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Pediatric reference data on activity of urinary *N*-acetyl-β-D-hexosaminidase and its isoenzymes

Beata Zalewska-Szajda[a,*], Katarzyna Taranta-Janusz[b], Sylwia Chojnowska[c], Napoleon Waszkiewicz[d], Krzysztof Zwierz[c], Anna Wasilewska[b]

[a] *Department of Imaging Diagnostics, Children's Clinical Hospital, Medical University of Bialystok, Bialystok, Poland*
[b] *Department of Pediatrics and Nephrology, Medical University of Bialystok, Bialystok, Poland*
[c] *Medical Institute, Łomza State University of Applied Sciences, Łomza, Poland*
[d] *Department of Psychiatry, Medical University of Bialystok, Bialystok, Poland*

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ABSTRACT

Purpose: The objective of the study was to establish age – dependent values of the urinary lysosomal exoglycosidases activities: *N*-acetyl-β-D-hexosaminidase (HEX) and its isoenzyme A (HEX A) as well as isoenzyme B (HEX B) in healthy children and adolescents.
Material and methods: The study was performed using a random sample of 203 healthy children and adolescents (girls = 99, boys = 104), aged six months to 17.9 years. The activities of HEX, HEX A and HEX B were determined by a colorimetric method. The activities of the urinary HEX and its isoenzymes were expressed in pKat/μg of creatinine (pKat/μg Cr).
Results: Median concentrations of urinary HEX, and its HEX A, HEX B isoenzymes in particular age groups were analyzed using ANOVA. Urinary HEX, HEX A and HEX B activities (pKat/μg Cr) were the highest in children below 3 years, in comparison to remaining age groups. There were statistically significant negative correlations between urinary HEX, HEX A as well as HEX B and age ($r = -0.24$, $p < 0.001$ (HEX); $r = -0.20$, $p < 0.01$ (HEX A); $r = -0.26$, $p < 0.001$ (HEX B), respectively. We constructed the reference values for urinary activity of HEX, HEX A and HEX B (pKat/μg Cr) in centiles according to age, in three-year intervals.
Conclusions: Reported data present, for the first time, reference values for urinary activities of HEX and its isoenzymes HEX A and HEX B in children and adolescent.

1. Introduction

Among above 50 enzymes produced by human renal cells, only several has diagnostic value [1]. N-acetyl- β-D-*hexo*saminidase HEX (EC 3.2.1.52) is a lysosomal acid hydrolase releasing N-acetylglucosamine or N-acetylgalactosamine from non-reducing ends of glycoconjugates (glycoproteins, glycolipids and glycosaminoglycans) [2,3]. HEX is a dimeric glycoprotein constructed with polypeptide chains α and β. The most active are HEX A (αβ) and HEX B (ββ) [4]. Mature HEX isoenzymes have molecular weights (MW) of 96–110 kDa, depending on tissue that served for isolation [5–7].

Determination of the urinary activities of the HEX and its isoenzymes HEX A and HEX B is a non-invasive method allowing on early detection and monitoring of the renal damage [1,8–10]. HEX is a well-known marker of renal proximal tubules dysfunction [1]. Renal [8] and colon cancers [11,12] significantly increased urinary HEX and its isoenzymes HEX A and HEX B activities while pancreatic adenoma increased only urinary HEX A activity [13]. Activities of HEX, HEX A and HEX B in serum [8,13], synovial fluid [14], cerebrospinal fluid [15], human milk [16] and saliva [17], reflect health condition of investigated person.

In diagnostics of renal diseases both significant decrease and increase in urinary HEX activity may be of high importance. Different theories exist regarding the potential mechanisms of kidney cell injury. The excessive reabsorption of ultrafiltered proteins by proximal tubular cells can lead to tubular damage and apoptosis/necrosis by exhaustion of the lysosomal degradation pathway and spillage of lysosomal enzymes into the cytoplasm. In response to excessive lysosomal protein degradation, the proximal tubular cells produce a variety of molecules, including lysosomal

* Corresponding author at: Department of Imaging Diagnostics, Children's Clinical Hospital, Medical University of Bialystok, Waszyngtona 17, 15-274 Bialystok, Poland.
E-mail address: sbszajda@gmail.com (B. Zalewska-Szajda).

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Table 1
Characteristics of the study population (Q1- lower quartile, Q3- upper quartile).

