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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 × *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