Department of Paediatrics
Faculty of Medicine in Hradec Králové
Charles University in Prague
Czech Republic

THE DIAGNOSTIC ROLE OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE AS A MARKER OF RENAL TUBULAR IMPAIRMENT IN CHILDREN

Sylva Skálová, M.D.

Ph.D. Thesis

Hradec Králové

May 2007

Lékařská fakulta Univerzity Karlovy Hradec Králové
Dětská klinika Lékařské fakulty a Fakultní nemocnice
Česká republika

DIAGNOSTICKÝ VÝZNAM N-ACETYL-BETA-D-GLUCOSAMINIDÁZY V MOČI JAKO UKAZATELE TUBULÁRNÍHO POŠKOZENÍ V DĚTSKÉM VĚKU

MUDr. Sylva Skálová

Disertační práce
v doktorském studijním programu pediatrie

Hradec Králové

Květen 2007

CONTENTS

List of abbreviations

ADH	antidiuretic hormone
ADHR	autosomal-dominant hypophosphatemic rickets
A1M	alpha-1-microglobulin
ANCA	antineutrophil cytoplasmic antibodies
ANF	atrial natriuretic factor
AQP-2	aquaporin 2
ATP	adenosine triphosphate
B2M	beta-2- microglobulin
BMA	bone mineral area
BMC	bone mineral content
BMD	bone mineral density
BMDvol	volumetric bone mineral density
BMI	body mass index
Ca	calcium
CaSR	calcium-sensing receptor
Cl	chloride
CLCKNB	chloride channel
CLCN5	ClC-5 chloride channel
CST3	human cystatin C
cAMP	cyclic adenosine monophosphate
CMV	cytomegalovirus
Da	dalton
DCT	distal convoluted tubule
dDAVP	1-deamino-8-D-arginine vasopressin
ECF	extracellular fluid
ENaC	epithelial sodium channel
FECa	fractional excretion of calcium
FEK	fractional excretion of potassium
FENa	fractional excretion of sodium
$FENaHCO_3$	fractional excretion of bicarbonate
FEP	fractional excretion of phosphate
FGF-23	fibroblast growth factor 23
FRP-4	frizzled related protein 4
GBM	glomerular basement membrane
GFR	glomerular filtration rate
HCO^{3-}	bicarbonate
H^+	hydrogen ion
HN	hydronephrosis
IH	idiopathic hypercalciuria
K	potassium
MAG3	^{99m}Tc mercaptoacetyltriglycine
MEPE	matrix extracellular phosphoglycoprotein
mEq	miliequivalent
Mg	magnesium
mmol	milimole
mOsm	miliosmole
NAG	N-acetyl-beta-D-glucosaminidase
Na	sodium
NCCT	sodium-potassium 2 chloride contransporter

NDI	nephrogenic diabetes insipidus
NE	nocturnal enuresis
NKCC2	loop diuretic-sensitive $Na^+/K^+/2Cl^-$ cotransporter
Npt	sodium gradient-dependent phosphate transporter
NSAIDs	nonsteroidal anti-inflammatory drugs
P	phosphorus
P-Ca	plasma calcium concentration
P-Cr	plasma creatinine concentration
P-K	plasma potassium concentration
P-Na	plasma sodium concentration
P-P	plasma phosphate concentration
PHEX	phosphate-regulating gene with homologies to endopeptidases on the X chromosome
PO_4	phosphate
PTH	parathyroid hormone
RAA	renin-angiotensin-aldosterone axis
RN	reflux nephropathy
ROMK1	rectifying K^+ channel
RTA	renal tubular acidosis
S-Ca	serum calcium concentration
S-Cr	serum creatinine concentration
S-K	serum potassium concentration
S-Na	serum sodium concentraton
S-P	serum phosphorus concentration
SDS	standard deviation score
SFU	Society for Fetal Urology
TmP/GFR	tubular maximum for phosphate corrected for GFR
TSC	thiazide-sensitive Na/Cl co-transporter
TTKG	transtubular gradient of potassium
U-Ca	urine calcium concentration
U-Ca/24h	24-hour urinary calcium excretion
U-Ca/U-Cr	urinary calcium/creatinine ratio
U-Cr	urine creatinine concentration
U-K	urine potassium concentration
U-Na	urine sodium concentration
$U\text{-}NaHCO_3$	urine bicarbonate concentration
U-NAG	urinary N-acetyl-beta-D-glucosaminidase activity
U-NAG/Cr	urinary N-acetyl-beta-D-glucosaminidase activity/creatinine ratio
U-P	urine phosphorus concentration
UTI	urinary tract infection
VPR2	vasopressin V2 receptor
VUR	vesicoureteral reflux
XLH	X-linked hypophosphatemic rickets
$1,25(OH)_2D3$	calcitriol

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION

1.1. Anatomy and physiology of kidney

The kidneys are a pair of bean-shaped organs located below the ribs near the middle of the back. The kidneys lie in the retroperitoneal space slightly above the level of umbilicus. They range in length and weight, respectively, from approximately 6 cm and 24 g in a full-term newborn to 12 cm or more and 150 g in an adult (2,26,34). The kidneys are protected by three layers of connective tissue: the renal fascia (fibrous membrane) surrounds the kidney and binds the organ to the abdominal wall; the adipose capsule (layer of fat) cushions the kidney; and the renal capsule (fibrous sac) surrounds the kidney and protects it from trauma and infection. The kidney has an outer layer, the cortex, which contains the glomeruli, proximal and distal convoluted tubules, and collecting ducts; and an inner layer, the medulla, which contains the strait portions of the tubules, the loops of Henle, the vasa recta, and the terminal collecting ducts (Fig. 1). The kidneys regulate the volume and concentration of fluids in the body by producing urine. Urine is produced in a process called glomerular filtration, which is the removal of waste products, minerals, and water from the blood. The kidneys maintain the volume and concentration of urine by filtering waste products and reabsorbing certain substances and water from the blood (2, 26,34).

The major functions of the kidney are:

- to maintain body fluid and electrolyte homeostasis
- to remove waste products of metabolism
- to provide endocrine functions (vitamin D activation, erythropoetin production)
- to perform metabolic functions (gluconeogenesis)
- to regulate regional blood flow (via angiotensin II)
- to regulate blood pressure and electrolyte balance (via renin-angiotensin axis).