Variable	GIRLS n = 99			BOYS n = 104			*P* value
	Median	Q1	Q3	Median	Q1	Q3	
Age (years)	9.10	7.09	11.09	9.09	7.05	12.07	NS
Weight (kg)	30.98	23.90	41.80	38.50	30.50	51.80	NS
Height (cm)	137.70	126.30	148.30	144.50	132.30	158.40	NS
HEX (pKat/mL)	120.63	91.28	180.46	114.42	91.28	256.19	NS
HEX A (pKat/mL)	24.84	9.03	42.90	20.32	11.85	40.08	NS
HEX B (pKat/mL)	96.93	70.96	128.54	87.90	69.83	119.50	NS
Creatinine (μg/mL)	1028.238	608.450	1465.356	936.905	612.214	1227.363	NS
HEX (pKat/μg Cr)	0.123	0.088	0.217	0.133	0.090	0.234	NS
HEX A (pKat/μg Cr)	0.021	0.009	0.054	0.025	0.010	0.053	NS
HEX B (pKat/μg Cr)	0.093	0.071	0.148	0.097	0.074	0.165	NS

NS – not significant

exoglycosidases. Although many studies suggested that lysosomal exoglycosidases play a role in the progression of renal diseases, the pathogenetic roles of their direct interaction in renal fibrosis remain unclear. Observed changes can be due to a defective kidney filtration barrier, cellular lysis of proximal tubule cells, and due to increased lysosomal exocytosis. It was reported that particularly high activities of HEX were confirmed in cells of proximal convoluted tubules, and negligible activities were released by secretion and exocytosis from their lysosomes to urine, without disturbing plasma membrane [18–20].

The activities of HEX, HEX A and HEX B detected in serum, urine or saliva of healthy people are important for further interpretation of values obtained in children and adolescent with different health disturbances. Analysis of available reference values indicate shortage of data concerning HEX and its isoenzymes activity in biological fluids of children and adolescent [17,21–23]. Therefore we decided to determine reference values for urinary HEX and its isoenzymes HEX A and HEX B in urine of healthy children and adolescent.

The additional aim of our research was to establish the relations between the age, gender and urinary HEX as well as HEX A and HEX B activities.

2. Materials and methods

2.1. Participants

The study was performed using a random sample of 203 healthy Caucasian children and adolescents (girls = 99, boys = 104), aged from six months to 17.9 years. The study group was divided into six age groups in 3-year intervals: 0.5–2.9 yrs – 28 subjects; 3–5.9 yrs – 29 subjects; 6–8.9 yrs – 36 subjects; 9–11.9 yrs – 35 subjects; 12–14.9 yrs – 43 subjects and 15–17.9 yrs – 32 subjects.

The material and data were obtained from participants of the OLAF study [24] as well as from healthy children of the Hospital staff.

Informed consent was obtained from parents of all participants and children older than 16 years of age. The study protocol was approved by Local Committee of Bioethics, Medical University of Bialystok (01 September 2014; ANZ-06042-134-41724/14) while the OLAF study was approved by The Children's Memorial Health Institute Ethics Committee.

The medical past and present history of the study participants was taken from the parents. For all participants careful clinical histories were taken and physical examinations were performed. Body weights and heights were measured using a balance beam scale and pediatric wall – mounted stadiometer. Inclusion criteria

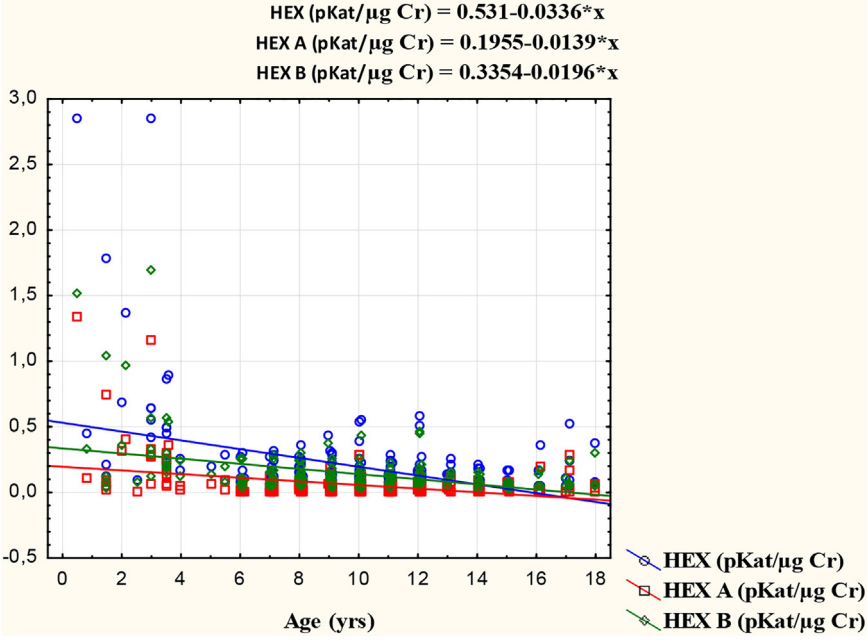

Fig. 1. Linear regression analysis showing the relationship between urinary HEX and its isoenzymes activity (pKat/μg Cr) and age.

