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Original research article

Activity of lysosomal exoglycosidases in the urine of healthy normotensive and pre-hypertensive children

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ABSTRACT

Purpose: We investigated the relationship between blood pressure (BP) and the activity of lysosomal exoglycosidases: N-acetyl-β-hexosaminidase (HEX), its isoenzymes A (HEX A) and B (HEX B), α-fucosidase (FUC), β-galactosidase (GAL), β-glucuronidase (GLU) and α-mannosidase (MAN) in pre-hypertensive (high normal blood pressure - HNBP) and normal blood pressure (NBP) children.
Material and methods: The study was carried out with urine samples collected from 176 children, aged 6–17.9 years, divided into 2 groups: 42 HNBP and 134 NBP subjects. The children were stratified depending on systolic and diastolic BP (SBP; DBP): HNBP (SBP and/or DBP greater than or equal to the 90th percentile, but less than the 95th percentile) for sex, age, and height; and NBP (SBP and DBP less than the 90th centile). The activities of lysosomal exoglycosidases were determined by the colorimetric method, and expressed in pKat/mL and pKat/μgCr.
Results: The activity of urinary HEX A in HNBP group was significantly higher than in NBP ($p < 0.05$). The HNBP group showed significant positive correlation between HEX, HEX A (pKat/mL) and SBP. AUC for HEX A was 0.616, cut-off value −29.351 pKat/mL (sensitivity 51.2%, specificity 71.8%), and 0.589, cut-off value −0.054 pKat/μgCr (sensitivity 31.7%, specificity 86.3%).
Conclusions: This is the first report of the relationship between BP and the activity of urinary lysosomal exoglycosidases: HEX, HEX A and HEX B, FUC, GAL, GLU, and MAN in healthy children and adolescents. It seems that HEX A (pKat/mL) can be used as a useful tool in identifying children with HNBP.

1. Introduction

Arterial hypertension, which is common in adults, represents a growing problem in children. Children with high normal blood pressure (HNBP) are at a greater risk of being hypertensive adults [1,2] than children with normal blood pressure (NBP). It has been postulated that strategies for preventing hypertension should involve a population-based approach and should be focused on individuals at high risk for hypertension. Detection of hypertensive tendencies in the early stages of life provides the greatest long-term potential for early detection, prevention, and reduction of the overall concerns associated with hypertension-associated complications [3]. Growing evidence, based on experimental and clinical research, indicates the potential involvement of chronic inflammation and oxidative stress in the complex pathogenesis of hypertension [4]. Since chronic inflammation and oxidative stress are connected with degradation of tissues, it may be postulated that development of hypertension is accompanied by an increase in the activity of degradative enzymes, including lysosomal exoglycosidases. Lysosomal exoglycosidases are involved in the degradation of oligosaccharide chains of glycoproteins (HEX, HEX A, HEX B, FUC, GAL, MAN), glycolipids (HEX, HEX A, HEX B, FUC, GAL) and glycosaminoglycans as well as proteoglycans (HEX, HEX A, HEX B, GAL, GLU) [5,6]. Lysosomal exoglycosidases are specific for only one, i.e.α or β anomeric forms of glycosidic bound. Inherited deficiency or reduction in the activity of appropriate lysosomal exoglycosidase induces accumulation of non-degraded oligosaccharides in lysosomes, causing storage disease. In storage diseases, non-degraded oligosaccharides enlarge lysosomes that disturb structure and function of the

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affected cells [7].The increase in the specific activity of lysosomal exoglycosidases is observed in the inflammatory and damaging states where increased degradation of damaged tissue glycoconjugates takes place [8–12]. Activity of lysosomal exoglycosidases was detected in kidneys [13], liver [14], spleen [15], intestine [10], lungs [16] brain [17], placenta [18], skeletal muscles and skin fibroblasts [19]. Lysosomal exoglycosidases are active in serum, urine [9,10,20–22], synovial fluid [12], cerebrospinal fluid [23], and the saliva [11].

In our previous studies, diagnostic significance of the increased activities of lysosomal exoglycosidases in urine was stated in children and adolescents with tubular injury due to ureteropelvic junction obstruction [24] and in renal agenesis [25]. Elevated specific activity of HEX was also found in saliva of children with diabetes [26].

It is worth noting that lysosomal exoglycosidases have gained considerable attention as renal tubular damage markers due to their clinical relevance as sensitive and specific biomarkers for predicting the development and progression of early-stage kidney diseases [24,27].

There is also accumulating evidence that urinary lysosomal enzymes are associated not only with nephropathy, but also with vascular complications, including retinopathy [28], neuropathy [29], and macrovascular disease [30].