Renal blood flow represents about 20% of cardiac output. The majority of this 20% (approximately 90%) is distributed to the renal corte (22). The blood supply to each kidney usually consists of a main renal artery, that arises from the aorta. The renal artery enters the kidney and the renal vein emerges from the kidney at an indentation in the middle of the organ called the hilum. The renal artery supplies oxygen and blood to the kidney. Blood flows from the kidney through the renal vein after waste products have been removed. The main renal artery divides into segmental branches within the medulla and these into interlobar arteries that pass through the medulla to the junction of the cortex and medulla. At this point, the interlobar arteries branch to form the arcuate arteries, which run parallel to the surface of the kidney. Interlobular arteries originate from the arcuate arteries and give rise to the afferent arterioles of the glomeruli (Fig. 1,2). Specialized muscle cells in the wall of the afferent

arteriole and the macula densa within the distal tubule next to the glomerulus form the juxtaglomerular apparatus that controls the secretion of renin. The afferent arteriole (vas afferens) divides into the glomerullar capillary network which then merges into the efferent arteriole (vas efferens). The efferent arterioles of glomeruli next to the medulla (juxtamedullary glomeruli) are larger than those in the outer cortex and provide blood supply (vasa recta) to the tubules and medulla (13,22).

The internal redistribution of blood flow within the kidney as well as the overall renal blood flow itself is affected by the intrarenal production of prostaglandins. The autoregulation of regional blood flow is influenced by angiotensin II. Atrial natriuretic peptides play an important role as vasodilators and natriuretic agents (22).

The major homeostatic functions of the kidney are carried out by the processes of glomerular ultrafiltration, tubular reabsorption, and secretion (32).

The formation of urine occurs in the basic units of the kidney, called nephrons. Each human kidney contains over 1 million nephrons. Nephrons consist of a network of capillaries (glomerulus), a renal tubule, and a membrane that surrounds the glomerulus and functions as a filter (Bowman's capsule) (Fig. 1,2). In humans, formation of nephrons is completed at birth, but functional maturation with tubular growth and elongation continues during the first decade of life (13). The glomeruli are where urine production begins. The blood runs through afferent arteriole (vas afferens) into the glomerular capillary network in the glomerulus, where the process of filtration occurs. Afterwards, the blood is driven away from the glomerulus via efferent arteriole (vas efferens) (35) (Fig. 2). The glomerular network of specialized capillaries serves as the filtering mechanism of the kidney (13). The glomerular capillaries are lined by endothelial cells having very thin cytoplasm that contains many holes (fenestrations). The glomerular basement membrane (GBM) forms a continuous layer between the endothelial and mesangial cells on one side and the epithelial cells on the other. The membrane has three layers: a central electron-dense lamina densa; the lamina rara interna, which lies between the lamina densa and the endothelial cells; and the lamina rara externa, which lies between the lamina densa and the epithelial cells. Bowman capsule, which surrounds the glomerulus, is composed of a basement membrane, which is continuous with the basement membranes of the glomerular capillaries and the proximal tubules; and the parietal epithelial cells, which are continuous with the visceral epithelial cells (13). As the blood passes through the glomerular capillaries, the plasma is filtered through the glomerular capillary walls. The ultrafiltrate, which is cell-free, contains all the substances in the plasma (electrolytes, glucose, phosphate, urea, creatinine, peptides, low molecular weight proteins) with the exception of proteins having a molecular weight of 68,000 or more (i.e. albumin and the globulins). The filtrate is collected in Bowman space and enters the tubules, where its composition is modified by solute and fluid secretion and absorbtion in accordance with tightly regulated homeostatic mechanisms until it leaves the kidney as urine. Urine formation therefore occurs in the renal tubules, which travel from the outer tissue of the kidney (the cortex), to the inner tissue (the medulla), and return

to the cortex. The renal tubular system consists of proximal tubule, the loop of Henle, distal tubules, and collecting ducts. Extensions of the cortex project into the medulla and divide the tissue into renal pyramids. The renal pyramids extend into funnel-like extensions (calyces), where the collection of urine occurs. Minor calyces merge to form major calyces and major calyces merge to form the renal pelvis, the upper portion of the ureter. Each section of the renal tubule performs a different function (Fig. 3). As the tube leads away from Bowman's capsule into the cortex, it forms the proximal convoluted (highly coiled) tubule. In this section, waste products and toxic substances (e.g., ammonia, nicotine) are forced out of the blood through a permeable membrane, while other substances (e.g., glucose, amino acids, vitamins, minerals) are reabsorbed. Urine then travels through the loop of Henle, a long U-shaped extension of the proximal convoluted tubule. It consists of a descending limb and an ascending limb (Fig. 2) (13, 34, 35). Some sections of the loop are permeable to water and impermeable to substances in the urine (e.g., salt, ammonia), and some sections are impermeable to water and permeable to other substances. The next section is the distal convoluted tubule. Normally, this section is water permeable. Substances, that remain in the urine are reabsorbed, increasing the concentration of the urine. After passing through the distal convoluted tubule, the urine consists almost entirely of waste products. Most of the water and other useful substances have been reabsorbed. Next, urine enters the collecting tubule. Urine from several nephrons empties into each collecting tubule. These tubules form the calyces, and the calyces form the renal pelvis (upper portion of the ureter). Urine travels from the kidneys through the ureters to the bladder, where it is stored until it is eliminated from the body through the urethra. The hypothalamus detects the level of substances in the blood and controls the secretion of hormones. Antidiuretic hormone, aldosterone, and atrial natriuretic factor (ANF) are hormones that change the permeability of the distal convoluted tubule and the collecting tubule, regulating urine volume and helping to maintain blood pressure. For example, when water content in the blood is low, the secretion of antidiuretic hormone (ADH) increases and the kidneys reabsorb more water. This increases the concentration of the urine and decreases urine output. When water content in the blood is high, ADH production ceases and the kidneys reabsorb less water. This decreases the concentration of the urine and increases urine output (13, 32, 34, 35).

1.2. Anatomy and physiology of renal tubules

As mentioned before, the renal tubular system consists of proximal tubule, the loop of Henle, distal tubules and collecting ducts. Each section of the renal tubule performs a different function.

