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Lysosomal exoglycosidases in the urine of children and adolescents

Egzoglikozydazy lizosomalne w moczu dzieci i młodzieży

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Abstract

Standard indices of renal function, i.e. creatinine, glomerular filtration rate (GFR) and endogenous creatinine clearance, still fail to accurately characterise disease processes in kidneys and are not sufficient for proper therapy selection, efficacy prediction and treatment efficacy monitoring for renal diseases. Therefore, markers that allow for an inexpensive, easy and reproducible kidney function assessment should be sought. Lysosomal exoglycosidases, such as N-acetyl-β-D-hexosaminidase (HEX), its isoenzymes A (HEX A) and B (HEX B), α-fucosidase (FUC), β-galactosidase (GAL), β-glucuronidase (GLU) and α-mannosidase (MAN), excreted in minimal amounts in the urine of healthy children and adolescents, yet showing a significant increase in pathological conditions, may be useful in the diagnosis of kidney disease. Such conclusions were a result of our research on the activity of lysosomal exoglycosidases in the urine of healthy children and adolescents compared to children and adolescents with hypertension, ureteropelvic junction obstruction, and congenital or acquired solitary functioning kidney, as well as based on the findings on the urinary activity of lysosomal exoglycosidases in children and adolescents with various kidney diseases, which were obtained by other authors.

Keywords: children and adolescents, normal values, kidney diseases, lysosomal exoglycosidases, urine

Streszczenie

Klasyczne wskaźniki oceniające funkcję nerek, tj. stężenie kreatyniny, wskaźnik przesączania kłębuszkowego (GFR) i klirens kreatyniny endogennej, wciąż nie dość dokładnie charakteryzują procesy chorobowe toczące się w nerkach i nie są wystarczające dla właściwego doboru metody, prognozowania skuteczności i monitorowania efektywności leczenia chorób nerek. W związku z tym istnieje potrzeba poszukiwania markerów pozwalających na ocenę funkcji nerek w sposób tani, łatwy i powtarzalny. Przydatne w diagnostyce chorób nerek mogą być aktywności egzoglikozydaz lizosomalnych: N-acetylo-β-D--heksozoaminidazy (HEX), jej izoenzymów A (HEX A) i B (HEX B), α-fukozydazy (FUC), β-galaktozydazy (GAL), β-glukuronidazy (GLU) i α-mannozydazy (MAN), wydalanych w minimalnych ilościach do moczu zdrowych dzieci i młodzieży, wykazujących istotny wzrost w stanach patologicznych. Takie wnioski są możliwe dzięki analizie wyników badań własnych nad aktywnością egzoglikozydaz lizosomalnych w moczu zdrowych dzieci i młodzieży, a także u dzieci i młodzieży z nadciśnieniem tętniczym, zwężeniem podmiedniczkowym moczowodu oraz z wrodzonym lub nabytym brakiem nerki, jak również dzięki analizie wyników badań innych autorów dotyczących aktywności egzoglikozydaz lizosomalnych w moczu dzieci i młodzieży w różnych chorobach nerek.

Słowa kluczowe: dzieci i młodzież, wartości prawidłowe, choroby nerek, egzoglikozydazy lizosomalne, mocz

INTRODUCTION

The primary function of the kidney is to produce urine. Kidneys are also a "factory" of hormones: renin (involved in the regulation of blood pressure) and erythropoietin (which stimulates the production of erythrocytes). Prostaglandins and kinins (which have dilating effects on blood vessels) are also synthesised in the kidneys. The active form of vitamin D is also produced in these organs[1–4]. The kidneys are sensitive to the effects of hormones involved in the regulation of blood pressure, e.g. vasopressin (also called antidiuretic hormone, ADH) and atrial natriuretic peptide (ANP)[5,6]. They are also involved in water-electrolyte regulation and acid-base homeostasis[5].