It was proved that changes in the activity of urinary lysosomal HEX and its association with blood pressure (BP) in young adults may be the evidence of early hypertensive disease [31]. Alderman et al. [32] found that urinary N-acetyl-beta-glucosaminidase is frequently elevated in persons with high blood pressure, even though there is no other evidence of renal damage. However, the causes of increased HEX activity in the urine of hypertensive persons remain unclear. Despite that, the development of methods suitable to estimate the risk of high blood pressure is highly desirable and will aid the prevention of cardiovascular events with target organ damage. Furthermore, we did not find in the literature any reports concerning the relation between the activity of urinary lysosomal exoglycosidases other than N-acetyl-beta-glucosaminidase and high-normal blood pressure of healthy children.

It is also known that a panel of biomarkers, as a combination of markers increasing and decreasing with severity of hypertension, would be more helpful than a single marker. We assumed that determination of the activities of lysosomal exoglycosidases in body fluids (including urine), which is cheap, easy to perform, and a sensitive screening test, may be used to investigate the effects of hypertension on the kidney functions in children.

The first aim of the present study was to investigate urinary activities of the following lysosomal exoglycosidases: N-acetyl-β-hexosaminidase (HEX), HEX isoenzymes A (HEX A), HEX isoenzymes B (HEX B), α-fucosidase (FUC), β-galactosidase (GAL), β-glucuronidase (GLU), and α-mannosidase (MAN) in NBP and HNBP children. The second aim was to evaluate and predict subtle degrees of renal injury in HNBP children.

2. Materials and methods

2.1. Participants

The studied population comprised of 176 children (school pupils), aged 6–17.9 years (girls = 88, boys = 88), divided into 2 groups: 134 NBP and 42 HNBP children.

Urine and data were obtained from participants of the OLAF study [33] and from the healthy children of the staff at the University Children's Hospital in Bialystok. Patients who met all of the following inclusion criteria were included in the study: (1) no clinical and laboratory signs of infection, (2) lack of antibiotic treatment within the last 4 weeks and (3) signed informed consent.

Exclusion criteria were: chronic diseases that influence BP (renal disease, diabetes, arthritis, history of hypertension) and treatment with medications that influence BP (antihypertensive or antiarrhythmic medication, stimulant medication for attention deficit hyperactivity disorder, systemic steroids, thyroid or growth hormone supplementation).

The medical history of the study participants, including past and present diseases as well as medications used, was obtained from the parents. In order to exclude the influence of possible diseases on BP and activity of urinary lysosomal exoglycosidases, experienced physicians assessed the general health status of each participant. Height and body weight were measured and Body Mass Index (BMI) was calculated.

After overnight fasting, samples of urine were taken in hospital department from each participant to disposable polyethylene containers without preservatives. Urine samples stored in a refrigerator were delivered to the laboratory within 4 h of collection, centrifuged at $4000 \times g$ for 20 min, poured into Eppendorf tubes and frozen at −80 °C until the assay was performed.

The study was conducted with the consent of children aged over 16 and parents of all remaining children, with the consent of the Local Bioethics Commission at the Medical University of Bialystok (dated 1st September 2014; approval number ANZ-06042-134-41724/ 14). The OLAF study was approved by The Children's Memorial Health Institute Ethics Committee.

2.2. Blood pressure

We measured BP using an automated oscillometric device (Datascope Accutor Plus) that has been validated for use in children [34]. Four cuff sizes of the device were available (child's cuff, small adult cuff, adult cuff, and large adult cuff). The appropriate cuff size (bladder width at least 40% of arm circumference and length 80–100% of arm circumference) was determined by measuring the mid-upper arm circumference. BP was measured in triplicate at 3-min intervals after a 5–10-min rest in the sitting position with the arm and back supported. The mean of the second and third measurements were used for analysis. Trained staff took all measurements.

NBP was defined as systolic blood pressure (SBP) and diastolic blood pressure (DBP) that is less than the 90th percentile for sex, age, and height. Average SBP or DBP levels that were greater than or equal to the 90th percentile, but less than the 95th percentile, had been designated as HNBP, also known as pre-hypertensive blood pressure [35].

2.3. Creatinine determination

Urinary creatinine concentrations were determined using ABX Pentra Enzymatic Creatinine CP kit, biochemical analyser ABX Pentra 400, and expressed in µg/mL.

2.4. HEX, HEX A, HEX B, FUC, GAL, GLU and MAN activity determination

HEX, HEX B, FUC, GAL and MAN activities in urine (pKat/mL) were determined using the method of Zwierz et al. [36] and GLU activity in urine (pKat/mL) with the method of Marciniak et al. [37] as modified by Szajda et al. [38].

The reaction mixture for determining HEX, HEX B, FUC, GAL, GLU and MAN activities was composed of 10 µl of urine and 30 µl of the appropriate substrate solution: 20 mM solution of p-nitrophenyl-*N*-acetyl-ß-D-glucosaminide (for HEX), 4-nitrophenyl-α-L-fucopyranoside (for FUC), 4-nitrophenyl-β-D-galactopyranoside (for GAL), 4-nitrophenyl-β-D-glucuronide (for GLU), 4-nitrophenyl-α-D-mannopyranoside(for MAN) (Sigma, St. Louis, MO, USA). Additionally 40 µl of 100 mM citrate-phosphate buffer pH 4.7 (for HEX), and pH 4.3 (for FUC, GAL, and MAN) or 200 mM sodium acetate buffer pH 4.5 (for GLU) were added. The microplates were incubated for 60 min at 37 °C with constant shaking. The enzymatic reactions were stopped by adding 200 µl of 200 mM borate buffer, pH 9.8.

