ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXII, N 1, 38 SECTIO D 2007

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Excretion of glycosaminoglycans with urine in healthy population with respect to age and gender

Glycosaminoglycans (GAG) are polysaccharide chains formed by repetition of identical disaccharide units, where one of the components is always an aminosaccharide, and the others – uronic acid. The GAG can be divided into seven types: hialuronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, keratan sulfate, heparin and heparan sulfate. All of them, except hyaluronic acid, link to proteins forming proteoglycans. GAG are degradation products of high molecular weight proteoglycans (PG). GAG can perform, mechanical-structural function and take part in the processes of adhesion, migration, proliferation, differentiation and maturation of cells. The origin of GAG in urine is widely discussed and it is not recognized as a product of connective tissue but as biologically activity GAG in urinary system (6, 9). According to some authors, GAG in urine may reflect the turnover of GAG in the glomerulus (3). 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 (7, 10). 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, 5).

Urinary GAG has been suggested as a clinical marker in various diseases, including lupus nephritis, urinary bladder damage and bladder tumors, mucopolisaccharidoses, renal amyloidosis, primary glomerulonephritis, chronic pyelonephritis, chronic obstructive pulmonary disease and diabetic nephropathy (2, 3, 7).

The purpose of this investigation was to find out whether the age and gender affect GAG's excretion with urine in healthy individuals.

MATERIAL AND METHODS

The material for our research was 12-hours urine collection, conducted from 7 p. m. to 7 a.m. of next day. The urine came 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. Patients were classified according to sex and arranged in three age groups of those under 20 years of age, those between 20–39 and those over 39 years of age.

The urine was centrifugated at 1500 x g for 10 minutes in order to separate cell elements. Appropriately apportioned material was frozen at -20C. It was stored in such conditions until its analysis. Creatinine concentration in urine was estimated according to Jaffe`s reaction, with the use of the colorimetric method, and Biofarm (Poland) kits. 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 (4, 8). Urinary GAG were measured spectrophotometrically using the Blyscan Sulfated Glycosaminoglycan Assay, produced by Biocolor Ltd. (Belfast). GAG excretion was expressed as: total GAG excretion in 12h speciments (μg/12h), concentration (μg/ml of urine) and concentration calculated per urine creatinine (μg/mg creatinine).

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 for a comparison between two groups. All hypotheses were verified at the significance level of $p<0.05$.

RESULTS

The results of GAG excretion in female and male groups in 12-h specimens estimation as concentration and 12-h excretion and calculated per mg creatinine are shown in Table 1, and Figure 1. The average 12-hour GAG excretion in the female urine was 2266 μg/12h and the average excretion in males was 2657 μg/12h. No statistically significant difference were found between medium levels of GAG urine concentration in both groups. Medium values of GAG concentration, calculated per urine creatinine did not differ significantly between the studied groups, either.

The results concerning GAG excretion with urine according to age division are presented in Table 2 and Figure 2. Depending on the age, GAG excretion values in all the examined groups of patients were 2420 μg/12h, 2102 μg/12h and 2841 μg/12h, respectively. No statistically significant differences were found between the studied groups.

The results obtained in the study do not point to any statistically significant change between the examined groups of patients with respect to age or gender as expressed either in terms of concentration, 12-hour excretion or creatinine-calculated values.

Table 1. GAG urine excretion in 12-h sample depend on sex in healthy volunteers

Sex	Women			Men			P<0.05
parameters	N	X	SD	N	X	SD	
Gag concentration (μg/ml of urine)	21	4.69	1.84	21	4.26	1.86	0.4594
Total gag excretion (μg/12 h)	21	2.266	1.132	21	2.657	1.435	0.3331
Gag concentration/ per urine creatinine (μg/mg creatinine)	21	3.81	1.66	21	3.49	1.48	0.5113

p – statistically significant, n – number of casus, x – mean, SD – standard deviation

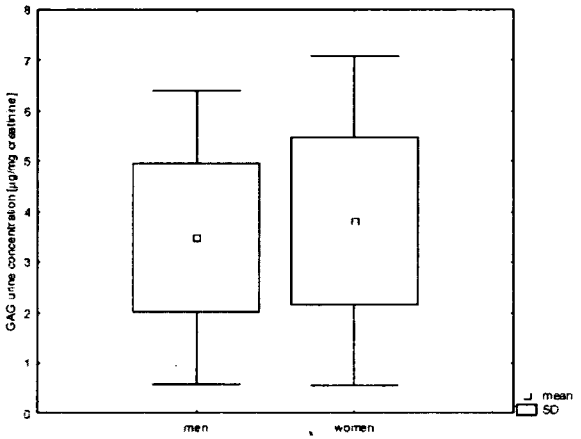

Fig. 1. Total GAG urine concentration in 12-h sample depend on sex (µg/mg urine creatinine)