The most distinctive characteristic of the proximal tubule is its "brush (striated) border". The luminal surface of the epithelial cells of this segment of the nephron is covered with densely packed microvilli forming a border readily visible under the light microscope. The microvilli greatly increase the luminal surface area of the cells, presumably facilitating their resorptive function. The cytoplasm of the cells is densely packed with mitochondria in keeping with the energetic requirements of the cells resorptive

activity. Agonal resorption of the contents of the proximal tubular contents after interruption of circulation in the capillaries surrounding the tubule often leads to disturbance of the cellular morphology of the proximal tubule cells, including the ejection of cell nuclei into the tubule lumen. This has led some observers to describe the lumen of proximal tubules as "dirty looking", and to contrast this with the "clean" appearance of distal tubules, which have quite different properties (36). The proximal tubule as a part of the nephron can be divided into an initial convoluted portion, and a following straight (descending) portion. Differences in cell outlines exist between these segments, and therefore presumably in function too. Some investigators on the basis of particular functional differences have divided the convoluted part into two segments designated S1 and S2. As a logical extension of this nomenclature they have designated the straight segment as S3. In relation to the morphology of the kidney as a whole the convoluted segments of the proximal tubules are confined entirely to the cortex. Straight segments descend into the outer medulla. They terminate at a remarkably uniform level and it is their line of termination that establishes the boundary between the inner and outer stripes of the outer zone of the renal medulla (32,34-36).

The proximal tubule is characterized by iso-osmotic reabsorption of the glomerular ultrafiltrate (22). Under physiologic conditions, two thirds of the glomerular ultrafiltrate is reabsorbed from the proximal tubule; a number of solutes, such as glucose and aminoacids, are completely reabsorbed, and potassium is nearly completely reabsorbed. Most phosphate is also reabsorbed from the proximal tubule, and calcium is reabsorbed in parallel with sodium reabsorption. The straight portion of the proximal tubule is responsible for secreting organic acids, including drugs such as penicillin (34-36).

The loop of Henle (sometimes known as the nephron loop) is a U-shaped tube that consists of a descending limb and ascending limb. It begins in the cortex, receiving filtrate from the proximal convoluted tubule, extends into the medulla, and then returns to the cortex to empty into the distal convoluted tubule. Its primary role is to concentrate the salt in the interstitium, the tissue surrounding the loop (34-36). The loop of Henle serves a major role, as 25% of filtered sodium chloride is absorbed in this segment. Differential permeabilities change the isotonic fluid entering the loop of Henle from the proximal tubule into a hypotonic fluid delivered to the distal tubule (Fig. 4). Preferential sodium chloride absorption is the principal mechanism by which the countercurrent multiplier is activated, and by which the medullary interstitial hypertonicity, required for urinary concentration, is accomplished. This principally occurs in the thick ascending limb. Salt and water movement across the thin limbs is primarily driven by osmotic gradients (Fig. 4).

The distal tubule is made up of the distal convoluted tubule, which is not permeable for water and continues to carry out the dilution of luminal fluid by way of active sodium chloride absorption, and the collecting ducts, which are the primary sites of antidiuretic hormone activity. The distal convoluted tubule is similar to the proximal convoluted tubule in structure and function (22, 34-36). These distal

segments are the site of potassium secretion, which is virtually all the potassium excreted within the nephron system. Hydrogen ion secretion, which is responsible for the final acidification of the urine, also occurs at the distal convolution. The processes of sodium reabsorption and potassium and hydrogen ion secretion are all stimulated by aldosterone (22).

1.2.1. Renal handling of sodium and chloride

Sodium (Na) and its associated anions (mostly chloride and bicarbonate) are confined to the extracellular fluid (ECF) compartment and are the principle determinants of ECF osmolality. Because water moves freely across cell membranes, and because the osmolality of ECF is kept constant, it follows that the volume of ECF is directly related to the total body content of Na. Sodium is therefore essential in maintaining extracellular fluid balance, and, thus, volume status. The kidney is capable of effecting large changes in sodium excretion in a variety of normal and pathologic states (22,24). There are four main sites of sodium transport. Approximately 60% of sodium is absorbed in the proximal tubule by coupled transport with glucose or amino acids, 25% in the ascending loop of Henle (bumetanide-sensitive sodium-potassium 2 chloride contransporter, NCCT) and collecting tubule (epithelial sodium channel, ENaC) (22, 24, 34-36).

The reabsorption of Na ion takes place against electrical and chemical (concentration) gradients and requires expenditure of metabolic energy. Such a process is described as the active transport. The energy for the bulk of Na reabsorption derives from aerobic metabolism. There is a direct, linear relationship between the rate of Na reabsorption and oxygen consumption by the kidney (19). The exact mechanisms of Na transport throughout the nephron continue to be investigated. In the proximal tubule, Na in the tubular lumen travels down its concentration gradient, across the luminal (apical) membrane, into the tubular epithelial cell. Within the cell, the Na concentration is kept low by pumps in the basal and lateral membranes, that extrude Na into the peritubular space, from which it can enter the peritubular capillaries. These pumps, involving the enzyme Na,K-ATPase, represent the "active" (energy-consuming) component of Na transport. For the most part, Cl reabsorption follows Na as a consequence of the negative luminal potential created by outward Na movement (19).

Water is reabsorbed "passively", by moving down a gradient of osmolality between tubular fluid (lower) and peritubular (higher). This gradient is established by the reabsorption of Na and its attendant anions. Because the water permeability of the proximal tubule is high, only a small osmotic gradient is required to effect water movement. Similar Na reabsorptive processes probably operate in the distal tubule and collecting ducts. Unlike these other segments, however, the thick ascending limb of the loop of Henle has a positive luminal potential. Among the mechanisms proposed to account for this is active transport of Cl with secondary, passive Na reabsorption (19).