To determine the HEX B activities, 40 µL of 100 mM of phosphate-citrate buffer at pH 4.7 and 10 µL of the urine were added to microplate wells, followed by incubation for 180 min at 50 °C to inactivate a thermolabile HEX A. Then, 30 μL of 20 mM solution of p-nitrophenyl-*N*-acetyl-ß-D-glucosaminide (Sigma, St. Louis, MO, USA) was added and the mixtures were incubated for additional 60 min at 37 °C. The enzymatic reactions were terminated by adding 200 μL of 200 mM borate buffer at pH 9.8. The activity of lysosomal exoglycosidases corresponding to the amounts of released p-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 a 3-hour incubation at 50 °C, and HEX B is thermostable, HEX A activity was calculated from the difference between the activity of total HEX and HEX B.

The level of urinary activities of HEX, HEX A, HEX B, FUC, GAL, GLU and MAN were expressed in Kat/mL of urine and standardized by comparison to urinary creatinine concentrations and expressed in pKat/μg of creatinine (pKat/μg Cr).

2.5. Statistical analysis

Data analysis was performed using computer program Statistica version 12.0 (StatSoft Inc., Tulsa, OK, USA). The nonparametric statistics was chosen, as the population of study participants was relatively small. Statistical analysis was performed using the Mann–Whitney *U* test. Correlations between variables were evaluated by the Pearson or Spearman test as appropriate. $P < 0.05$ was considered statistically significant. Receive Operating Characteristic (ROC) curve was used to determine the cut off values of estimated exoglycosidases urinary activity that gave the best sensitivity and specificity.

3. Results

Clinical characteristic of NBP and HNBP children is summarized in Table 1. The age of children did not differ significantly ($p > 0.05$). There were more boys than girls in both NBP and HNBP groups ($p > 0.05$). The body weights, BMI and BMI Z-scores of children from HNBP group was higher compared to NBP ($p < 0.05$). SBP and DBP were significantly higher in HNBP group ($p < 0.001$) compared to healthy NBP children.

We found that only HEX A (pKat/mL) activity was significantly higher in HNBP children when compared to NBP group ($p < 0.05$). No statistically significant differences were found in all other estimated HEX, HEX A, HEX B and FUC, GAL, GLU, MAN activities (pKat/μg Cr), as well as creatinine concentration between NBP and HNBP groups ($p > 0.05$).

In all the studied children, we found significant positive correlation between urinary HEX as well as its isoenzyme HEX A (pKat/mL) and SBP ($r = 0.17$, $p < 0.05$; $r = 0.21$, $p < 0.05$; respectively) (Table 2). We found significantly negative correlation between urinary FUC, GAL (pKat/μg Cr) and SBP ($r = -0.16$, $p < 0.05$; $r = -0.17$, $p < 0.05$; respectively) as well as negative correlation between urinary GAL (pKat/μg Cr) and DBP ($r = -0.16$, $p < 0.05$). There was a correlation tendency (on the border of significance) between urinary GLU (pKat/mL) as well as MAN (pKat/μg Cr) activity and SBP ($r = 0.148$, $p = 0.052$; $r = -0.15$, $p = 0.056$; respectively). No significant correlations were found between the estimated exoglycosidases with BMI and BMI Z-score ($p > 0.05$).

When we compared (Table 3) the results between boys and girls in NBP and HNBP groups no statistically significant differences were found between the estimated exoglycosidases activity (pKat/mL and pKat/μg Cr) and clinical parameters ($p > 0.05$). However, we found statistically significant positive correlation between urinary HEX A (pKat/mL and pKat/μg Cr) and SBP in boys and statistically significant positive correlation between urinary HEX, HEX B, FUC, GAL, GLU, as well as MAN (pKat/mL) and SBP in girls ($p < 0.05$).

Table 1
Characteristics of the study group. Comparison of HNBP with NBP, (Q1- first quartile, Q3- third quartile).