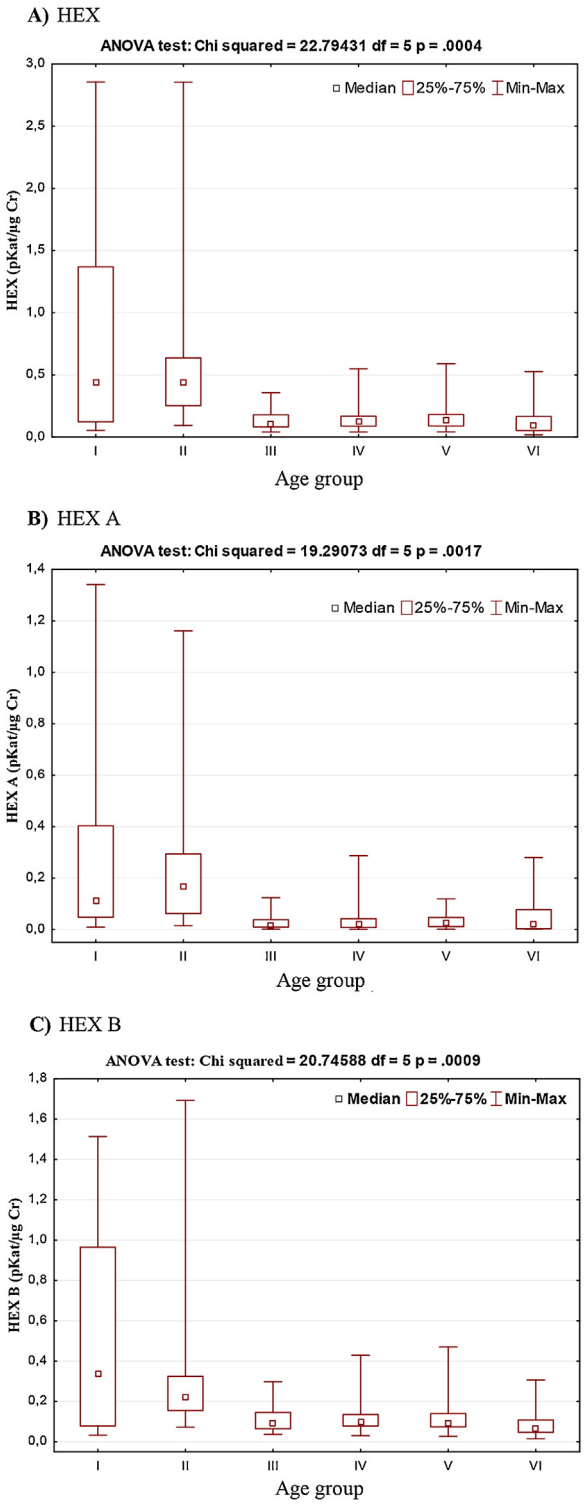

Fig. 2. Comparison of urinary HEX (A) and its isoenzymes activity (B and C) (pKat/µg Cr) between six age groups: **I** 0.5–2.9 years, **II** 3.0–5.9 years, **III** 6.0–8.9 years, **IV** 9.0–11.9 years, **V** 12.0–14.9 years, **VI** 15.0–17.9 years; ANOVA analysis of variance.

of the study participants: healthy children and adolescents aged 0.5-18 years. Exclusion criteria: any signs of infection, chronic diseases and medications that may have influence on renal function. After an overnight fasting, first morning voided urine samples were collected from each patient. The samples were transported to the laboratory within 4 h and then frozen at a temperature −80 °C.

2.2. HEX and HEX B activities

HEX and HEX B activities in urine (pKat/mL) were determined using the method of Marciniak's et al. [25] as modified by Szajda et al. [26]. For the determination of urinary HEX activity, to a microplate wells were added: 10 µL of urine, 30 µL of substrate (20 mM solution of 4-nitrophenyl-*N*-acetyl-ß-D-glucosaminide −Sigma, St. Louis, MO, USA) and 40 µL of 100 mM citrate-phosphate buffer pH 4.7. The microplates were incubated for 60 min at 37 °C with constant shaking. The enzymatic reaction was stopped by adding 200 µL of 200 mM borate buffer, pH 9.8.

For determination the HEX B activities, to a microplate wells were added 40 µL of 100 mM of phosphate-citrate buffer at pH 4.7, than 10 µL of the urine and incubated for 180 min at 50 °C to deactivate a thermolabile HEX A. Then 30 µL of 20 mM solution of 4-nitrophenyl-N-acetyl-ß-D-glucosaminide (Sigma, St. Louis, MO, USA) were added and the mixtures were incubated for 60 min at 37 °C. The enzymatic reactions were terminated by adding 200 µL 200 mM borate buffer at pH 9.8. The activities of lysosomal exoglycosidases corresponding to the amounts of released 4-nitrophenol, were measured at 405 nm, using the microplates reader EL_X800^{TM} and computer program KC junior (Bio-Tek Instruments, Winooski, VT, USA).

As thermolabile HEX A is inactivated during 3 h incubation at 50 °C at pH 5.0, and HEX B is thermostable, HEX A activity was calculated from the difference between activity of total HEX and HEX B:

HEX A = HEX − HEX B

The concentration of urinary activities of HEX, HEX A and HEX B were expressed in pKat/mL. Urinary activities of HEX, HEX A and HEX B were standardized by comparison to urinary creatinine concentrations.