Under normal circumstances, the urinary excretion of sodium approximates the sodium intake (80-250 mmol/24 hours for an adult) minus 1-2 mmol/kg/24 hours required for normal metabolic processes. However, in states of volume depletion (dehydration, blood loss) or decreased effective circulating

blood volume (septic shock, hypalbuminemia, congestive heart failure), there may be a dramatic decrease in urinary sodium excretion to as low as 1 mmol/L. Changes in volume status are detected by baroreceptors in the atria, afferent arteriole, and the carotid sinus and by the macula densa, which detects changes in chloride delivery (24). The major hormonal mechanisms mediating sodium balance include the renin-angiotensin-aldosterone (RAA) axis, ANF, and norepinephrine. Angiotensin II and aldosterone increase sodium reabsorption in the proximal tubule and distal tubules, respectively. Norepinephrine, released in response to volume depletion, does not directly act on tubular transport mechanisms but impacts on sodium balance by decreasing renal blood flow and thus decreasing the filtered load of sodium, as well as stimulating renin release. With more severe volume depletion antidiuretic hormone ADH is also released. Sodium excretion is promoted by ANF and suppression of renin (24). Recent observations concerning aquaporins shed further light on the renal handling of water and sodium. Aquaporins are proteins that mediate transmembrane water transport in a variety of tissues, including the kidney. In response to increased serum osmolarity (as detected by osmoreceptors in the hypothalamus) and/or severe volume depletion, ADH is released into the systemic circulation. ADH then binds to its receptor, vasopressin V2 (AVPR2), on the basolateral membrane of the collecting tubule cell. Binding of ADH to its receptor activates a cyclic adenosine monophosphate (cAMP)-dependent cascade that results in movement of preformed water channels, aquaporins 2 (AQP-2) to the luminal membrane of the collecting duct, rendering it permeable to water (12, 22, 33). The synthesis of AQP-2 in kidney collecting duct principal cells is therefore stimulated by ADH, and low levels of AQP-2 were observed in association with extreme polyuria (12,28).

1.2.2. Renal handling of potassium

Extracellular potassium homeostasis is very tightly regulated, becuase small changes in plasma potassium concentrations have dramatic effects on cardiac, neural, and neuromuscular function (22). Over 90 % of plasma K undergoes glomerular filtration. Essentially all filtered potassium is fully reabsorbed in the proximal tubule and the loop of Henle. The bulk of K in the final urine is added to tubular fluid by secretion in the late distal tubule and the cortical collecting duct. Tubular epithelial cells in these segments take up K from peritubular fluid by a mechanism involving Na,K-ATPase. This gives rise to an intracellular transport pool of K. Potassium secretion is favored by the negative intratubular potential created by distal Na reabsorption, and by the concentration gradient between intracellular K and tubular fluid. In addition to these passive forces that influence K secretion, an active transport mechanism may exist. There is no evidence either for a coupled exchange between Na^+ absorption and K^+ secretion, or for competition between intracellular K^+ and H^+ for tubular secretory pathways (19). Therefore, urinary excretion of potassium is completely dependent on tubular secretion by potassium channels present in the principal cells of the collecting tubule (13, 22).

Potassium excretion is augmented by an increase in distal tubular fluid flow rate (as with saline or

osmotic diuresis, diuretic drugs, or postobstructive diuresis). This promotes K secretion by maintaining a steep K concentration gradient between the cell and the tubular fluid. In addition, increased quantities of Na are presented to distal reabsorptive sites. The negative intratubular potential created by increased Na reabsorption also promotes K secretion (19).

Factors that promote potassium secretion include aldosterone, increased sodium delivery to the distal nephron, and increased urine flow rate (13).

Mineralocorticoids stimulate K secretion, possibly by stimulating Na,K-ATPase in the basolateral membrane. This would increase K uptake and raise intracellular K concentration, thereby enhancing K secretion. Aldosterone is known to act on the distal nephron segments to enhance the reabsorption of sodium and excretion of potassium. Occupancy of the high affinity cytosol receptors appears to directly correlate with both sodium and potassium transport. Therefore, the greater the plasma aldosterone concentration the greater the reabsorption of sodium and excretion of potassium. The major site of action of aldosterone appears to be the cortical and medullary collecting tubule. This has been worked out by both perfusion studies of isolated rabbit tubules, as well as micropuncture studies on rat tubules. Aldosterone is known to induce certain proteins such as Na-K-ATPase and citrate synthetase. The increase in Na-K-ATPase appears to be secondary to stimulation of luminal sodium reabsorption since it is blocked by amiloride. In contrast, spironolactone not amiloride blocks induction of citrate synthetase (19).

1.2.3. Renal handling of calcium

Calcium intake and skeletal calcium requirements vary widely from day to day and across various stages of life cycle. Therefore, the homeostatic system is constantly adjusting to deliver sufficient calcium, magnesium and phosphate from intestine and kidney into the extracellular fluid and blood and then to bone to meet changing skeletal growth requirements without disturbing the serum ionized calcium (Ca^{2+}) concentration. The serum Ca^{2+} fraction controls cellular biological functions, and therefore the homeostatic system maintains serum Ca^{2+} at the expense of bone mineral content. Serum Ca^{2+} may increase from calcium influx from the intestinal absorption or bone resorption and decrease with Ca efflux into bone mineralization sites, secretion into the intestinal lumen, or filtration at the renal glomerulus and secretion along selected segments of the nephron. The serum changes in $Ca^{2+}$are being regulated by the parathyroid hormone (PTH) via the calcium-sensing receptor (CaSR), which detects ambient serum Ca^{2+} and regulates minute-to-minute PTH secretion (7).

Complexed and ionized calcium together are termed the ultrafilterable calcium, and are freely filtered by the glomerulus with a calcium concentration being cca 1.5 mmol/L. The quantitiy of calcium filtered each day is over 270 mmol(=10 g) and is far greater than calcium content of the entire extracellular fluid compartment and far more than net calcium absorption, which is cca 4.0 mmol (= 160 mg)/24 hours. To maintain neutral calcium balance, 98% of the filtered calcium is reabsorbed along the renal tubule. The substantial filtration followed by selective reabsorption allows very precise control of