Variables	NBP n = 134 Median (Q1–Q3)	HNBP n = 42	*P value*
Age (years)	10.04 (8.05–12.03)	10.56 (8.07–12.07)	NS
Height (cm)	140.60 (129.50–154.00)	146.25 (135.00–160.40)	NS
Weight (kg)	33.60 (26.50–45.05)	40.60 (33.35–51.10)	*0.017*
BMI (kg/m²)	16.92 (15.38–18.99)	19.04 (17.25–20.62)	*0.003*
BMI (Z–score)	0.102 (-0.208 – 0.827)	0.475 (-0.049 – 1.206)	*0.003*
SBP(mmHg)	104.00 (98.00–110.00)	119.50 (110.00–128.00)	*0.00001*
DBP (mmHg)	59.00 (54.00–64.00)	71.50 (66.00–73.00)	*0.00001*
HEX (pKat/mL)	116.94 (91.28–156.75)	120.41 (94.66–198.52)	NS
HEX A (pKat/mL)	17.84 (8.42–32.73)	29.91 (14.67–49.67)	*0.014*
HEX B (pKat/mL)	97.49 (73.21–121.76)	87.33 (73.21–140.95)	NS
FUC (pKat/mL)	75.47 (64.18–91.28)	85.07 (65.31–102.57)	NS
GAL (pKat/mL)	81.68 (69.83–95.79)	82.25 (66.44–107.08)	NS
GLU (pKat/mL)	74.34 (61.93–95.79)	78.86 (61.93–108.21)	NS
MAN (pKat/mL)	78.86 (67.57–93.53)	86.20 (68.70–111.60)	NS
Creatinine (μg/mL)	998.67 (752.62–1440.96)	1137.00 (908.82–1465.35)	NS
HEX (pKat/μgCr.)	0.121 (0.082 – 0.169)	0.111 (0.088 – 0.165)	NS
HEX A (pKat/μgCr.)	0.017 (0.008 – 0.041)	0.028 (0.012 – 0.063)	NS
HEX B (pKat/μgCr.)	0.093 (0.068 – 0.142)	0.084 (0.068 – 0.118)	NS
FUC (pKat/μgCr.)	0.078 (0.054 – 0.102)	0.078 (0.053 – 0.095)	NS
GAL (pKat/μgCr.)	0.082 (0.057 – 0.110)	0.076 (0.060 – 0. 095)	NS
GLU (pKat/μgCr.)	0.077 (0.053 – 0.110)	0.079 (0.056 – 0.096)	NS
MAN (pKat/μgCr.)	0.082 (0.056 – 0.111)	0.071 (0.062 – 0.103)	NS

NBP – normal blood pressure; HNBP – high normal blood pressure; NS – not significant; BMI – body mass index; SBP – systolic blood pressure; DBP - diastolic blood pressure; HEX – N-acetyl-β-hexosaminidase, HEX A – HEX isoenzyme A; HEX B – HEX isoenzyme B, FUC – α-fucosidase, GAL – β-galactosidase, GLU – β-glucuronidase; MAN – α-mannosidase.

Table 2
Correlations between estimated urinary exoglycosidases activities (expressed in Kat/mL as well as pKat/μg Cr) and blood pressure values in all studied children.

	SBP		DBP	
	r	*p*	r	*p*
HEX (pKat/mL)	***0.174***	***0.022***	0.063	0.409
HEX A (pKat/mL)	***0.207***	***0.006***	0.018	0.813
HEX B (pKat/mL)	0.111	0.145	0.082	0.283
FUC (pKat/mL)	0.092	0.229	0.061	0.421
GAL (pKat/mL)	0.064	0.405	0.021	0.776
GLU (pKat/mL)	0.148	0.052	0.072	0.345
MAN (pKat/mL)	0.092	0.228	0.100	0.190
Creatinine (μg/mL)	***0.204***	***0.007***	***0.199***	***0.008***
HEX (pKat/μgCr.)	−0.024	0.748	−0.100	0.190
HEX A (pKat/μgCr.)	0.112	0.142	−0.061	0.425
HEX B (pKat/μgCr.)	−0.112	0.141	−0.117	0.127
FUC (pKat/μgCr.)	***−0.162***	***0.033***	−0.149	0.050
GAL (pKat/μgCr.)	***−0.166***	***0.029***	***−0.160***	***0.036***
GLU (pKat/μgCr.)	−0.109	0.153	−0.114	0.135
MAN (pKat/μgCr.)	−0.146	0.056	−0.140	0.067

SBP – systolic blood pressure, DBP – diastolic blood pressure, r – correlation coefficient, *p* – significance level, HEX – N-acetyl-β-hexosaminidase, HEX A – HEX isoenzyme A; HEX B – HEX isoenzyme B, FUC – α-fucosidase, GAL – β-galactosidase, GLU – β-glucuronidase; MAN – α-mannosidase.

In HNBP children (Table 4A) we observed statistically significantly positive correlation between urinary HEX, as well as HEX A (pKat/mL) and SBP ($r = 0.35$, $p < 0.05$; $r = 0.4$, $p < 0.01$; respectively). There

Table 3
Correlations between estimated urinary exoglycosidases activities (expressed in Kat/mL as well as pKat/μg Cr) and blood pressure values depending on sex.