2.3. Creatinine determination

Creatinine concentrations were determined using kits ABX Pentra Enzymatic Creatinine CP, biochemical analyzer ABX Pentra 400, and expressed in µg/mL.

2.4. Statistical analysis

The data were analyzed with Statistica (version 12.0, StatSoft, Tulsa, OK, USA) program. Kolmogorov–Smirnov test was used to determine normality of variables. Discrete variables were expressed as counts (percentage) and continuous variables as median and quartiles, unless stated otherwise. The comparison between groups was done using chi-square and Fisher exact tests for categorical variables and *t* test for continuous variables for normally distributed data, or Mann–Whitney or analysis of variance; (ANOVA) tests were used for non-normally distributed data. Correlations between urinary HEX, HEX A and HEX B activities and creatinine ratio as well as other variables were evaluated by Pearson's or Spearman's test, as appropriate. $P < 0.05$ was considered statistically significant. Age-specific reference values for HEX, HEX A and HEX B activities to creatinine ratio were generated by the Lambda-Mu-Sigma (LMS) method using LMS Chart Maker software [27], which characterizes the age dependent distribution of a target parameter based on a quantile regression fit by three different components: the median (M), the variance (S) and the skewness of the distribution, which is evaluated by an exponential factor (L) from a Box-Cox transformation [28].

Centile curves for age were obtained as:

$$C_{100\alpha}(t) = M(t)\,[1 + L(t)\,S(t)Z\alpha]^{1/L(t)}$$

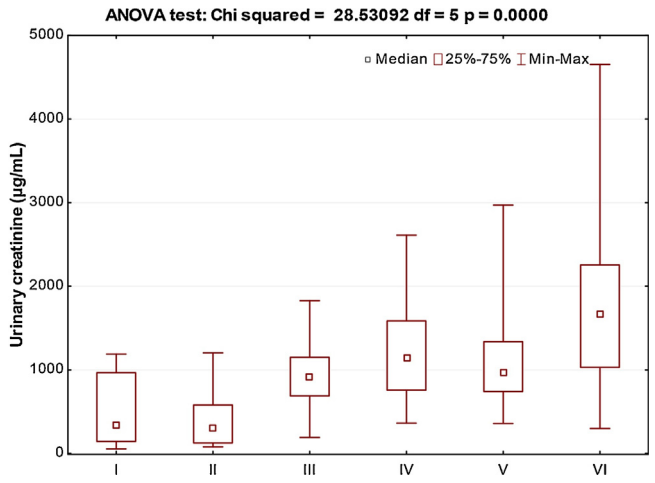

Fig. 3. Urinary creatinine excretion (µg/mL) between six age groups: **I** 0.5–2.9 years, **II** 3.0–5.9 years, **III** 6.0–8.9 years, **IV** 9.0–11.9 years, **V** 12.0–14.9 years, **VI** 15.0–17.9 years; ANOVA analysis of variance.

where $Z\alpha$ is the normal equivalent deviate for tail area α and $C_{100\alpha}(t)$ is the centile corresponding to $Z\alpha$

3. Results

The study group of 203 individuals was divided into six age groups in three-year intervals: 0.5–2.9; 3–5.9; 6–8.9; 9–11.9; 12–14.9 and 15–17.9 years old; 48.8% were girls and 51.2% boys of similar age ($p > 0.05$). There were no statistically significant differences in median values of all estimated HEX, HEX A and HEX B activities (pKat/mL and pKat/µg Cr) between girls and boys ($p > 0.05$). Groups' characteristics are shown in Table 1.

We observed statistically significant positive correlation between urinary HEX, HEX A, HEX B (pKat/mL) and age ($r = 0.26$, $r = 0.02$, $r = 0.3$, $p < 0.001$, respectively). To reduce the variations due to dilution, as a common practice we corrected the pKat/mL values according to urinary creatinine concentration. Following adjustment for creatinine, urinary HEX, HEX A, HEX B excretion showed trend toward decreasing values with increasing age. Fig. 1 presents significant negative correlation between age and urinary (pKat/µg Cr): HEX ($r = -0.24$, $p < 0.001$), HEX A ($r = -0.20$, $p < 0.005$) and HEX B ($r = -0.26$, $p < 0.001$), respectively. Therefore, we assessed urinary HEX, HEX A, HEX B (pKat/µg Cr) in particular age groups in three-year intervals.

We observed statistically significant differences in median activity in urine HEX, HEX A and HEX B (pKat/µg Cr) in particular age groups (ANOVA, $p < 0.05$). The highest urinary activity of HEX, HEX A and HEX B were found in children <3 years (HEX – 0.59, HEX A – 0.28, HEX B – 0.32 pKat/µg Cr) and the lowest in the oldest children >15 years (HEX – 0.091, HEX A – 0.017, HEX B – 0.068 pKat/µg Cr) Fig. 2A–C. Urinary creatinine excretion also revealed significant differences depending on age (ANOVA, $p < 0.05$) (Fig. 3).