calcium excretion. Approximately 70% of filtered Ca is reabsorbed in the proximal tubule through predominantly passive mechanisms. About 20% of filtered Ca is reabsorbed in the loop ofr Henle. Only little Ca is reabsorbed in the thin descending and thin ascending limbs of the loop. However, the thick ascending limb of the loop of Henle is the site of paracellular Ca reabsorption driven by the Na-K-2 Cl transporter. Loop diuretics, such as furosemide impair Ca reabsorption in this segment by decreasing lumen-positive voltage created by the transporter. The basolateral membrane of these cells contains CaSR. An increase in peritubular Ca stimulates the CaSR, which reduces lumen-positive voltage and thereby reduces Ca reabsorption. Also along this segment is the tight junction protein paracellin 1. Mutations of paracellin 1 result in a selective defect in paracellular Ca and Mg reabsorption. The distal convoluted tubule reabsorbs 8% of filtered Ca and is the major site of physiologic regulation of urine Ca excretion. Active Ca reabsorption against electrochemical gradient involves entry across the apical membrane through the highly Ca-selective renal epithelial Ca channel 1. The channel is selectively more permeable to Ca than Na and is induced by $1,25(OH)_2D3$, estradiol, and low Ca diet. Cytosolic Ca diffusion is facilitated by Ca binding to calbindin, and active extrusion across the distal nephron plasma membrane is accomplished by the Na-Ca exchanger and a Ca-ATPase. While Ca generally follows Na in this segment, reabsorption and excretion of Ca and Na can be dissociated. The collecting duct absorbs less than 5% of the filtered load. As a result of the reabsorption of Ca along the nephron, the final urine contains only 2% of the filtered Ca load (17). Factors affecting Ca reabsorption include PTH, calcitriol, thiazide and loop diuretics, phosphate or sodium administration (17).

1.2.4. Renal handling of phosphate

About 600 g of phosphate are found in adult humans; 85% of the total is contained in the hydroxyapatite crystals in bone, and 15% is present in soft tissues, with only 0.1% in the extracellular fluids (17). Unlike the tight control of serum Ca, serum phosphate levels fluctuate widely, depending on sex, age, dietary intake, rate of growth, and levels of several hormones. An adequate phosphate concentration in serum is required to maintain the $CaxPO_4$ ion product sufficient to support mineralization of bone. Low serum phosphate levels may create a suboptimal $CaxPO_4$ ion product and impair skeletal mineralization. A pathologically high $CaxPO_4$ ion product in serum and extracellular fluids promotes ectopic or extraskeletal soft tissue calcifications. Approximately 85% of serum phosphate is ultrafilterable, and urine phosphate excretion is 25-30 mmol (750-1000 mg) per day. Thus, cca 12% of glomerular filtrate is excreted in the urine (17). 85% of phosphate reabsorption occurs in the proximal tubule. The rate-limiting step in the phosphate reabsorption is located in the apical domain of the proximal tubule cells, which is also the site of active sodium-phosphate co-transport. The transporter moves phosphate against trans-brush border electrochemical phosphate gradients and follows a transcellular pathway that is dependent on low intracellular Na concentration. Three genes encode related sodium gradient-dependent phosphate transporters (Npt1-Npt3), and the apical brush

border membrane Npt2 accounts for 85% of proximal tubule phosphate reabsorption. Npt2, but not Nptl is regulated, and a major regulator of Npt2 is phosphatonin - fibroblast growth factor 23 (FGF-23). Elevated levels of FGF-23 have been found in phosphate-wasting disorders, such as X-linked hypophosphatemic rickets, autosomal dominant hypophosphatemic rickets, or tumor-induced osteomalacia. Other phosphatonins that regulate phosphate Npt2, and thus the phosphate urinary excretion, include MEPE (matrix extracellular phosphoglycoprotein) and FRP-4 (frizzled related protein 4) (5, 17, 23, 31). Beyond the proximal tubule, a small fraction of phosphate reabsorption occurs in the distal convoluted tubule. Tubular maximum for the reabsorption of phosphate is regulated and is approximately equal to the normal amount of phosphate filtered by the glomerulus. Thus, any appreciable increase in filtered phosphate increases urinary phosphate excretion (17, 31). The major factors affecting phosphate reabsorption are dietary phosphate intake, PTH, FGF-23, calcitonin, calcitriol and thiazide diuretics. Low phosphate intake stimulates reabsorption, whereas high phosphate intake inhibits reabsorption and increases urine phosphate (17).

1.2.5. Renal handling of magnesium

Adult tissues contain about 1.04 mol (25 g) of magnesium, of which 66% is located in the skeleton, 33% is intracellular and 1% is within the extracellular compartment. Magnesium blood levels are regulated largely by the quantitativce influx and efflux of Mg across intestine, bone and kidney rather than by an elaborate hormonal system that has evolved for control of Ca. Ionized and complexed Mg are 70% of total serum Mg and constitute the ultrafilterable Mg. Urine Mg averages about 24 mmol/day, indicating that 95% of the filtered magnesium is reabsorbed before excretion (17). In contrast to the 70% reabsorption of filtered Ca in the proximal tubule, this segment reabsorbs only 15% of the Mg ultrafiltrate. About 70% of Mg is reabsorbed in the cortical thick ascending limb of the loop of Henle, and no reabsorption of Mg occurs in the medullary thick ascending limb of the loop of Henle. Mg may also stimulate CaSR, resulting in decreased Mg reabsorption. Paracellin 1 in the tight junctions also regulates Mg reabsorption. The distal convoluted tubule reabsorbs 10% of Mg through a transcellular transport process (17). Factors affecting the reabsorption of Mg include PTH, metabolic alkalosis, phoshate depletion, hyper- and hypocalcemia and loop diuretics.

Hypermagnesemia leads to decreased tubular reabsorption of Mg, while hypomagnesemia results in increased Mg reabsorption (17).

1.2.6. Normal urinary acidification

Urinary acidification involves two processes: bicarbonate reabsorption and hydrogen ion excretion. To maintain the acid-base balance of the extracellular fluid (ECF) the kidney must excrete net acid at a rate equal to the rate of extrarenal net acid production (approximately 0.3 to 1.0 mEq/kg day). The kidney maintains the pH of the ECF by regulating the plasma bicarbonate (HCO^{3-}) concentration. It does so by two processes: (a) reclamation of filtered HCO^{3-} and (b) generation of new HCO^{3-} by means of net acid excretion (19). Bicarbonate reabsorption results in reclamation of the filtered bicarbonate but does not

