ANNALES
UNIVERSITATIS MARIE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LVIII, N 1, 65 SECTIO D 2003

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Urinary excretion of N-acetyl-β-D-glucosaminidase and α_1-microglobulin in children with proliferative blood diseases

In physiological conditions, the activity of lysosome N-acetyl-β-D-glucosaminidase enzyme (NAG) in urine can be stated as quite insignificant. Besides, the concentration of low-molecule protein: α_1-microglobulin (α1M) is minimum. Examination of the urinary excretion of these parameters is of widely recognized value and it is anon-invasive method of estimating the condition of renal proximal tubules. This examination is also a good indicator of toxicity of some drugs and other pathogenic factors. Increased urinary excretion of NAG and α1M were stated, among others, during the therapy of cisplatin (12), methotrexate (12, 14), iphosphamide (12) and other cytostatic drugs. Increased urinary excretion of NAG and α1M in the course of diabetes is also, besides albuminuria, a very early indicator of diabetes nephropathy development (7). Recently, it has been stated that there is a relationship between the urinary excretion of NAG and α1M and the values of arterial pressure. This fact can come in useful in early diagnostics of renal damage in spontaneous hypertension (1). The urinary excretion of NAG and α1M as indicators of tubular damage found its application in the diagnostics of the most typical urinary tract diseases. It may be assumed that indication of these markers may come in useful in the estimation of nephropathy arising in the course of viral hepatitis B and C (2, 8).

The purpose of the study was to estimate the urinary excretion of NAG and α1M in children with proliterative blood diseases who are treated by means of cytostatic drugs.

MATERIAL AND METHODS

The study comprised 73 children (28 girls and 45 boys) aged from 4.1 to 18.6 (average 11.7 ± 3.56 years). These are the children who were treated in the past or are treated currently according to BFM program (reports:I, M and II) because of proliferative blood diseases, including leucaemia – 70 children, and non-Hodgkin lymphoma – 3 children hospitalized in the Department of Hematology and Oncology at University Childrens`s Hospital in Lublin. Depending on the pattern of treatment the patients were given the following drugs: adriblastin, arabinosid cytosine, L-asparaginase, cyclophosphamid, daunorubicin, mercaptopurin, metho-

threxate, thioguanine, vincristine, prednisone. The basic parameters of liver functions (bilirubin, aminotransferases) and renal functions (creatinine, urea), viral hepatitis B markers and HCV antibodies were indicated in blood among all those children. A routine analysis of urine was also done. There were no features of renal failure or pathological changes in urine stated among the children. An insignificantly increased activity of aminotransferases was noticed, only among 3 children.

The examined children were divided into groups. Group I – 22 children who are treated currently or whose treatment was completed within last year. Group II – 51 children whose treatment was completed over two years ago. In group I none of the patients was a carrier of HBs antigen. Only among 5 children the HCV antibodies were present. Among 51 children from group II the antigenemia HBs, remaining for 2 to 11 years, was noticed among 29 children (56.9%). Besides the presence of HCV antibodies was stated among 38 (74.5%) children including 22 (43.1%) children among whom the markers of HBV and HCV infection were noticed (Tab.1). It is worth noticing that there are differences occurring among the children from Groups I and II. The differences relate to the percentage of viral hepatititis infections that were stated. What is worth attention is the fact that there is lack of antigenemia HBs among the children from Group I and a high percentage of uninfected children (77.3%) in Group I in comparison with the children from Group II.

Table 1. Antigenemia HBs and antibody anti HCV (a-HCV) in the studied children

	Group I (22 children)		Group II (51 children)	
	n	%	n	%
HBs (-) a-HCV (-)	17	77.3	6	11.8
HBs (+)	-		29	56.9
a-HCV (+)	5	22.7	38	74.5
HBs (+) a-HCV (+)	-		22	43.1

The activity of NAG in urine was indicated by means of colorimetric method (Boehringer Mannheim Biochemica, cat. N^{O} 875406), whereas the urinary concentration of α1M was indicated by immunoturbidometric method (Boehringer Mannheim Biochemica, cat. N^{o} 1360566) using Cobas-Mira-S analiser. The measurement was carried out in the second morning portion of urine. The results were presented as a NAG/creatinine (U/g) and α1M/creatinine (mg/g) ratio. The control group consisted of 70 healthy children (35 girls and 35 boys) at the age from 4 to 16 years (the average 10.06±3.97 years). The statistical analysis was carried out by means of a t-Student test, assuming that the level of statistical significance is $p<0.05$.

RESULTS

The average value of NAG/creatinine ratio was significantly different in each group ($p<0,001$) but at the same time it was higher among children who currently are or recently have been treated by means of cytostatic drugs. The average value of α1M/creatinine ratio was also higher among children who are currently treated by means of cytostatic drugs in comparison with those children whose treatment was completed long time ago. However, the difference was not of statistic significance (Tab. 2).