As already described above, for each period 3rd, 10th, 25th, 50th, 75th, 90th and 97th centiles were estimated of analyzed patients. Results were found in Table 2A–C.

4. Discussion

Urinalysis is one of the basic medical laboratory investigations that includes evaluation of physical and chemical properties of urine as well as microscopy assessment of urinary sediment. Urinalysis detects pathological changes in urinary system and kidneys, infections of urinary tract, presence of deposits, decompensated diabetes, early changes in vascular system in course of hypertension, diabetes and other diseases. Urinalysis is a simple,

Table 2
Urinary HEX and its isoenzymes (pKat/µg Cr) activities – reference values for children and adolescents, according to age.

A) HEX

Age (years)	LMS			Centiles						
	L	M	S	3rd	10th	25th	50th	75th	90th	97th
0.5–2.9	−0.137	0.828	0.958	0.165	0.266	0.446	0.828	1.631	3.186	6.591
3.0–5.9	−0.429	0.460	0.739	0.154	0.208	0.293	0.460	0.807	1.555	3.831
6.0–8.9	−0.173	0.123	0.557	0.047	0.062	0.085	0.123	0.181	0.264	0.391
9.0–11.9	−0.281	0.123	0.512	0.052	0.067	0.088	0.123	0.177	0.255	0.380
12.0–14.9	−0.459	0.126	0.534	0.055	0.069	0.090	0.126	0.188	0.288	0.488
15.0–17.9	0.679	0.099	0.596	0.012	0.033	0.062	0.099	0.142	0.184	0.229

B) HEX A

Age (years)	LMS			Centiles						
	L	M	S	3rd	10th	25th	50th	75th	90th	97th
0.5–2.9	−0.176	0.273	1.110	0.046	0.076	0.135	0.273	0.611	1.408	3.702
3.0–5.9	−0.048	0.144	1.057	0.021	0.038	0.071	0.144	0.298	0.586	1.168
6.0–8.9	−0.088	0.016	1.023	0.002	0.004	0.008	0.016	0.032	0.064	0.132
9.0–11.9	0.129	0.019	1.051	0.002	0.004	0.009	0.019	0.039	0.068	0.115
12.0–14.9	0.262	0.023	1.110	0.001	0.003	0.010	0.023	0.046	0.078	0.124
15.0–17.9	0.068	0.020	1.210	0.001	0.004	0.008	0.020	0.046	0.090	0.172

C) HEX B

Age (years)	LMS			Centiles						
	L	M	S	3rd	10th	25th	50th	75th	90th	97th
0.5–2.9	0.039	0.468	0.943	0.074	0.135	0.246	0.468	0.878	1.527	2.605
3.0–5.9	−0.373	0.277	0.732	0.091	0.124	0.176	0.277	0.478	0.881	1.921
6.0–8.9	−0.280	0.099	0.553	0.039	0.051	0.069	0.099	0.146	0.218	0.339
9.0–11.9	−0.301	0.099	0.503	0.043	0.055	0.072	0.099	0.142	0.204	0.303
12.0–14.9	−0.684	0.098	0.496	0.047	0.058	0.072	0.098	0.144	0.227	0.436
15.0–17.9	0.881	0.070	0.488	1.081	0.028	0.048	0.070	0.094	0.116	0.138

non-invasive, cheap and open-access method, that may provide significant information concerning our health [29].

One of the non-invasive methods for evaluation of the renal function is determination of enzymes produced by kidneys and excreted with urine. It was documented that only enzymes not-denatured in acid urinary environment, excreted in minimal amounts with urine of healthy persons may be of diagnostic importance [1]. *N*-acetyl-β-D-h*exo*saminidase (HEX) is the enzyme that fulfills the above conditions, because it is present in small quantities in urine of healthy persons, and have highest activity in proximal renal tubules in comparison to remaining renal regions [1,11,13]. Both in renal medulla and cortex HEX A and HEX B activities with distinct dominance of HEX A were found [30].

The HEX A in body fluids is an inflammatory indicator, secreted into extracellular space by the inflammatory cells such as mast cells, basophils, macrophages, eosinophils, and neutrophils as well as an indicator of metabolic and secretory activity of the cells [31,32]. HEX B is an indicator of cell damage [31].

Significant increase in urinary HEX activity occurs in acute and chronic glomerulonephritis [33], uremia [34], vesicoureteral reflux [35], exacerbation of chronic pyelonephritis [36], nephrotic syndrome [37], damage to renal tubules in children with pyelo-ureteral stenosis [21], in children with solitary kidney [22], after injuries and renal transplantation [38]. Urinary HEX activity may be the marker for evaluation the efficiency of alcoholic disease treatment [39]. Moreover, increased urinary HEX activity in cigarette smokers may indicate on microinjuries to renal tubules [40]. Increase in urinary HEX activity is also an early symptom of rejection of renal transplant [41]. It is worthy of note that, urinary HEX activity in the early stage of diabetic nephropathy in children is twice higher when compared to healthy people [42] and that Liubimova et al. reported HEX as a more sensitive marker of renal tubules damage by cytostatic drugs, than urinary urea and creatinine [43,44].