Standard indices of renal function include creatinine, glomerular filtration rate (GFR) and endogenous creatinine clearance. Cystatin C, neutrophil gelatinase associated lipocalin-1 (NGAL-1), kidney injury molecule 1 (KIM-1), albuminuria and albumin/creatinine ratio, which allow for early and more accurate diagnosis of renal diseases, and thus rapid implementation of appropriate therapy, are also useful in the diagnosis[7]. However, these markers are still imperfect and prone to errors. They fail to accurately characterise the disease process in the kidneys, and do not provide sufficient basis for the choice of therapeutic approach as well as predicting or monitoring therapeutic efficacy. Therefore, other renal function indicators need to be sought. Urinary activity of lysosomal exoglycosidases may be one of such markers[8].

The aim of the study was to discuss the role of urinary lysosomal exoglycosidases, such as N-acetyl-β-D-hexosaminidase (HEX), its isoenzymes A (HEX A) and B (HEX B), α-fucosidase (FUC), β-galactosidase (GAL), β-glucuronidase (GLU) and α-mannosidase (MAN), as potential markers of kidney disease in children and adolescents.

LYSOSOMAL EXOGLYCOSIDASES

Lysosomal exoglycosidases[8]: HEX, FUC, GAL, GLU and MAN, are involved in the modification of glycoconjugate oligosaccharide chains in the Golgi apparatus and endoplasmic reticulum as well as in the degeneration of glycoconjugates (along with aminohydrolases and endoglycosidases). Exoglycosidases cleave individual sugar residues from the non-reducing end of glycoprotein oligosaccharides, proteoglycans and glycolipids in lysosomes, the primary digestive organelles of a cell[9]. Lysosomal exoglycosidases located inside the lysosomal vacuoles partially diffuse and are partially bound to the membrane, forming a specific multienzyme system (Fig. 1)[10].

URINARY LYSOSOMAL EXOGLYCOSIDASES

Kidney diseases are a major problem in modern medicine since they are asymptomatic for a long time, which delays the diagnosis. Therefore, it is necessary to search for appropriately sensitive, non-invasive and inexpensive methods for renal assessment to diagnose kidney diseases at an early (asymptomatic) stage.

Urinalysis, which is one of the basic laboratory tests, assesses physical and chemical properties of the urine as well as involves microscopic evaluation of urine sediment. It is performed to detect pathologies within the urinary tract and the kidneys, such as infections, deposits, uncontrolled diabetes as well as in patients with hypertension and/or diabetes, and other entities. It is a simple, non-invasive, inexpensive and widely available method, which provides important health information despite its simplicity[11].

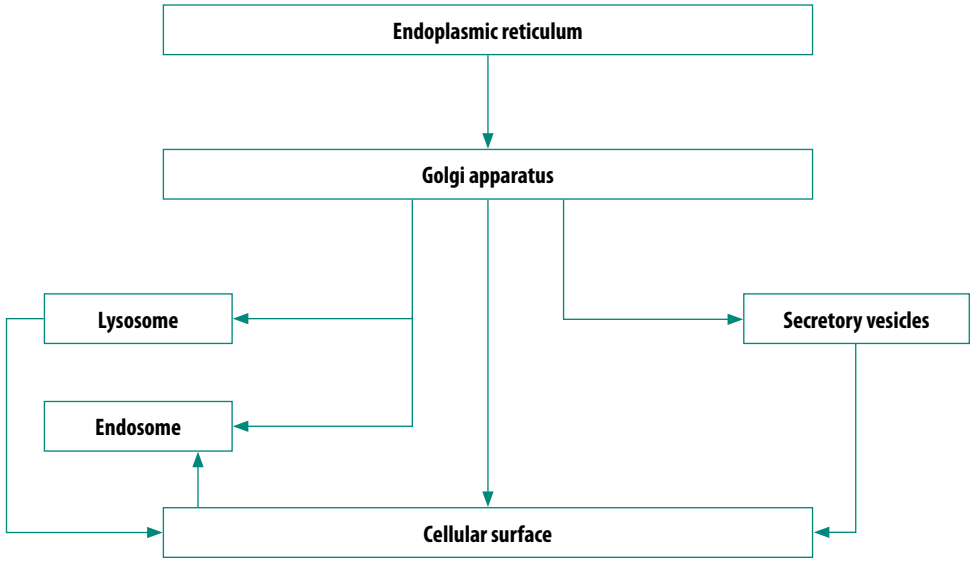

Fig. 1. Transport of lysosomal enzymes[10]