Table 2. GAG urine excretion in 12-h sample depend on age in healthy volunteers

Age [years] parameters	<20 (n=8)		20-39 (n=17)		>39 (n=17)		P<0.05
	X	Sd	X	Sd	X	Sd	
Gag concentration (µg/ml)	4.45	1.65	4.48	1.92	4.49	1.95	0.999
Gag total excretion (µg/12 h)	2.420	1.195	2.102	911	2.841	1.591	0.2533
Gag concentration/per urine creatinine (µg/mg creatinine)	4.03	1.81	3.55	1.4	3.57	1.67	0.7544

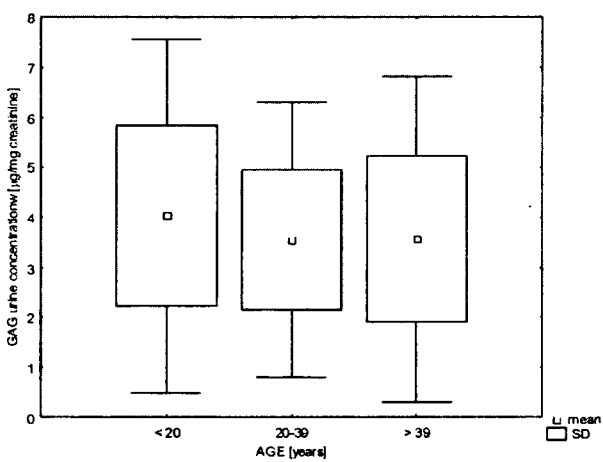

Fig. 2. Total GAG urine concentration in 12-h sample depend on age (µg/mg urine creatinine)

DISCUSSION

The values reflecting GAG excretion as examined and explained by various authors show significant discrepancies. According to Stone (11), 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. That makes it difficult or even impossible to compare the authors' own results with literature data and at the same time requires a precise elaboration of the control (11). On the other hand, the incongruity or incompatibility of the results makes it hard to accept this study as a clinical diagnostic tool.

As argued by Wład (12), 24-hour GAG excretion with urine is determined by a number of different factors such as sex, age, hormones, time of day or even year. The highest values are observed in spring and summer, which is said to be related to metabolic stimulation of bone and increased PG turnover taking place in summer (9, 12). A correlation between GAG excretion and hormones has also been found. Estrogens increase GAG synthesis while progesterone precipitates their excretion (5). Studies showed a correlation between the structure and content of GAG in the connective tissue and age. The most intense process of GAG excretion with urine is found in children during the most pronounced bone cartilage formation and it slows down with age. The content of CS decrease with age, whereas those of KS and HA increase (9, 12). According to Stone (11), GAG excretion, increased during childhood and puberty, does not exhibit significant sex or age correlations.

In this study, no statistically significant differences were observed between the values of GAG excretion in male and female urine. In the available literature, Eun-Yong obtained similar results (3). According to other authors, the amount of GAG excreted with the urine of men is larger than that in women so there is a suggestion to convert the obtained GAG values to urine-excreted creatinine. Such a conversion has also been used in our study. The study also presents a lack of correspondence between GAG excretion and age in the examined cohort. The reason for the absence of correlation between the values of GAG expression in patients under 20 years of age is probably the fact that the study concentrated mainly on 18-year-olds, the time when puberty has already been completed. The results of the analysis suggest that future studies should treat patients in this age sector as a homogenous group without any further subdivisions.

CONCLUSIONS

The results of our study show that: the age in the study groups does not seem to affect significantly the urinary excretion of GAG; the sex in the study groups does not seem to affect significantly the urinary excretion of GAG.