result in net acid secretion (24). The proximal tubule lowers the luminal pH from 7.3 to approximately 6.7 and thus reabsorbs the major portion of HCO^{3-}. The collecting tubule provides the final urinary acidification with titration of ammonia, phosphate and other titratable buffers (19). The normal filtered load of HCO^{3-} is about 4500 mEq per day. Less than 0.1 per cent of filtered HCO^{3-} appears in the final urine. Approximately 85% of the filtered bicarbonate is reabsorbed in the proximal tubule. In infants, bicarbonate reabsorption is less efficient, and renal bicarbonate excretion may occur at serum concentrations less than 22 mmol/L. Bicarbonate itself is not directly absorbed through a specific transporter, but instead is absorbed by an indirect process. Proximal tubular reabsorption of bicarbonate begins with secretion of a hydrogen ion in exchange for a sodium ion. The hydrogen ion in the tubular lumen binds with bicarbonate and, under the influence of carbonic anhydrase, is converted to carbon dioxide and water. Carbon dioxide then diffuses into the proximal tubular cell, where a series of chemical reactions result in the creation of a bicarbonate molecule (which then enters the peritubular capillary) and a hydrogen ion, which can participate in further buffering of bicarbonate in the tubular lumen. The remaining 15% of bicarbonate is reabsorbed distally. Secretion of the daily acid load (approximately 1 mmol/kg/24 hours produced during normal cellular processes) is accomplished by hydrogen ion secretion (mediated by a H^+ATPase present in the intercalated cells of the collecting tubule), ammoniagenesis, and formation of titratable acids (formed when H^+ ions are buffered by organic acids such as phosphate) (24).

1.3. Pathologic states affecting the renal tubules

Various disease states can affect the renal tubular functions. These can be divided into inherited and acquired disorders. Furthermore, the tubular dysfunction may be a primary disorder, where the tubule is primarily affected; or a secondary disorder, where another underlying disease is present and a tubular affection might occur.

1.3.1.Renal tubular acidosis (RTA)

Renal tubular acidosis is a disease state characterized by a normal anion gap metabolic acidosis resulting from either impaired bicarbonate reabsorption or impaired urinary acid/hydrogen ion excretion (9,21,24, 30). Both inherited and acquired primary and secondary forms exist. There are three main forms of RTA: proximal (type II) RTA, distal (type I) and hyperkalemic (type IV) RTA. Mixed lesions, which include elements of type I and II RTA, and which occur primarily in patients with inherited carbonic anhydrase deficiency, have been designated as type III by some authors.

1.3.1.1. Proximal (type II) RTA

Proximal RTA results from impaired proximal tubule bicarbonate reabsorption. Isolated forms of inherited or acquired proximal RTA occur, although they are generally rare. Isolated autosomal dominant as well as autosomal recessive forms exist. More typically, proximal RTA occurs as a component of global proximal tubule dysfunction or Fanconi syndrome. The latter condition is characterized by low molecular weight proteinuria, glycosuria, phosphaturia, aminoaciduria,

dehydration, anorexia, vomiting, failure to thrive and proximal RTA. Both autosomal dominant and autosomal recessive forms of primary Fanconi syndrome occur. In addition, secondary Fanconi syndrome may occur as a component of inherited or acquired disease states (24)

1.3.1.2. Distal (type I) RTA

Distal RTA occurs as the result of impaired distal urinary acidification (hydrogen ion secretion). Primary or secondary causes can result in damaged, or impaired functioning of one or more transporters or proteins involved in the acidification process, including the H^+/ATPase, the HCO_3^-/Cl^- anion exchanges, or the components of the aldosterone pathway. Urine pH can't be reduced below 5.5, despite the presence of severe metabolic acidosis. Loss of calcium bicarbonate results in hyperchloremia and hypokalemia. Patients with distal RTA share common features with those of proximal RTA. However, distinguishing features of distal RTA include nephrocalcinosis and hypercalciuria (3,24).

1.3.1.3. Hyperkalemic (type IV) RTA

Type IV RTA occurs as the result of impaired aldosterone production (hypoaldosteronism) or impaired renal response to aldosterone (pseudohypoaldosteronism). Since aldosterone has a direct effect on the H^+ATPase responsible for hydrogen secretion, acidosis results. In addition, aldosterone is a potent stimulator for potassium secretion in the collecting tubule. Loss of aldosterone results in hypokalemia. This further affects acid-base status by inhibiting ammoniagenesis and therefore hydrogen ion secretion. Patients with RTA IV present with growth failure, polyuria, dehydration and hyperkalemia (24).

1.3.2. Nephrogenic diabetes insipidus (NDI)

Nephrogenic diabetes insipidus is a rare, inherited kidney disorder, characterized by insensitivity of the distal renal nephron to the antidiuretic effect of ADH - vasopressin. As a consequence, the kidney loses its concentrating ability and produces large volumes of hypotonic urine (50-100 mOsm/kg H20), which may lead to severe dehydration and electrolyte imbalance (hypernatremia and hyperchloremia). In NDI, the proper amount of water cannot be reabsorbed and is instead voided in large quantities as dilute urine, leaving NDI patients chronically thirsty and in danger of dehydration. NDI may be either acquired or hereditary (congenital). Inherited NDI is very rare. Affected patients may begin showing symptoms in the first few days of life. There are three types of inherited NDI: X-linked NDI, autosomal recessive NDI and autosomal dominant NDI. Acquired NDI occurs more frequently than inherited NDI, but is still a rare disorder. It is usually less severe than inherited NDI and usually occurs in adults. This is because acquired NDI is generally due to a treatment or pathology that develops later in life. NDI can be acquired at any time. Acquired NDI could be a result the of use of certain prescription drugs, of a physical condition or occurs due to an underlying systemic disease or disorder (24,47).

1.3.2.1. X-linked NDI

X-linked NDI is the most common type of inherited NDI. It affects males more often than females. Males are certain to be seriously affected by NDI if they inherit the gene, whereas females are usually affected mildly or not at all. Rarely, girls may be affected as severely as boys. Females who carry this gene, whether or not they show symptoms, will pass it on to their daughters and their sons in 50% of the cases (24,47).

1.3.2.2 Autosomal recessive NDI

Autosomal recessive NDI is a very rare type of inherited NDI. It affects males and females equally. For a child to have the disease, both parents must carry this gene. Parents who are both carriers of this form of NDI have a 25% chance with each pregnancy of having another affected child (47).

1.3.2.3. Autosomal dominant NDI

Autosomal dominant NDI is an extremely rare type of NDI. It affects both males and females. For a child to inherit this type of NDI, only one parent needs to carry the gene (24,47).