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Lysosomal exoglycosidases, such as N-acetyl-β-D-hexosaminidase (HEX), α-fucosidase (FUC), β-galactosidase (GAL), β-glucuronidase (GLU) and α-mannosidase (MAN)[8], which are produced in the renal tubular epithelium and excreted in minimal amounts in the urine of healthy individuals, yet show a significant increase in pathological conditions, which do not denature in acidic urine pH, and are found in specific renal cells and subcellular structures, may be useful in the diagnosis of kidney diseases[12].

The activity of lysosomal exoglycosidases has become a common research subject due to their multiple functions and their presence in tissues and bodily fluids[13–17]. HEX is the most active and the best described lysosomal exoglycosidase[8,18,19]. FUC, GAL, GLU and MAN are less known and need to be thoroughly evaluated for the utility of the assessment of their activity in tissues and bodily fluids. Studies published so far suggest that it may be useful to determine the activity of lysosomal exoglycosidases in urine. Lysosomal exoglycosidases are among the few enzymes whose activity may be assessed in urine[11,20]. Determining their urinary activity may help diagnose ureteropelvic junction obstruction with tubular damage in children[21], a solitary functioning kidney (SFK)[22], type 1 diabetes mellitus[23], thyroid, kidney and colon cancer, pancreatic adenocarcinoma[15,16,24–27] as well as alcohol abuse[28] in adults. Significantly increased urinary HEX levels are found in acute and chronic glomerulonephritis[29], uraemia[30], vesicoureteral reflux[31], chronic pyelonephritis[32], nephrotic syndrome[33], renal tubular damage in children with ureteropelvic junction obstruction[21], children with a SFK[22], kidney injuries and transplantations[34]. It is worth noting that HEX activity in the urine of children with early-stage diabetic nephropathy is 2-fold higher compared to healthy children[35]. Liubimova et al. found HEX to be a more sensitive marker of cytotoxic drug-induced renal tubular damage than urea or albumin/creatinine ratio[36,37]. Urinary activity of HEX may help assess therapeutic outcomes in alcoholism[38]. Increased urinary activity of HEX is also seen in tobacco smokers, which may indicate renal tubular microinjuries[39]. Furthermore, increased urinary activity of HEX is an early indicator of kidney transplant rejection[40].

Due to their high molecular weight, lysosomal enzymes are not filtrated through a properly functioning filtration membrane of renal tubules. Therefore, increased urinary activity of lysosomal exoglycosidases does not originate from blood, but is due to renal tubular damage. Under physiological conditions, trace activity of lysosomal exoglycosidases is detectable in the urine[15,16,21,22,24,25], which is due to natural exfoliation of the renal tubular epithelium or release of exoglycosidases from renal tubular cells in the urine[41].