	SBP		DBP	
	Boys r (p)	Girls r (p)	Boys r (p)	Girls r (p)
HEX (pKat/mL)	0.113 (0.299)	***0.227 (0.034)***	0.057 (0.601)	0.044 (0.681)
HEX A (pKat/mL)	***0.327 (0.002)***	0.103 (0.343)	0.103 (0.347)	−0.064 (0.554)
HEX B (pKat/mL)	0.015 (0.887)	***0.219 (0.042)***	0.063 (0.561)	0.084 (0.440)
FUC (pKat/mL)	−0.009 (0.932)	***0.223 (0.038)***	−0.012 (0.907)	0.129 (0.235)
GAL (pKat/mL)	−0.069 (0.527)	***0.240 (0.025)***	−0.029 (0.785)	0.058 (0.592)
GLU (pKat/mL)	0.065 (0.550)	***0.277 (0.009)***	0.102 (0.349)	0.049 (0.649)
MAN (pKat/mL)	−0.004 (0.965)	***0.219 (0.042)***	0.019 (0.856)	0.194 (0.073)
Creatinine (μg/mL)	0.131 (0.231)	***0.273 (0.010)***	***0.218 (0.044)***	0.156 (0.149)
HEX (pKat/μgCr.)	0.011 (0.919)	−0.055 (0.611)	−0.081 (0.457)	−0.112 (0.301)
HEX A (pKat/μgCr.)	***0.231 (0.033)***	−0.038 (0.722)	−0.005 (0.958)	−0.131 (0.226)
HEX B (pKat/μgCr.)	−0.112 (0.305)	−0.096 (0.376)	−0.133 (0.224)	−0.094 (0.385)
FUC (pKat/μgCr.)	−0.119 (0.274)	−0.196 (0.069)	−0.147 (0.178)	−0.138 (0.203)
GAL (pKat/μgCr.)	−0.155 (0.154)	−0.161 (0.138)	−0.176 (0.105)	−0.128 (0.238)
GLU (pKat/μgCr.)	−0.111 (0.310)	−0.113 (0.296)	−0.084 (0.440)	−0.123 (0.256)
MAN (pKat/μgCr.)	−0.127 (0.267)	−0.017 (0.117)	−0.177 (0.104)	−0.08 (0.413)

SBP – systolic blood pressure; DBP – diastolic blood pressure; r – correlation coefficient; *p* – significance level; HEX – N-acetyl-β-hexosaminidase, HEX A – HEX isoenzyme A; HEX B – HEX isoenzyme B, FUC – α-fucosidase, GAL – β-galactosidase, GLU – β-glucuronidase; MAN – α-mannosidase.

were no statistically significant correlations between the estimated markers and BP values in NBP groups ($p > 0.05$).

To exclude the influence of diuresis and personal attributes of investigated children on urinary activity of lysosomal exoglycosidases (Table 1) we presented our results in relations to urinary creatinine concentration [21,22]. The studied children reveal statistically significant positive correlation between urinary creatinine (μg/mL) and SBP ($r = 0.20$, $p < 0.01$), as well as DBP ($r = 0.20$, $p < 0.01$) (Table 2). The kidneys play an essential role in the integrated blood pressure regulation system [39], and creatinine concentration reflects their functioning [40], which may be a confirmation of the existence of a relationship between creatinine and BP in our (Table 2) and other studies [40].

Children with NBP (Table 4B) presented significantly negative correlation between urinary activity of FUC, GAL (pKat/μg Cr) and SBP ($r = -0.19$, $p < 0.05$; $r = -0.18$, $p < 0.05$; respectively) as well as urinary HEX, its isoenzyme HEX A, FUC, GAL, GLU, MAN (pKat/μg Cr) and DBP ($r = -0.18$, $p < 0.05$; $r = -0.20$, $p < 0.05$; $r = -0.24$, $p < 0.05$; $r = -0.25$, $p < 0.05$; $r = -0.21$, $p < 0.05$; $r = -0.24$, $p < 0.05$; respectively). Similarly we search for correlations between urinary lysosomal exoglycosidases expressed in pKat/μg Cr with BP levels in HNBP group, however our analysis revealed only strong tendency to positive correlation between urinary HEX A (pKat/μg Cr) activity and SBP, on the border of statistical significance ($r = 0.30$, $p = 0.054$).

ROC analyses were performed in order to define the diagnostic profile of urinary activity of HEX A expressed in pKat/mL and pKat/μg Cr in identifying children with HNBP among all estimated healthy children (Fig. 1). The area under the curve (AUC) for HEX A (pKat/mL) was 0.616 with the best cut-off value 29.351 pKat/mL presenting with 51.2% sensitivity and 71.8% specificity, and for pKat/μg Cr. AUC was 0.589 with the best cut-off value 0.054 pKat/μg Cr (sensitivity 31.7%, specificity 86.3%) (Table 5).

4. Discussion

Since hypertension is increasingly recognized as beginning in childhood, we hypothesized that changes in urinary exoglycosidases may start early in life and may also be the evidence of existing early pre-hypertensive disease. We analyzed the activity of urinary lysosomal exoglycosidases: HEX, HEX A, HEX B, FUC, GAL, GLU and MAN changes in 176 children under the age of 18, in relation to age, as well as SBP and DBP values.