1.3.3. Bartter/Gitelman syndromes

Bartter's and Gitelman's syndromes represent two of the autosomal recessive syndromes producing normotensive hypokalaemic metabolic alkaloses. The inherited hypokalaemic alkaloses are typified by a constellation of metabolic abnormalities, including metabolic alkalosis, hypokalemia, chloride wasting, hypomagnesemia, and hyper- or hypocalciuria (4). The molecular basis of these disorders involves effects on molecular transporters/channels including an inward rectifying K^+ channel (ROMK1), the thiazide-sensitive Na/Cl cotransporter (TSC), the loop diuretic-sensitive $Na^+/K^+/2Cl^-$ cotransporter (NKCC2), and the chloride channel (CLCKNB). Both syndromes present early in life, frequently before the twenties. Children affected by these inherited disorders suffer from growth and developmental retardation. Generally, patients experience muscle weakness and cramps, attributable to profound hypokalaemia. Both adults and children describe a range of symptoms, including muscle cramps, muscle weakness, polyuria, nocturia, nephrogenic diabetes insipidus, failure to thrive, salt-craving , enuresis, constipation, seizures, tetany and joint pains, due to chondrocalcinosis (4, 24). Classically, patients are normotensive or hypotensive with elevated plasma renin and aldosterone concentrations.

1.3.3.1. Bartter's syndrome

Bartter's syndrome occurs with a familial tendency and is an autosomal recessive disorder. Bartter's syndrome is linked to a defect in the renal genes, such as NKCC2, ROMK, or CLCKNKB.

Mutation of the NKCC2, located on chromosome 15, leads to reduced Na^+ and Cl^- reabsorption in the thick ascending limb, with subsequent salt wasting and hypovolemia . As approximately 30% of the filtered Ca^{2+} load is coupled to Na^+ transporter activity, this would also account for the observed

hypercalciuria through the lack of a lumen positive potential to generate a net calcium reabsorption. Magnesium, which is mainly reabsorbed in the thick ascending limb, would presumably be maintained through increased distal tubule reabsorption via Na^+/Mg^{2+} exchangers (4).

Loss of ROMK function leads to the inability of K^+ to recycle out of the cells of the thick ascending limb into the lumen, resulting in a reduced lumenal K^+ concentration. This in turn leads to an inhibition of $Na^+/K^+/Cl^-$ activity with resultant salt wasting from the thick ascending limb. The consequent reduced volume causes an increased renin release, secondary hyperaldosteronism, and increased Na^+ reabsorption from the distal nephron in exchange for K^+ and H^+ ions, which are secreted (4).

Loss or impaired CLCKNB activity from the basolateral membrane of the thick ascending limb leads to a rise in intracellular Cl^- resulting in a reduced Na^+ reabsorption and thus salt wasting and hypovolaemia. These ionic changes stimulate the renin-angiotensin system, resulting in Na^+ reabsorption and K^+ secretion distally. The reduced lumen positive potential from the excess intracellular Cl^- leads to a reduction in Ca^{2+} reabsorption and hypercalciuria (4).

1.3.3.2. Gitelman's syndrome

Gitelman's syndrome has mainly autosomal recessive inheritance. Defects of the TSC in the distal convoluted tubule lead to reabsorptive failure of sodium Na^+ and Cl^-, and subsequent water loss with ensuing hypovolemia. The reduced vascular volume stimulates the renin-angiotensin system, resulting in elevation of renin and aldosterone concentrations. These pathophysiological changes cause an increase in apical Na^+ reabsorption via epithelial cortical collecting duct Na^+ channels and stimulation of the basolateral Na^+/K^+-ATPase to allow flow of Na^+ down its electrochemical gradient. The increased aldosterone concentrations stimulate cortical and medullary collecting ducts H^+-ATPase pumps leading to an increased apical H^+ ion secretion. K^+ and H^+ ion excretion increases as K^+ enters from the basolateral membrane via the Na^+/K^+-ATPase pumps, resulting in hypokalaemic metabolic alkalosis. The resultant low intracellular Na^+ increases DCT Ca^{2+} reabsorption via basolateral Na^+/Ca^{2+} exchangers, causing hypocalciuria. Magnesium loss, via apical Mg^{2+} /Na^+ exchangers, increases due to the net negative transepithelial potential. This reduced Mg^{2+} may also stimulate PTH release, which further increases Ca^{2+} reabsorption. The presence of hypocalciuria, hypomagnesemia, presentation later in life, milder symptomatology and relative lack of a concentrating defect (in keeping with a defect of the distal convoluted tubule) are all features which may occur in patients with Gitelman's and not Bartter's syndrome (4).

1.3.4. Cystinuria

Cystinuria is an autosomal recessive defect in reabsorptive transport of cystine and the dibasic amino acids ornithine, arginine, and lysine from the luminal fluid of the renal proximal tubule and small intestine. The only phenotypic manifestation of cystinuria is cystine urolithiasis, which often recurs

throughout a patient's lifetime. The high-affinity transporter is present in the apical brush-border membrane of the jejunum and is responsible for absorption of cystine and dibasic amino acids. Cystinuria is divided into 3 subtypes: Rosenberg I, II, and III. Cystinuria type I is the most common variant. Patients with cystinuria usually present with renal colic. Uncommon presentations include hematuria, chronic backache, and urinary tract infection. Twenty-five percent of symptomatic patients report their first stone in the first decade of life, and another 30-40% have their first experience as teenagers (39).

1.3.5. X-linked nephrolithiasis (Dent disease)

Dent's disease (X-linked nephrolithiasis) is a proximal tubulopathy that has been associated with inactivating mutations in the CLCN5 gene encoding the ClC-5 chloride channel expressed in tubular epithelial cells (20). The earliest manifestations of the disorder occur in childhood or in adult. It is characterized by tubular proteinuria (due to defective reabsorption of some low molecular weight proteins, such as beta-2 microglobulin), hypercalciuria, calcium nephrolithiasis, nephrocalcinosis and chronic renal failure. The molecular disorder usually affects a chloride channel, the CLCN5. Mutations in the gene coding for this channel have also been found in related syndromes, named recessive X-linked nephrolithiasis or X-linked hypophosphatemia or idiopathic proteinuria of low molecular weight found in Japanese children (20, 41).