URINARY LYSOSOMAL EXOGLYCOSIDASES IN HEALTHY CHILDREN AND ADOLESCENTS

Assessing the activity of enzymes produced in the kidneys and released in the urine is one of the non-invasive methods for renal function assessment in children and adolescents. Only enzymes that do not denature at acidic urinary pH, are excreted in the urine in minimal amounts in healthy individuals and in significantly increased amounts under pathological conditions, are of diagnostic importance[19]. Enzymes that meet the above criteria include HEX, which shows the highest activity in the proximal renal tubules[19,24,27]; its isoenzymes HEX A and HEX B, with a clear predominance of HEX A activity, reaching the highest levels in the renal medulla and cortex[16,19] as well as FUC, GAL, GLU and MAN, whose small amounts are found in the urine of healthy individuals[21,22,25]. Publications on the effects of diseases on the activity of lysosomal exoglycosidases, such as HEX, HEX A, HEX B, FUC, GAL, GLU and MAN, have provided reference levels for the obtained results[21,22,24–27]. However, studies on normal levels of HEX, HEX A, HEX B, FUC, GAL, GLU and MAN activity in the urine of children, adolescents and healthy adults are missing. Therefore, we decided to assess changes in urinary activity of HEX and its isoenzymes, i.e. HEX A and HEX B, in a group of 230 children and adolescents[42]. Having classified children into 6 age groups with 3-year time intervals, we showed statistically significant differences ($p < 0.05$) in the urinary activity of HEX and its isomers (pKat/μg Cr) between the youngest children (0.5–2.9 years) and adolescents (15.0–17.9 years). An inverse correlation was found between age and urinary activity of HEX and its isoenzymes when expressed as creatinine: HEX: $r = -0.24$, $p < 0.001$; HEX A: $r = -0.20$, $p < 0.01$ and HEX B: $r = -0.26$, $p < 0.001$[42]. The obtained findings partly correspond to those presented by Agirbasli et al.[43], who demonstrated that urinary activity of HEX in young people (18–32 years) depends on age, sex, physical exercise, and blood pressure. Reviewing the available literature, we found a study demonstrating a relationship between urine activity of HEX and the age of healthy children and adolescents[44,45]. A negative correlation between urinary activity of HEX, HEX A and HEX B (pKat/μg Cr) and age was also found in children and adolescents with SFK and hydronephrosis[21,22].

We showed[46] that urinary activity of FUC, GAL, GLU and MAN in 203 children and adolescents, as in the case of HEX and its isoenzymes, is independent of sex, but depends on age and is statistically significantly higher ($p < 0.001$) (pKat/μg Cr) in the youngest children (0.5–2.9 years) compared to adolescents (15.0–17.9 years).

Furthermore, we showed a negative correlation between the age of children and adolescents and urinary activity (pKat/μg Cr) of FUC ($r = -0.36$, $p < 0.0001$); GAL ($r = -0.36$, $p < 0.0001$); GLU ($r = -0.35$, $p < 0.0001$) and MAN ($r = -0.35$, $p < 0.0001$)[46].

Our study is the first such a large analysis on reference values for urine activity of HEX, its isoenzymes HEX A and HEX B as well as FUC, GAL, GLU and MAN in healthy

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children and adolescents[42,46]. The relationship between urinary activity of lysosomal exoglycosidases in children/adolescents and age and the percentile chart within normal values of the 3rd, 10th, 25th, 50th, 75th, 90th and 97th percentile may be of practical importance for differentiating between healthy and unhealthy children and adolescents[42,46].

URINARY LYSOSOMAL EXOGLYCOSIDASES IN CHILDREN AND ADOLESCENTS WITH ARTERIAL HYPERTENSION

A new concept of the pathogenesis of hypertension has been developed in recent years. It was initially believed that intravascular inflammation results from increased blood pressure; however, recent studies point to the important role of chronic inflammation in the pathogenesis of arterial hypertension. Inflammatory processes are associated with increased levels (in the tissues and bodily fluids) of multiple proteins, including adhesion molecules, cytokines, acute phase proteins[47] as well as lysosomal enzymes, including lysosomal exoglycosidases, such as HEX, its isoenzymes HEX A and HEX B, FUC, GAL, GLU and MAN[13–16,25,27,48].