Our study demonstrates that only activity of urinary HEX A is statistically significantly higher in pre-hypertensive children when compared to normotensive controls. In addition, urinary activity of HEX, its isoenzyme HEX A expressed in pKat/mL positively correlated with SBP.

In recent years, the novel concept of hypertension pathogenesis has gained support. Initially, an opinion dominated that increased intravascular inflammatory activity resulted from increased vascular BP. However, recently a dominant hypothesis prevails that chronic inflammatory processes play crucial role in the pathogenesis of hypertension. Inflammatory processes are connected with an increase (in tissues and body fluids) in the concentration of many proteins including adhesive molecules, cytokines, acute phase proteins [41] as well as the activity of lysosomal enzymes and among them the lysosomal exoglycosidases: HEX, HEX A, HEX B, FUC, GAL, GLU and MAN [8–12].

Although glycoconjugates (glycoproteins, glycolipids, proteoglycans) play a significant role in the structure and function of endothelial cell - glycocalyx, smooth muscles and extracellular matrix [24], information concerning the role of glycoconjugates in arterial walls is limited [42,43]. Hermelin et al. [42] found a statistically significant decrease in HEX and GLU activity in the lysosomal fraction from arterial media-intima in old rats in comparison to young rats, which suggests slow-down glycoconjugate metabolism in arterial media-intima of rats with aging. On the other hand, Markle [43] reported significant increase in the activity of HEX, GAL, GLU and MAN in the rat aortic tissue depending on the growth and aging.

Thus we decided to evaluate the relationship between the activity of urinary exoglycosidases and BP values in children and adolescents. In the literature, there is no data on the association between the urine exoglycosidase activities and BP in the pediatric population.

In the present study, we showed the relationship between the urinary activity of exoglycosidases and BP in NBP and HNBP healthy children. Children with NBP and HNBP presented significant differences in SBP and DBP ($p < 0.001$). Weight and BMI were significantly different in the NBP and HNBP groups ($p < 0.05$) (Table 1). The relationship between the body weight and essential hypertension is well known. Excess weight is thought to correlate with increased intraglomerular capillary pressure, resulting in glomerular hyperfiltration, which is a condition for end-organ damage. Although Girişgen et al [44] did not find a statistically significant correlation between the estimated urinary lysosomal enzyme and BMI, their findings are consistent with our present data that the activity of all the assessed exoglycosidases was not correlated with BMI. However, we report a statistically significant positive correlation between the urinary creatinine

Table 4
Correlations of estimated urinary exoglycosidases activities (**4A** - pKat/mL; **4B** - pKat/μg Cr.) with blood pressure values.

4A

	NBP		HNBP	
	coefficient	p-value	coefficient	p-value
HEX (pKat/mL)				
SBP (mmHg)	0.089	0.310	***0.347***	***0.024***
DBP (mmHg)	0.034	0.693	−0.115	0.467
HEX A (pKat/mL)				
SBP (mmHg)	0.078	0.375	***0.399***	***0.008***
DBP (mmHg)	−0.103	0.243	−0.188	0.231
HEX B (pKat/mL)				
SBP (mmHg)	0.093	0.290	0.210	0.180
DBP (mmHg)	0.118	0.180	−0.060	0.704
FUC (pKat/mL)				
SBP (mmHg)	0.035	0.687	0.106	0.500
DBP (mmHg)	−0.004	0.962	−0.009	0.952
GAL (pKat/mL)				
SBP (mmHg)	0.043	0.620	0.115	0.466
DBP (mmHg)	−0.012	0.887	0.046	0.771
GLU (pKat/mL)				
SBP (mmHg)	0.128	0.145	0.165	0.294
DBP (mmHg)	0.022	0.796	0.030	0.850
MAN (pKat/mL)				
SBP (mmHg)	0.053	0.543	−0.013	0.934
DBP (mmHg)	0.010	0.906	0.081	0.607

4B

	NBP		HNBP	
	coefficient	p-value	coefficient	p-value
HEX (pKat/μgCr.)				
SBP (mmHg)	−0.095	0.279	0.160	0.309
DBP (mmHg)	***−0.183***	***0.037***	0.011	0.942
HEX A (pKat/μgCr.)				
SBP (mmHg)	−0.006	0.943	0.298	0.054
DBP (mmHg)	***−0.195***	***0.026***	−0.121	0.444
HEX B (pKat/μgCr.)				
SBP (mmHg)	−0.117	0.185	−0.003	0.980
DBP (mmHg)	−0.152	0.084	0.138	0.383
FUC (pKat/μgCr.)				
SBP (mmHg)	***−0.194***	***0.027***	−0.120	0.446
DBP (mmHg)	***−0.238***	***0.006***	0.072	0.649
GAL (pKat/μgCr.)				
SBP (mmHg)	***−0.177***	***0.044***	−0.122	0.437
DBP (mmHg)	***−0.246***	***0.004***	0.151	0.339
GLU (pKat/μgCr.)				
SBP (mmHg)	−0.130	0.141	−0.096	0.543
DBP (mmHg)	***−0.211***	***0.016***	0.141	0.370
MAN (pKat/μgCr.)				
SBP (mmHg)	−0.138	0.116	−0.192	0.221
DBP (mmHg)	***−0.236***	***0.006***	0.198	0.207