REFERENCES

1. Akcay T., Konukoglu D., Dincer Y.: Urinary glycosaminoglycans excretion in urolithiasis. Arch. Dis. Child., 80, 271, 1999.
2. De Lima C. et al.: Reduced urinary excretion of sulfated polysaccharides in dibetic rats. Biochim. Biophys. Acta., 1741, 30, 2005.
3. Eun-Young L., Soo-Hyun K., Soo-Kyung W.: Isolation, identyfication, and quantification of urinry glycosaminoglycans. Am. J. Nephrology, 23, 152, 2003.
4. Ferndale R. W., Buttle D. J., Barrett: Imrowed quantification and discrimination of sulfated glycosaminoglycans by use of DMB. Biochem. Biophys. Acta, 883, 173, 1986.

5. Hesse A., Wuzel H., Wahlensieck W.: Significance of glycosaminoglycans for the formation of calcium oxalate stones. American J. Kidney. Diseases, 4, 414, 1991.
6. Hurst R. E.: Structure, function, and pathology of proteoglycans and glycosaminoglycans in urinary tract. World J. Urol., 12, 3,1994.
7. Hurst R. E.: A deficit of proteoglycans on the bladder uroepithelium in interstitial cystitis. European Urology Supplements, 2, 10, 2003.
8. Jong J. G. N., Wevers R. A., Liebrand-van Sambeek R.: Measuring urinary glycosaminoglycans in the presence of protein: An iproved screening procedure for mucopolysaccharidoses based on dimetylmethylene blue. Clin. Chem., 38(6), 803, 1992.
9. Kaznowska-Bystryk I., Chlebus D.: Wydalanie glikozaminoglikanów z moczem- znaczenie diagnostyczne. Diagn. Lab., 36, 93, 2000.
10. Nickel J., Cornish J.: Ultrastructural study of antybodystabilized bladder surface: a new prospective on the elusive glycosaminoglycans layer. World J. Urol., 12, 11, 1994.
11. Stone J. E.: Urinary analysis in the diagnosis of mucopolysaccharide disorders. Ann. Clin. Biochem., 35, 207,1998.
12. Wład H.: Glikozoaminoglikany surowicy i moczu i ich znaczenie w diagnostyce klinicznej. Pol. Tyg. Lek., 19,1075, 1988.

SUMMARY

The purpose of this investigation was to find out whether the age and gender affect GAG excretion with urine in healthy individuals. Patients were classified according to sex and arranged in three age groups of those under 20 years of age, those between 20–39 and those over 39 years of age. The object of analysis was a 12-hr urine collection. The content of GAG was expressed in terms of an overall amount of GAG excreted (μg/12 h), GAG concentration in the examined sample of urine (μg/ml), GAG concentration converted to creatinine (μg/mg of creatinine). The average 12-hour GAG excretion in the female urine was 2266 μg/12h and the average excretion in males was 2657 μg/12h. Depending on the age, GAG excretion values in all the examined groups of patients were 2420 μg/12h, 2102 μg/12h and 2841 μg/12h, respectively. The results obtained in the study do not point to any statistically significant change between the examined groups of patients with respect to age or gender as expressed either in terms of concentration, 12-hour excretion or creatinine-calculated values.

Wydalanie GAG z moczem w grupie osób zdrowych zależnie od płci i wieku

Celem pracy było ustalenie wpływu wieku i płci na wydalanie GAG z moczem u zdrowych osób. Pacjentów podzielono ze względu na płeć oraz na trzy grupy wiekowe: poniżej 20 lat, pomiędzy 20 a 39 i powyżej 39 lat. Przedmiotem badań była 12-godzinna zbiórka moczu. Zawartość GAG wyrażono jako: całkowitą ilość wydalanych GAG (μg/12 h), stężenie GAG w badanej próbce moczu (μg/ml), stężenie GAG przeliczone na kreatyninę (μg/mg kreatyniny). Wydalanie 12-godzinne GAG w moczu u kobiet wynosiło średnio 2266 μg/12h, zaś u mężczyzn 2657 μg/12h. Uwzględniając wiek, w pierwszej grupie wiekowej wydalanie GAG wynosiło średnio 2420 μg/12h, w przedziale wiekowym 20–39 lat – 2102 μg/12h, a u osób powyżej 39 roku życia odpowiednio 2841 μg/12h. Uzyskane wyniki wskazują na brak istotnych statystycznie różnic pomiędzy badanymi grupami wiekowymi oraz między grupą kobiet i mężczyzn, wyrażonych zarówno jako stężenie, wydalanie 12-godzinne, jak i przeliczonych na wartość kreatyniny.