Childhood-onset hypertension is now commonly observed. This seems to support the hypothesis that changes in the activity of lysosomal exoglycosidases may already occur at an early age, providing evidence for prehypertension. Glycoconjugates (glycoproteins, glycolipids, proteoglycans) are known to play an important role in the structure and function of the endothelium, smooth muscles and extracellular matrix[21]; however, knowledge on the role of lysosomal exoglycosidases in arterial endothelium and walls is still limited[49,50]. Hermelin et al.[49] pointed to significantly reduced activity of HEX and GLU in the lysosomal fraction of the intima–media complex in old vs. young rats, which indicates age-related reduction in glycoconjugate metabolic rate in the internal arterial membranes. Markle[50], found that the increase in the activity of HEX, GAL, GLU and MAN in rat aortic tissue depended on rat size and age. Data on the relationships between urinary activity of lysosomal exoglycosidases and blood pressure in children and adolescents are missing in the literature. Therefore, we conducted a study in 176 children and adolescents less than 18 years of age to assess urinary activity of lysosomal exoglycosidases: HEX, its isoenzymes HEX A and HEX B, FUC, GAL, GLU and MAN in relation to age and systolic/diastolic blood pressure[51]. We showed a correlation between urinary activity of lysosomal exoglycosidases and normal/increased blood pressure in healthy children and adolescents. There were differences in systolic and diastolic blood pressure between children and adolescents with normal vs. increased blood pressure ($p < 0.001$). Body weight and body mass index (BMI) were also significantly lower in the group of healthy children and adolescents with normal blood pressure ($p < 0.05$). Excess body weight is considered to be correlated with increased blood pressure, causing glomerular hyperfiltration and kidney damage. Girişgen et al.[52] found no significant correlation between urinary activity of lysosomal exoglycosidases and BMI. Findings presented by other authors are consistent with the results of our work[51]. We found a significant, positive correlation between urinary activity (pKat/mL) of HEX ($r = 0.17$, $p < 0.05$) and its isoenzyme HEX A ($r = 0.21$, $p < 0.05$) and systolic blood pressure in all children and adolescents included in the study. We also found statistically significant negative correlations between urinary activity (pKat/μg Cr) of FUC ($r = -0.16$, $p < 0.05$) and GAL ($r = -0.17$, $p < 0.05$) and systolic blood pressure as well as a negative correlation between urinary activity (pKat/μg Cr) of GAL ($r = -0.16$, $p < 0.05$) and diastolic blood pressure. We additionally observed a tendency towards a correlation (on the border of statistical significance) between the activity of GLU (pKat/mL) and MAN (pKat/μg Cr) and systolic blood pressure[51]. Of all evaluated lysosomal exoglycosidases, only HEX A urinary activity (pKat/mL) showed significant differences between healthy children and adolescents with normal blood pressure and children/adolescents with increased blood pressure ($p < 0.05$)[51]. Although the relationship between HEX and arterial hypertension was demonstrated in clinical trials in adults, the obtained results are still unsatisfactory. A study in a group of 84 patients with uncomplicated essential hypertension showed increased urine activity of HEX in patients with hypertension compared to those with normal blood pressure[53]. Alderman et al.[54] showed that the assessment of urinary activity of lysosomal exoglycosidases may be a useful marker of renal damage in hypertensive patients. Another study[55] suggested that urinary activity of HEX may be an independent marker for the diagnosis of early kidney failure in hypertensive patients. Comparing the results of urinary activity of lysosomal exoglycosidases between boys and girls, we found a significant, positive correlation between HEX A activity (pKat/mL and pKat/μg Cr) and systolic blood pressure in boys as well as a significant positive correlation between the activity of HEX, HEX B, FUC, GAL, GLU and MAN (pKat/mL) and systolic blood pressure in girls ($p < 0.05$)[51]. In their study in patients with hypertension, Schmieder et al.[53] showed that high serum activity of HEX was associated with increased systolic blood pressure. The authors found a significant negative correlation between urinary activity of FUC and GAL (pKat/μg Cr) and systolic blood pressure ($p < 0.05$) as well as between the activity of HEX, HEX A, FUC, GAL, GLU and MAN (pKat/μg Cr) and diastolic blood pressure in children and adolescents with normal blood pressure ($p < 0.05$)[51]. De Muro et al.[56] found a significant relationship between HEX activity and hypertension