Table 2. Urinary excretion of NAG and α1M in the studied children

	N	NAG U/g			α1M mg/g		
		X	SD	P	X	SD	P
Group I	22	3.50	± 1.72	< 0.001	2.88	± 0.05	> 0.9
Group II	51	2.64	± 0.90		2.31	± 0.58	
Control group	70	2.67	± 1.6	I/k > 0.9 II/k > 0.9	2.8	± 0.4	I/k > 0.9 II/k > 0.9

A comparison of the results of the examined children NAG and α1M indicators among children with the presence of HBs antigenemia or HCV antibodies was carried out only among the patients from Group II. The purpose was to eliminate the influence of treatment and a selection of more homogeneous clinical material. There were 4 subgroups A, B, C, D distinguished among the examined children from Group II (Tab. 3). A significantly higher NAG/creatinine ratio was stated among children with HCV antibodies (group B) in comparison with HBs antigen carriers (group A) ($p<0.001$). Similarly, a higher value of this indicator in urine was stated in comparison with the children from group D, among whom no presence of markers of hepatitis viralis infection was stated ($p<0.001$) (Tab. 3). However, the average value of α1M/cretinine ratio was significantly higher in the group of children with the presence of both viral hepatitis markers (group C) in comparison with the group of children with HBs antigenemia (group A) ($p<0.001$) – Tab. 3.

Table. 3. Urinary excretion of NAG and α1M in group II children's according to markers of hepatitis viralis (HV)

	N	NAG U/g			α1M mg/g		
		X	SD	P	X	SD	P
A. HBs+ HCV-	7	2.07	0.35	$^{A}/_{B} < 0.001$ $^{A}/_{C} > 0.9$	1.59	0.41	$^{A}/_{B} > 0.9$ $^{A}/_{C} < 0.001$
B. HBs- HCV+	16	3.61	0.55	$^{A}/_{D} > 0.9$	1.68	1.03	$^{A}/_{D} > 0.9$
C. HBs+ HCV+	22	2.2	0.25	$^{B}/_{C} > 0.9$	3.56	1.30	$^{B}/_{C} > 0.9$
D. HBs-HCV-	6	2.19	0.37	$^{B}/_{D} < 0.001$ $^{C}/_{D} > 0.9$	2.63	0.71	$^{B}/_{D} > 0.9$ $^{C}/_{D} > 0.9$

DISCUSSION

Nephrotoxicity of cytostatic drugs is commonly known. In their works, numerous authors describe the growth of urinary excretion of NAG and α1M among children who are treated with cytostatic drugs. Our research showed that among children who are currently or quite recently have been treated by means of cytostatics, the average urinary excretion of NAG and α1M was higher than among children who completed chemotherapy over two years ago. These results are consistent with other authors' observations in which they say that the tubular damage caused by cytostatic treatment has usually a passing character. However, it should be noticed that among some patients who undergo the treatment, this state can sometimes precede the permanent handicap of kidney functions. Hovi et al. stated a decrease in glomerular filtration after 1 to 9 years in spite of the lack of other features of kidney damage (6). Similarly, Dusek et al. (3)

described the presence of hematuria, glucosuria and albuminuria after treatment by means of cisplatin, methotrexate and other cytostatic drugs among 38% children. Erni at al. (4) stated that after such treatment the level of creatinine in blood rises. Our previous research (14) showed increased values albumin in urine, which is the result of renal glomerular damage among 66% of children currently treated with cytostatic drugs, among 40% of children who completed the treatment a year ago and among 44% of children whose treatment was completed long ago. The increased urinary excretion of NAG and α1M among children who suffer from leucaemia and are treated by means of methotrexate was described by Hungarian authors, among others (11). In Poland it was described by the centre in Białystok (13). It was concluded that all cytostatic drugs applied among children with proliferative blood diseases are toxic to the renal proximal tubules. Besides, the increased urinary excretion of NAG shows the structurally proximal tubules damage. However, the increased urinary excretion of α1M may also be caused by exceeding the maximum ability of tubular reabsorption of protein which freely trickles through gromerular beasament membrane. Both sensitive indicators of early renal damage examined by us confirm the passing nephrotoxicity of cytostatic drugs applied in proliferative blood diseases.