HNBP – high normal blood pressure; NBP – normal blood pressure; SBP – systolic blood pressure; DBP – diastolic blood pressure; HEX – N-acetyl-β-hexosaminidase, HEX A – HEX isoenzyme A; HEX B – HEX isoenzyme B, FUC – α-fucosidase, GAL - β-galactosidase, GLU – β-glucuronidase; MAN – α-mannosidase.

and both SBP as well as DBP values. Also in all the studied children we found a statistically significant positive correlation between the urinary HEX as well as its isoenzyme HEX A (pKat/mL) and SBP. We found a statistically significantly negative correlation between the urinary FUC, GAL (pKat/μg Cr) and SBP as well as a negative correlation between the urinary GAL (pKat/μg Cr) and DBP. There was a correlation tendency (on the border of significance) between the urinary GLU (pKat/mL) as well as MAN (pKat/μg Cr) activity and SBP (Table 2).

Among all the exoglycosidases estimated by us only the urinary activity of HEX A expressed in pKat/mL statistically significantly differ between the NBP and HNBP groups ($p < 0.05$) (Table 1). The relationship between HEX and BP was confirmed in clinical adult studies; however, the results are still equivocal. In the study conducted on 84 patients with uncomplicated essential hypertension, urinary HEX activity tended to be slightly higher in hypertensive patients than in the normotensive group [45]. Moreover, Alderman et al. [32] reported that the urinary determination of lysosomal enzyme in hypertensive patients is a marker of renal damage. In another study [46], urinary N-acetyl-β-D-glucosaminidase activity was suggested to be an independent promising candidate marker for use in assessing the progression of early renal impairment in patients with hypertension.

Also, comparing the results between boys and girls (Table 3) we found a statistically significant positive correlation between the urinary HEX A (pKat/mL and pKat/μg Cr) and SBP in boys and a statistically significant positive correlation between the urinary HEX, HEX B, FUC, GAL, GLU, as well as MAN (pKat/mL) and SBP in girls ($p < 0.05$).

In children with HNBP we observed a positive correlation tendency between the urinary HEX, its isoenzyme HEX A (pKat/mL) and SBP

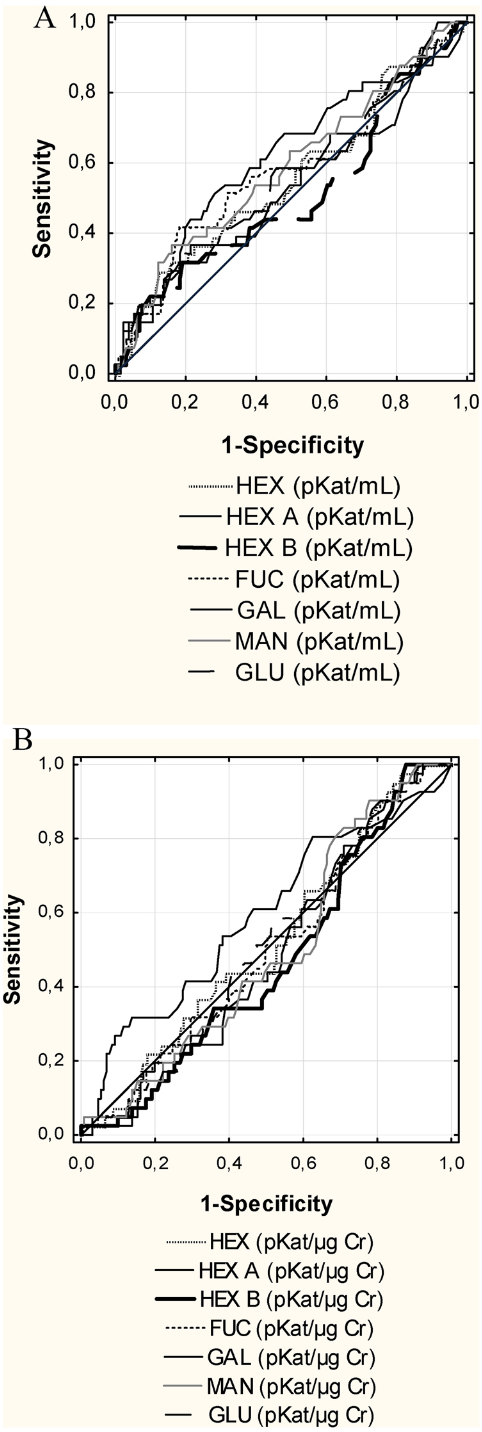

Fig. 1. Receiver Operating Characteristic (ROC) curves for urinary HEX, HEX A, HEX B, FUC, GAL, MAN and GLU (1 A - pKat/mL; 1B - pKat/µg Cr) in prehypertensive children (HNBP) among all estimated healthy children.
HEX – N-acetyl-β-hexosaminidase, HEX A – HEX isoenzyme A; HEX B – HEX isoenzyme B, FUC – α-fucosidase, GAL – β-galactosidase, MAN – α-mannosidase; GLU – β-glucuronidase.