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in diabetic patients. There are also publications whose authors found no differences in urine or serum activity of HEX in patients with mild hypertension and juvenile borderline hypertension compared to controls[57,58]. The discrepancies in the published research indicate the need for further studies on the urinary and serum activity of lysosomal exoglycosidases in children and adolescents with mild hypertension and juvenile borderline hypertension. To the best of our knowledge, our study[51] was the first attempt to assess correlations between urinary activity of lysosomal exoglycosidases, i.e. HEX, HEX A, HEX B, FUC, GAL, GLU and MAN, and blood pressure in normotensive and hypertensive children. Our observations[51] suggest that HEX A (pKat/mL) may be considered a useful tool for identifying children and adolescents with increased arterial blood pressure. This is supported by the ROC (receiver operating characteristic) analysis, which showed a relatively good diagnostic value of urine HEX A activity expressed as pKat/mL (area under curve, AUC = 0.616), with a sensitivity and specificity of 51.2% and 71.8%, respectively, as well as a lower diagnostic value when expressed as creatinine (pKat/μg Cr), with AUC = 0.589, sensitivity of 31.7%, and specificity of 86.3%. It should be emphasised that urinary activity of HEX and HEX A expressed as pKat/mL is positively correlated with systolic blood pressure[51].

URINARY LYSOSOMAL EXOGLYCOSIDASES AS A MARKER OF RENAL TUBULAR IMPAIRMENT IN CHILDREN WITH URETEROPELVIC JUNCTION OBSTRUCTION

Recent decades have witnessed significant advances in the knowledge on the pathophysiology of ureteropelvic junction obstruction based on animal studies[59,60]. Regardless of the underlying causes, ureteropelvic junction (UPJ) obstruction leads to limited or blocked urinary outflow from the kidney. Initially, the renal pelvis is able to overcome the blockade by increasing the strength of contractions, which is reflected by significantly increased pressure in the pelvicalyceal system. However, persistent obstruction causes pelvic decompensation, leading to hydronephrosis and progressive renal damage. This results in multiple cellular changes, such as apoptosis, mesenchymal/myocytic transformation, which is manifested in progressive glomerular and tubular impairment and damage[61]. These processes are associated with the release of various multifactorial biochemical markers, of which the well-known N-acetyl-β-D-hexosaminidase is particularly interesting.

Knowledge from animal studies allowed for the discovery of new, potentially diagnostically and prognostically useful biomarkers that can be measured in the urine, none of which has been fully implemented in clinical practice[62]. Research is continued to find appropriately sensitive and, at the same time, non-invasive enzymatic biomarkers for the assessment of tubular function[19]. The majority of renal pathologies involve glomeruli, proximal and distal tubules as well as vascular endothelium. Increased activity of lysosomal exoglycosidases suggests cellular damage, but may also reflect enhanced lysosomal activity without cellular disorders[63]. According to our knowledge, there are several studies investigating the role of HEX in children with unilateral UPJ obstruction. However, none of these papers attempted to assess the urine activity profile of lysosomal exoglycosidases as biomarkers of UPJ obstruction. We found that the urinary activity of lysosomal exoglycosidases was significantly higher in children with UPJ obstruction compared to heathy children[21]. The conducted research confirmed the diagnostic importance of determining urinary activity of HEX as a marker of renal tubular damage in polycystic kidney disease and diabetes[64,65]. Skalova et al.[66] found significantly increased urinary activity of HEX in children with diabetic hydronephrosis vs. controls. Another study found that urine activity of HEX, HEX B in particular, may be a specific marker of proximal tubular damage after transplantation[67]. Taha et al.[68] found that urinary activity of HEX may be a non-invasive monitoring tool for children diagnosed with UPJ obstruction. Our study[21] showed that the highest urinary activity of lysosomal exoglycosidases, expressed as pKat/μg Cr, is found before surgery (excision of obstructed UPJ and reattaching the ureter to the renal pelvis, creating a wide junction between the two), and significantly drops afterwards. Although the activity of all lysosomal exoglycosidases, expressed as pKat/μg Cr, dropped after surgical treatment, it was still significantly higher compared to controls[21], which could result from diluted urine due to the functioning of both kidneys and the ongoing kidney regeneration. The assessment of urinary activity of HEX as an enzyme determining the quality of renal tubules proved useful for health status assessment in conservatively treated children with UPJ obstruction; it was found in this group that HEX may be a biomarker allowing for determining the risk of UPJ obstruction compared to the control group[69]. Our study showed a negative correlation between the activity of lysosomal exoglycosidases, expressed as pKat/μg Cr, and age and serum creatinine levels. It is worth noting that the activity of all investigated lysosomal exoglycosidases (expressed as pKat/μg Cr), except for HEX A, positively correlated with the urinary albumin/creatinine ratio[21]. Rustom et al.[70] found a strong correlation between proteinuria and HEX activity in patients with glomerulonephritis, hypertensive nephropathy, and chronic pyelonephritis. Another study found a positive correlation between transforming growth factor β1 (TGF-β1) and proteinuria in congestive nephropathy[71]. The lack of correlation between urinary activity of lysosomal exoglycosidases and glomerular filtration