Chronic HBs antigenemia can also lead to kidney damage and occurrence of glomerulonephritis, especially of glomerulonephritis membranous. The membranoproliterative glomerulonephritis was also described (8). The presence of deposits of virus antigens was discovered within the glomerular cells as well as in the structures of tubules. It has been proved that immunological complex circulating in blood take part in the pathomechanism of glomerulonephritis. However, transcription of the virus within nephron cells including the cells of tubular epithelium is also possible (5, 9, 10). It has been observed that the presence of HBV DNA in the tubular cells among the patients with glomerulonephritis can be connected with a worse course of clinical nephropathy and even with the development of kidney failure (5, 10). There is a lack of data on the influence of the virus on tubular functions. Therefore, we have undertaken an attempt to estimate this phenomenon. (Among 56.9%of the patients from group II, who were examined by us the HBs antigenemia remaining for 2 to 11 years was observed. On the other hand, over half of the examined patients had HCV antibodies. On the basis of the statistical analysis we stated the significantly higher urinary excretion of NAG, especially within the group of children with HCV antibodies in comparison with the group of HBs antigen carriers as well as the group of children who were not infected by these viruses). The urinary excretion of α1M was significantly higher among the children who went through viral hepatitis B and C in comparison with the group of only HBs antigenenemia. The research related to the homogeneous group of children suffering from proliferative blood diseases whose treatment by means of cytostatic drugs was completed at least two years ago. It seems that not only the hepatitis viralis B infection, but also viral hepatitis C may be of important role in renal proximal tubules damage, especially that recently attention has been paid to the role of the virus of hepatitis C in the occurrence of nephropathy. It has been suggested that there is a relationship between the occurrence of membranoproliterative glomerulonephritis, glomerulone-phritis membranosa and HCV infection (2). In our research the presence of HCV antibodies was stated among over half of the examined children. Unfortunately, the lack of HCV RNA indicators did not allow us to isolate the patients who were the carriers of this virus.

CONCLUSION

1. Proximal tubular damage observed in children treated with cytostatic drugs is usually temporary.
2. HBV and HCV infection may cause proximal tubular damage.

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SUMMARY

The purpose of the study was to estimate the urinary excretion of NAG and α1M among children who suffer from proliferative blood diseases. The group of the examined children included those who went through a viral hepatitis (VH) and who are or were treated by means of cytostatic drugs. The study comprised 73 children aged from 4 to 18 (average 11.7 ± 3.5. There were 70 children with the diagnosis of leucaemia and 3 with the diagnosis of non-Hodgkin lymphoma. The examined group was divided according to the stage of treatment of a basic disease. Group I – 22 children who are treated currently or whose treatment has been completed recently. Group II – 51 children whose treatment was completed over two years ago. In group II there were 4 subgroups distinguished depending on positive antigenemia HBs and the presence of HCV antibodies. There were no clinical or biochemical features of damage of renal function observed among any of the children. The testing group consisted of 70 healthy children who were selected regarding age and sex. The urinary excretion of NAG and α1M was estimated in the second morning portion of urine and it was presented as NAG/creatinine and α1M/creatinine ratio. The results of the research underwent the statistical analysis by means of a t-Student test. It was stated that the urinary excretion of NAG and α1M was higher among children who

currently are or were treated by means of cytostatics drugs. It was also stated that the urinary excretion of NAG was higher among the children who went through viral hepatitis C in comparison with HBs antigen carriers. Similarly, the urinary excretion of α1M was higher among children with positive markers of viral hepatitis B and C markers in comparison with a group of HBs antigen carriers.

Wydalanie N-acetylo-β-D-glukozaminidazy i α1-mikroglobuliny w moczu dzieci chorych na choroby rozrostowe krwi

Celem pracy była ocena wydalania NAG i α1M u dzieci z chorobami rozrostowymi krwi, w tym po przebytym wirusowym zapaleniu wątroby (WZW), leczonych w chwili obecnej i w przeszłości lekami cytostatycznymi. Badaniem objęto 73 dzieci w wieku od 4 do 18 lat (średnio 11,7 ± 3,56), z rozpoznaniem białaczki – 70 dzieci i chłoniaka nieziarniczego – 3 dzieci. Badaną grupę podzielono pod względem etapu leczenia choroby podstawowej: grupa I - 22 dzieci leczonych obecnie lub z niedawno zakończonym leczeniem, grupa II – 51 dzieci z leczeniem ukończonym przed ponad 2 laty. W grupie II wyodrębniono 4 podgrupy dzieci zależnie od dodatniej antygenemii HBs i obecności p/ciał anty HCV. U żadnego dziecka nie obserwowano cech klinicznych ani biochemicznych uszkodzenia funkcji nerek. Grupę kontrolną stanowiło 70 zdrowych dzieci, dobranych pod względem wieku i płci. Wydalanie NAG i α1M oceniano w II rannej porcji moczu i przedstawiono jako wskaźnik NAG/kreatynina oraz α1M/kreatynina. Uzyskane wyniki badań poddano analizie statystycznej przy pomocy testu t-Studenta. Stwierdzono większe wydalanie NAG i α1M u dzieci obecnie lub niedawno leczonych cytostatykami, a także większe wydalanie NAG u dzieci po przebytym wzw C w porównaniu z nosicielami HBs. Podobnie większe było wydalanie α1M u dzieci z dodatnimi markerami przebytego wzw B i C w porównaniu z grupą nosicieli HBs.