($p < 0.05$, $p < 0.01$; respectively) (Table 4A). In the study by Schmieder et al. [45] hypertensive patients with high serum HEX activity had exaggerated increase in systolic pressure.

Additionally, in the children with NBP we found a statistically significant negative correlation between the urinary FUC, GAL (pKat/µg Cr) activities and SBP ($p < 0.05$) as well as the urinary HEX, HEX A, FUC, GAL, GLU, MAN (pKat/µg Cr) and DBP ($p < 0.05$) (Table 4B).

Table 5
Receiver Operating Characteristic (ROC) analyses for urinary activity of HEX A (pKat/mL and pKat/µgCr.) in identifying children with high normal blood pressure among all estimated healthy children.

	AUC	SE	−95%	+95%
HEX A (pKat/mL)	0.616	0.053	0.511	0.72
HEX A (pKat/µgCr.)	0.589	0.053	0.486	0.692

Similarly, De Muro et al. [47] detected a statistically significant relationship between HEX and the presence of hypertension in diabetic patients with normoalbuminuria.

What is noticeable, is that other authors did not find any differences in the urinary and serum activity of N-acetyl-β-D-glucosaminidase between patients with mild hypertension and juvenile borderline hypertension, in comparison to controls [48,49]. Discrepancy between the results of the above mentioned studies make us believe that new studies on the activity of lysosomal exoglycosidases in urine and serum of children with mild hypertension and with juvenile borderline hypertension are needed.

Our observations have led to the proposal that HEX A (pKat/mL) can be considered a useful tool for identifying children with HNBP among all estimated healthy children. Our proposal is supported by the data from our ROC analyses, which showed quite good diagnostic profile for HEX A expressed in pKat/mL (AUC 0.616) with 51.2% sensitivity, and 71.8% specificity (Fig. 1, Table 5).

Summing up, to our best knowledge, this study is the first to assess the correlations between the urinary activity of lysosomal exoglycosidases: HEX, HEX A, HEX B, FUC, GAL, GLU and MAN with BP values in pediatric HNBP and NBP subjects.

As the kidneys are one of the organs affected initially in the course of hypertension, increased urinary HEX excretion might be considered as a biochemical marker of early renal damage.

However, the excretion pattern of the urinary HEX must be interpreted with caution, as it may be a marker of the tubular functional status as well as tubular damage. Studies which will estimate lysosomal enzymes excretion in comparison with other markers of renal damage like albuminuria and/or glomerular filtration rate are needed.

In this perspective, our results, although preliminary, allow us to conclude that assessed urinary exoglycosidases activities seem not suitable for early diagnostics of hypertension. However, a special attention should be drawn to the correlation between different urinary exoglycosidases activities with SBP and/or DBP values not only in HNBP but also in NBP children. Validation of the difference in HEX A urine activity in a larger study clearly showing the added value for measuring the activity of HEX A as an appropriate biomarker for screening pre-hypertensive state and renal dysfunction, might be useful in moving those markers closer to routine clinical use.

4.1. Study limitations

Our study had several limitations. First, this study was a single-center study and might have involved self-selection bias. Second, the mechanism by which the elevated BP increases urinary HEX excretion remains unclear and is still under study.

Nevertheless, on the basis of the information gained from our study, we may speculate that further analysis of patterns of urinary exoglycosidases the activity may be a helpful adjunct for differential diagnosis of benign hypertension with/without organ target damage.

5. Conclusions

The identification of changes in urinary activity of lysosomal exoglycosidases, especially N-acetyl-β-hexosaminidases seem to be of considerable interest in diagnosing hypertension. The increasing urinary levels of HEX A (pKat/mL) activity may be related to the renal damage caused by hypertension. Longitudinal studies are needed to fully clarify the underlying causes of hypertension in children and adolescents.

Conflict of interest

The authors declare no conflicts of interest regarding the content herein.

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The authors contribution

Study Design: Zalewska-Szajda B., Taranta-Janusz K., Wasilewska A.
Data Collection: Zalewska-Szajda B., Chojnowska S.,
Statistical Analysis: Zalewska-Szajda B., Taranta-Janusz K.,
Data Interpretation: Zalewska-Szajda B., Taranta-Janusz K., Waszkiewicz N., Wasilewska A.
Manuscript Preparation: Zalewska-Szajda B., Taranta-Janusz K., Zwierz K,
Literature Search: Zalewska-Szajda B., Chojnowska S., Waszkiewicz N., Zwierz K.
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