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rate in our study may be explained by the fact that all children had normal renal function. Interestingly, no correlation was found between urinary activity of lysosomal exoglycosidases and scintigraphically evaluated renal function. This suggest the existence of factors other than the underlying disease, which may affect the activity of lysosomal exoglycosidases. Lysosomal exoglycosidases may be used as a panel for the diagnosis of UPJ obstruction, as confirmed by the ROC analysis, which demonstrated good diagnostic value of lysosomal exoglycosidases in identifying hydronephrosis in all evaluated children (AUC > 0.8). Furthermore, our results suggest a relatively good diagnostic value of urinary activity of HEX B, FUC, GAL, GLU and MAN in children presenting with early signs of renal damage due to UPJ obstruction (AUC > 0.7)[21]. The obtained findings indicate unusually high urinary activity of lysosomal exoglycosidases in patients with scintigraphically confirmed UPJ obstruction, which may suggest altered renal function in these patients.

URINARY LYSOSOMAL EXOGLYCOSIDASES AS A MARKER OF RENAL TUBULAR DAMAGE IN CHILDREN AND ADOLESCENTS WITH CONGENITAL OR ACQUIRED SOLITARY FUNCTIONING KIDNEY

Literature data indicate that there are usually no changes in the glomerular filtration rate at an early stage of chronic kidney disease. Renal function deteriorates with disease progression and hyperfiltration may occur in patients with normal glomerular filtration[72]. In our study[22], hyperfiltration was found in 2 patients only. However, it should be noted that the age of study participants ranged between 2 months and 18 years, with 30 out of 52 children presenting with albuminuria. The urinary albumin/creatinine ratio was significantly higher in the group of children with a SFK compared to children with two normally functioning kidneys. These findings indicate that children with a solitary kidney present with tubular damage, as confirmed by the increased activity of all investigated urinary lysosomal enzymes. According to Thomson et al.[73], proximal tubular hypertrophy may be due to activated renin–angiotensin–aldosterone system and glomerular hyperfiltration, since there is a relationship between glomerular hyperfiltration and kidney size in patients with SFK. Increased angiotensin II levels may cause glomerular hyperfiltration, inflammation as well as renal tissue damage and fibrosis. Long-term consequences of increased angiotensin II levels include proteinuria, arterial hypertension and reduced glomerular filtration rate. It is assumed that albuminuria is a predominantly haemodynamic effect of glomerular hypertension and hyperfiltration[74]. A large proportion of patients with congenital SFK develop albuminuria, which may be interpreted as a sign of hyperfiltration. Patients with congenital SFK and the resulting increased albumin excretion in the urine in the other kidney are at an increased risk of renal failure. Proteinuria may play a pathogenic role, contributing to tubulointerstitial damage. There are various theories on the potential proteinuria-related mechanisms underlying damage of renal tubular cells. Increased protein reabsorption in proximal renal tubules may lead to their damage and cell apoptosis due to exhaustion of the lysosomal degradation pathway and spillage of lysosomal enzymes into the cytoplasm. Only a few years ago it was assumed that measuring the activity of HEX and its isoenzymes may be useful in the diagnosis of Tay–Sachs disease[75]. Recently published studies have shown that HEX and its isoenzyme HEX B may be cellular damage markers[76] and