the morning – 12-hour and 24-hour samples. The contents of GAG were determined using the reaction with 1.9-dimethylomethylene blue in the urine coming from a morning collection, a 12-hour one and a 24-hour one. The contents of GAG were calculated as: overall volume of the secreted GAG (μg/12 h and μg/24 h); GAG concentrations in the examined urine sample (μg/ml); GAG concentrations calculated per creatinine (μg/mg creatinine); GAG concentrations calculated per urine osmolality (μg/mOsm). GAG excretion in a 12-hour urine sample was 2461 μg/12 h with the concentration of 4.48 μg/ml. GAG excretion calculated per creatinine shows positive correlation with GAG excretion in the urine calculated per urine osmolality.GAG excretion determined in the urine sample coming from a morning collection and calculated as calculated per urine creatinine values shows positive correlation with GAG excretion in a urine sample from a 24-hour collection, which indicates a comparable diagnostic value of the two urine samples.

Ocena wydalania glikozoaminoglikanów w różnych zbiórkach moczu

Wielu procesom chorobowym towarzyszą zmiany metabolizmu GAG i w konsekwencji zmiany wydalania tych związków z moczem. GAG w moczu były przedmiotem zainteresowań wielu autorów. Ponieważ wyniki tych badań często nie są zgodne, postanowiono przeprowadzić próbę oceny wydalania GAG z moczem z zastosowaniem polecanej metody oznaczania z błękitem 1,9-dwumetylometylenowym oraz porównać sposoby wyrażania wydalania GAG z moczem. Celem pracy było: ustalenie zakresu wydalania GAG z moczem w 12-godzinnej zbiórce moczu zdrowych osób; porównanie sposobu wyrażania wyników wydalania GAG z moczem jako stężenie, wydalanie oraz przeliczone na wartość kreatyniny i osmolalność; określenie korelacji między wydalaniem GAG z moczem w zbiórce porannej, 12-godzinnej, 24-godzinnej. Zawartość GAG oznaczano z wykorzystaniem reakcji z błękitem 1,9-dwumetylometylenowym w moczu pochodzącym z próbki porannej, zbiórki 12-godzinnej oraz z 24-godzinnej. Zawartość GAG wyrażono jako: całkowitą ilość wydalanych GAG (μg/12 h i μg/24h); stężenie GAG w badanej próbce moczu (μg/ml); stężenie GAG przeliczone na kreatyninę (μg/mg kreatyniny); stężenie GAG przeliczone na osmolalność moczu (μg/mOsm). Wydalanie GAG w 12-godzinnej zbiórce moczu wynosiło średnio 2461μg/12h, zaś stężenie 4,48 μg/ml. Wydalanie GAG z moczem wyrażone jak stężenie przeliczone na mg kreatyny koreluje z wartościami przeliczonymi na osmolalność moczu. Wydalanie GAG oznaczone w próbce moczu ze zbiórki porannej i wyrażone w przeliczeniu na wartość kreatyniny wykazuje dodatnią korelację z wydalaniem GAG w próbce moczu ze zbiórki 24-godzinnej, co wskazuje na porównywalną wartość diagnostyczną obydwu próbek moczu.