In publications reporting application of urinary HEX and its isoenzymes in diagnostics of different clinical states, activities of HEX and its isoenzymes in urine of healthy people are used as reference values [11–13,17]. Similar situation takes place in few reports on urinary activity of HEX and its isoenzymes in children [21,22]. Analyzing available reports, we found a shortage of publications that reported reference values for urinary HEX, HEX A and HEX B activities in children and adolescent. In our study urinary activities of HEX and its isoenzymes HEX A and HEX B of 203 children and adolescent were independent on gender (Table 1), but were age-dependent. After dividing the children and adolescent into 6 groups in 3 years intervals, we showed statistically significant differences ($p < 0.05$) in activities of HEX and its isoenzymes (pKat/μg Cr) in urine of youngest children (0.5–2.9 years), when compared to adolescent (15.0–17.9 years), (Fig. 2A–C). We found reverse correlation between age and activity of creatinine adjusted urinary: HEX: $r = -0.24$, $p < 0.001$; HEX A: $r = -0.20$, $p < 0.01$ and HEX B: $r = -0.26$, $p < 0.001$, respectively (Fig. 1). Our results are partially consistent with report of Agirbasli et al. [45] performed on young people (18–32 years old), who proved that urinary HEX excretion was dependent on age, gender, race and blood pressure. Possible explanation of it might be the fact that urinary creatinine excretion increases with age and reflects total muscle mass. Urinary creatinine concentration was used as a simple tool to normalize HEX, HEX A and HEX B (pKat/mL) measurements to account for the influence of urinary dilution on its concentration. In reviewing the literature, data was found on the association between urinary activity of HEX and age in the healthy pediatric population [46,47]; however similar findings concerning negative correlation of the urinary activity of HEX, HEX A, and HEX B (pKat/μg Cr) with patient's age were noted in children and adolescents with solitary functioning kidney and hydronephrosis [21,22].

Table 2A–C show dependence of the urinary activities of HEX, HEX A and HEX B in children and adolescent on age, taking into consideration centile network in range of normal values 3rd, 10th, 25th, 50th, 75th, 90th, and 97th centiles, which may be of practical importance in distinguishing normal from diseased children and adolescent.

5. Conclusions

Our research on reference activities of urinary HEX, HEX A and HEX B in children and adolescent obtained from a large group of participants, found their significant dependence on age but not on gender.

Declaration of interest

The authors report no conflicts of interest regarding the content herein. The research was supported by a grant (134-41724L) from the Medical University of Bialystok, Poland.