can be used for identifying renal damage[63]. Our results[22] confirm the hypothesis that renal tubular damage can be an important cause of single kidney damage. It should be noted that there is a very strong correlation between the albumin/creatinine ratio and urinary activity of lysosomal exoglycosidases expressed as pKat/μg Cr. Patients with tubular proteinuria in the acute stage of kidney disease show significantly increased urinary activity of HEX as opposed to patients with chronic tubular dysfunction[77]. Our findings[22] suggest that urinary activity of HEX and its isoenzymes A and B may be a useful marker of renal damage in patients with SFK. This hypothesis needs to be confirmed in further studies as increased urinary activity of lysosomal exoglycosidases may result not only from proximal tubular damage, but also from the increased activity of exoglycosidases in intact lysosomes[78]. The other investigated markers, i.e. α-fucosidase (FUC), β-galactosidase (GAL), β-glucuronidase (GLU) and α-mannosidase (MAN), play a key role in detecting carcinogenesis[15,16,24,27]. The obtained results indicate higher urinary activity of FUC, GAL, GLU and MAN in children with congenital and acquired SFK compared to healthy children with two normally functioning kidneys[22]. Interestingly, we additionally found a negative correlation between urinary activity of lysosomal exoglycosidases (pKat/μg Cr) in patients with congenital SFK and their age. This in line with the findings presented by Wikstad et al.[79] and Baudoin et al.[80], who found this correlation in adults with SFK. The measurement of urinary activity of lysosomal exoglycosidases is particularly useful for monitoring chronic diseases, especially since urine may be obtained non-invasively. It may be concluded, based on the published observations, that urinary activity of lysosomal exoglycosidases expressed as creatinine may be considered as a potentially useful, non-invasive marker of renal damage in patients with SFK. The above thesis is confirmed by our ROC analysis, which demonstrated good diagnostic value of all measured lysosomal exoglycosidases in SFK children with known albuminuria.

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Our studies showed that increased urinary activity of lysosomal exoglycosidases precedes clinical symptoms of kidney disease and is a more sensitive diagnostic marker compared to albuminuria[22].

CONCLUSIONS

Lysosomal exoglycosidases, such as N-acetyl-β-D-hexosaminidase (HEX), its isoenzymes A (HEX A) and B (HEX B), α-fucosidase (FUC), β-galactosidase (GAL), β-glucuronidase (GLU) and α-mannosidase (MAN), are lysosomal enzymes involved in the catabolism of oligosaccharide chains of glycoconjugates: glycoproteins, proteoglycans and glycolipids. Due to their stability in acidic environment, lysosomal exoglycosidases may be used as affordable and easily measurable diagnostic markers of kidney function. Usually, only small amounts of lysosomal exoglycosidases are found in the urine of healthy children, adolescents and adults. Urinary activity of lysosomal exoglycosidases depends on the severity of renal damage. Their increased activity is found in children and adolescents with UPJ obstruction and congenital/acquired SFK. Increased activity of lysosomal exoglycosidases is likely to be involved in the processes associated with the development of hypertension. This paper may be closed with a conclusion that the urinary profile of lysosomal exoglycosidases may be a sensitive biomarker for the diagnosis of kidney damage.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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