References

[1] Kępka A, Szajda SD, Jankowska A, Waszkiewicz N, Chojnowska S. Zwierz K: N-acetylo-beta-heksozoaminidaza – marker uszkodzenia cewek nerkowych bliższych. N-acetyl-beta-hexosaminidase-marker of damage to renal proximal tubules. Pol Merkuriusz Lek 2008;25(147):288–90.
[2] Winchester B. Lysosomal metabolism of glycoproteins. Glycobiology 2005;15 (6):1R–15R.
[3] Zwierz K, Zalewska A, Zoch-Zwierz W. Isoenzymes of N- acetyl-β-hexosaminidase. Acta Biochim Pol 1999;46(3):739–57.
[4] Zwierz K, Juszkiewicz J, Arciuch L, Gindzieński A. N-acetylo-β-D-heksozoaminidaza-enzym chorób Tay-Sachsa i Sandhoffa. Postępy Biochemii 1992;38(3):127–32.
[5] Arciuch LP, Bielecki D, Borzym M, Południewski G, Arciszewski K, Różański A, et al. Isoenzymes of N- acetyl- beta- hexosaminidase in complicated pregnancy. Acta Biochim Pol 1999;46(4):977–83.
[6] Stirling JL. Separation and characterisation of N-acetyl-β-glucosaminidases A and P from maternal serum. Biochim Biophys Acta 1972;271:154–62.
[7] Mahuran DJ. Characterization of human placental β–hexosaminidase I2. Proteolytic processing intermediates of hexosaminidase A. J Biol Chem 1990;265(12):6794–9.
[8] Borzym-Kluczyk M, Darewicz B, Knaś M, Szajda SD, Sulik M, Olszewska E, et al. N-acetyl-β-glucosaminidase and its isoenzymes in the renal tissue, serum and urine of patients with renal cancer. Contemp Oncol 2005;9(7):287–90.
[9] Csathy L, Pocsi I. Urinary N-acetyl-beta-D-glucosaminidase determination in newborns and children: methods and diagnostic applications. Eur J Clin Chem Clin Biochem 1995;33(9):575–87.
[10] Noonan CW, Sarasua SM, Campagna D, Kathman SJ, Lybarger JA, Mueller PW. Effects of exposure to low levels of environmental cadmium on renal biomarkers. Environ Health Perspect 2002;110(2):151–5.
[11] Szajda SD, Borzym-Kluczyk M, Snarska J, Puchalski Z, Zwierz K. N-acetyl-β-D-hexosaminidase and its isoenzymes A and B in blood serum and urine, as a potential colon cancer markers. Hepato Gastroenterol 2009;56:1287–98.
[12] Waszkiewicz N, Zalewska-Szajda B, Szajda SD, Kępka A, Waszkiewicz M, Roszkowska-Jakimiec W, et al. Lysosomal exoglycosidases and cathepsin D in colon adenocarcinoma. Polskie Archiwum Medycyny Wewnętrznej 2012;122 (11):551–6.
[13] Szajda SD, Snarska J, Jankowska A, Puchalski Z, Zwierz K. Isoenzymes A and B of N-acetyl-β-D-hexosaminidase in serum and urine of patients with pancreatic cancer. Hepato Gastroenterol 2008;55:695–8.
[14] Popko J, Zalewska A, Brycka R, Macias T, Knaś M, Zwierz K. Activity of N- acetyl-beta- hexosaminidase and its isoenzymes in joint fluid from a knee with an injured anterior cruciate ligament. Biol Sport 2002;19:43–9.
[15] Beratis NG, Mavrommatis T, Hatiris I, Kavaliotis J, Tsagaropoulou-Stiga H, Syrogiannopoulos GA. Increased activity of lysosomal acid hydrolases in the cell-free cerebrospinal fluid of bacterial meningitis. Pediatr Res 1997;41 (2):235–41.
[16] Dudzik D, Knaś M, Gocał M, Borzym-Kluczyk M, Szajda SD, Knaś-Karaszewska K, et al. Activity of N-acetyl-beta-D-hexosaminidase (HEX) and its isoenzymes A and B in human milk during the first 3 months of breastfeeding. Adv Med Sci 2008;53(2):300–4.
[17] Zalewska-Szajda B, Szajda S, Waszkiewicz N, Chojnowska S, Gościk E, Łebkowska U, et al. Activity of N-acetyl-β-D-hexosaminidase in the saliva of children with type 1 diabetes. Postępy Higieny i Medycyny Doświadczalnej 2013;22(67):996–9.
[18] Dance N, Price RG, Robinson D. Differential assay of human hexosaminidase A and B. Biochim Biophys Acta 1970;222(3):662–4.

[19] Kobata A. Use of endo- and exoglycosidases for structural studies of glycoconjugates. Anal Biochem 1979;100(1):1–14.
[20] Price RG, Dance N, Richards B, Cattell WR. The excretion of N-acetyl-β-glucosaminidase and β-galactosidase following surgery to the kidney. Clin Chim Acta 1970;27(1):65–72.
[21] Taranta-Janusz K, Zalewska-Szajda B, Chojnowska S, Wasilewska A. Urine exoglycosidases are potential markers of renal tubular injury in children with ureteropelvic junction obstruction. Acta Paediatr 2015;104(11):e518–23.
[22] Taranta-Janusz K, Zalewska-Szajda B, Gościk E, Chojnowska S, Dmochowska M, Pszczółkowska M, et al. New tubular injury markers in children with a solitary functioning kidney. Pediatr Nephrol 2014;29(9):1599–605.
[23] Chitayat D, Nakagawa S, Marion RW, Sachs GS, Shinnar S, Llena JF, et al. Elevation of serum beta-hexosaminidase and alpha-D-mannosidase in type 2 Gaucher disease: a clinical and biochemical study. J Inherited Metab Dis 1987;10(2):111–4.
[24] Kułaga Z, Litwin M, Grajda A, Kułaga K, Gurzkowska B, Góźdź M. OLAF Study Group. Oscillometric blood pressure percentiles for Polish normal-weight school-aged children and adolescents. J Hypertens 2012;30:1942–54.
[25] Marciniak J, Zalewska A, Popko J, Zwierz K. Optimization of an enzymatic method for the determination of lysosomal N-acetyl-beta-D-hexosaminidase and beta-glucuronidase in synovial fluid. Clin Chem Lab Med 2006;44(8):933–7.
[26] Szajda SD, Borzym-Kluczyk M, Snarska J, Puchalski Z, Zwierz K. N-acetyl-beta-D-hexosaminidase and its isoenzymes A and B in blood serum and urine, as a potential colon cancer markers. Hepatogastroenterology 2009;56(94–95):1287–98.
[27] Pan H, Cole TJ. LMSchartmaker, a program to construct growth references using the LMS method Version 2.42. 2000.
[28] Kulaga Z, Krzyżaniak A, Palczewska I, Barwicka K. Rapid westernization of Warsaw (Poland) children obesity? prevalence comparison with Australian and USA population. Ann Diagn Paediatr Pathol 2007;11(3–4):71–6.
[29] Abrami K, Tiwari SC. Urinalysis in clinical practice. J Indian Acad Clin Med 2001;2(1,2):39–50.
[30] Tassi C, Abbritti G, Mancuso F, Morucci P, Feligioni L, Muzi G. Activity and isoenzyme profile of N-acetyl-β-D-glucosaminidase in urine from workers exposed to cadmium. Clin Chim Acta 2000;299(1-2):55–64.
[31] Lew DB, Dempsey BK, Zhao Y, Muthalif M, Fatima S, Malik KU. Beta-hexosaminidase −induced activation of p44/42 mitogen −activated protein kinase is dependent on p21Ras and protein kinase C and mediates bovine airway smooth-muscle proliferation. Am J Respir Cell Mol Biol 1999;21(1):111–8.
[32] Waszkiewicz N, Zalewska-Szajda B, Chojnowska S, Szajda SD, Zalewska A, Konarzewska B, et al. The salivary β-HEX A% index as an excellent marker of periodontitis in smoking alcohol-dependent persons. Dis Markers 2013;35(5):457–63.
[33] Dance N, Price RG, Cattell WR, Lansdell J, Richards B. The excretion of N-acetyl-beta-glucosaminidase and beta-galactosidase by patients with renal disease. Clin Chim Acta 1970;27(1):87–92.
[34] Linko-Lopponen S. Fluorometric measurement of urinary N-acetyl-beta-D-glucosaminidase and its correlation to uremia. Clin Chim Acta 1986;160(2):123–7.
[35] Kunin CM, Chesney RW, Craig WA, England AC, DeAngelis C. Enzymuria as a marker of renal injury and disease: studies of N-acetyl-beta-glucosaminidase in the general population and in patients with renal disease. Pediatrics 1978;62(5):751–60.
[36] Filutkiewicz J. Wzrost wydalania enzymów lizosomalnych i fosfatazy alkalicznej w moczu jako wyraz zaostrzenia przewlekłego odmiedniczkowego zapalenia nerek. Pol Tyg Lek 1979;39:1901–4.
[37] Ellis BG, Tucker SM, Thompson AE, Price RG. Presence of serum and tissue forms of N-acetyl-beta-glucosaminidase in urine from patients with renal disease. Clin Chim Acta 1975;64(2):195–202.
[38] Bugge JF, Hartmann A, Osnes S, Bentdal O, Stenstrom J. Immediate and early renal function after living donor transplantation. Nephrol Dial Transplant 1999;14(2):389–93.
[39] Taracha E, Habrat B, Chmielewska K, Baran H, Szukalski B. Zastosowanie oznaczenia beta-heksozoaminidazy w moczu w diagnostyce nadużywania alkoholu przez osoby uzależnione od opiatów biorących udział w programie substytucyjnego leczenia matadonem. Psychiatr Pol 1999;33(2):215–23.
[40] Hultberg B, Isaksson A, Brattstrom L, Israelsson B. Elevated urinary excretion of beta-hexosaminidase in smokers. Eur J Clin Chem Clin Biochem 1992;30(3):131–3.
[41] Hultberg B, Isaksson A, Sterner G, Weibull H. Enzyme immunoassay of urinary beta-hexosaminidase isoenzymes in patients with renal transplants. Clin Chim Acta 1990;192(2):107–14.
[42] Romero R, Salinas I, Lucas A, Abad E, Reverter JL, Johnston S, et al. Renal function changes in microalbuminuric normotensive type II diabetic patients treated with angiotensin-converting enzyme inhibitors. Diabetes Care 1993;16(4):597–600.
[43] Liubimova NV, Kumykova ZhKh Kushlinskii NE, Kasumov ChM. Durnov LA. Biochemical parameters in the diagnosis of nephrotoxicity of antineoplastic chemotherapy in children. Vopr Onkol 1997;43(4):448–53.
[44] Liubimova NV, Kumykova ZhKh Kushlinskii NE, Mentkevich GL, Valentei LV, Durnov LA. The role of enzymuria in evaluation of the nephrotoxicity of antineoplastic chemotherapy in children. Bull Exp Biol Med 1997;124(10):446–50.
[45] Agirbasli M, Radhakrishnamurthy B, Jiang X, Bao W, Berenson GS. Urinary N-acetyl-beta-D-glucosaminidase changes in relation to age, sex, race, and diastolic and systolic blood pressure in a young adult biracial population. The Bogalusa Heart Study. Am J Hypertens 1996;9(2):157–61.
[46] Nishida M, Kawakatsu H, Komatsu H, Ishiwari K, Tamai M, Tsunamoto K, Kasubuchi Y, Sawada T. Values for urinary beta 2-microglobulin and N-acetyl-beta-D-glucosaminidase in normal healthy infants. Acta Paediatrica Japonica 1998;40(5):424–6.
[47] Skinner AM, Addison GM, Price DA. Changes in the urinary excretion of creatinine, albumin and N-acetyl-beta-D-glucosaminidase with increasing age and maturity in healthy schoolchildren. Eur J Pediatr 1996;155(7):596–602.