1.3.6. X-linked hypophosphatemic rickets (XLH)

X-linked hypophosphatemic rickets (XLH) is an X-linked dominant disorder characterized by growth retardation, rachitic and osteomalacic bone disease, hypophosphatemia, and renal defects in phosphate reabsorption and vitamin D metabolism. Autosomal-dominant hypophosphatemic rickets (ADHR) is similar disease with almost identical manifestations. The primary defects in XLH are now identified as inactivating mutations in a Zn-metalloendopeptidase (PHEX) and activating mutations in FGF23, respectively. In XLH, loss of PHEX function is proposed to result in an increase in uncleaved full-length FGF23 and/or inappropriate processing of MEPE. In ADHR, a mutation in FGF23 results in resistance to proteolysis by PHEX or other proteases, and an increase in half-life of full-length phosphaturic FGF23. This is proposed to result in abnormal renal-phosphate handling and mineralization with all clinical consequences (5,31).

1.3.7. Tubulointerstitial nephritis

Tubulointerstitial nephritis is an inflammatory process that affects the tubules of the kidneys and the tissues surrounding them (tubulointerstitial tissue). Tubulointerstitial nephritis may be acute or chronic, and it often results in kidney failure. It may be caused by various underlying diseases, drugs, or toxins that damage the kidneys. At the structural level in both acute and chronic tubulointerstitial diseases, clinical presentations are the result of the interplay of renal cells and inflammatory cells and their products. Lethal or sublethal injury to renal cells leads to expression of new local antigens,

inflammatory cell infiltration, and activation of proinflammatory and chemoattractant cytokines. These cytokines are produced by inflammatory cells (ie, macrophages, lymphocytes) and also by the renal cells (ie, proximal tubule, vascular endothelial cells, interstitial cells or fibroblasts). The outcome can be acute or chronic nephritis (24, 40).

1.3.7.1. Acute tubulointerstitial nephritis

In acute interstitial nephritis, the tubular damage leads to renal tubular dysfunction, with or without renal failure. Regardless of the severity of the damage to the tubular epithelium, the renal dysfunction is generally reversible, possibly reflecting the regenerative capacity of tubules with preserved basement membrane. The principal mechanism in acute tubulointerstitial nephritis is hypersensitivity reaction to drugs such as penicillins, NSAIDs, and sulfa drugs. Another mechanism is acute cellular injury caused by infection, viral or bacterial, often associated with obstruction or reflux (40).The acute tubulointerstitial nephritis might occur due to (a) hypersensitivity reactions (eg, drugs, penicillins, sulphonamide drugs, nonsteroidal anti-inflammatory drugs [NSAIDs]) (b) immunologic diseases (eg, associated with lupus, Goodpasture syndrome); (c) acute transplant rejection; (d) infections (bacterial - accompanied by obstruction or reflux, viral - cytomegalovirus [CMV], hantavirus, HIV, hepatitis B, fungal or parasitic such as *Leishmania, Toxoplasma*) (24,40).

1.3.7.2. Chronic tubulointerstitial nephritis

Conversely to acute tubulointerstitial nephritis, chronic tubulointerstitial nephritis is characterized by interstitial scarring, fibrosis, and tubule atrophy, resulting in progressive chronic renal insufficiency. The chronic tubulointerstitial nephritis might occur due to (a) drugs (eg, analgesics, lithium, cyclosporine, tacrolimus); (b) heavy metals (eg, lead, cadmium, mercury) (c) obstructive uropathy, nephrolithiasis, reflux disease; immunologic diseases, such as lupus, Sjögren syndrome, primary glomerulopathies, sarcoidosis, vasculitis, antineutrophil cytoplasmic antibody (ANCA)–associated vasculitides, Wegener granulomatosis or chronic transplant nephropathy or even due to neoplasia (eg, myeloma, leukemia, amyloidosis). Other causes of chronic tubulointerstitial nephritis might include atherosclerotic kidney disease (ischemic) due to cholesterol microembolism, metabolic diseases (eg, hypercalcemia, cystinosis, potassium depletion, nephropathy, hyperoxaluria), genetic disorders (eg, Alport syndrome, medullary cystic disease), or miscellaneous causes (eg, Balkan endemic nephropathy, Chinese herb/*Aristolochia* nephropathy) (40).

1.4. Evaluation of renal tubular functions

Tubular function tests involve evaluation of functions of the proximal tubule (*i.e* . tubular handling of sodium, glucose, phosphate, calcium, bicarbonate and aminoacids) and distal tubule (urinary

acidification and concentration) (3). The evaluation of renal tubular functions can be assessed by examining the:

- renal concentration capacity
- urinary electrolyte excretion in relation to glomerular filtration rate
- tests for urinary acidification
- tubular proteins
- tubular enzymes

Unfortunately, there is not one single reliable test to detect the degree of renal tubular impairment.

1.4.1. Renal concentration capacity tests

Urine osmolality normally varies from 50 mOsm/kg to approximately 1200 mOsm/kg.

The renal concentration capacity can be evaluated by means of **water deprivation test**, or **vasopressin/adiuretin test.** This test examines renal concentrating ability by the administration of a synthetic ADH analogue (desmopressin) and subsequent measurement of urine osmolality. After passing through the distal tubules, approximately 90 % of filtered water has been resorbed from the glomerular filtrate and urine is iso-osmolar or hypo-osmolar. Further water uptake with subsequent concentration of urine occurs when it passes through the collecting ducts in the renal pyramids. Antidiuretic hormone controls the permeability to water of the collecting ducts via its action at specific receptors. Impaired renal concentrating ability may arise because of reduced ADH effect (NDI, toxic or inflammatory processes) or because of adverse effects on osmotic gradient in pyramidal tissue (eg. circulatory disorders, hyponatremia, reduced glomerular filtration rate) (16). Failure of concentrating ability occurs with (a) renal disease, when approximately 2/3 of the nephrons are nonfunctional before concentrating ability is reduced; (b)diabetes insipidus.

1.4.1.1. Water deprivation test

The water deprivation test is currently considered as obsolete, yet for the sake of complexity, it deserves some attention. Withholding water for a period of 12-14 hours causes plasma hyperosmolality and pituitary release of ADH., that acts on the renal tubular epithelial cells causing reabsorption of water, thereby increasing urine specific gravity. If the tubules are nonfunctional, water will not be reabsorbed and the specific gravity will remain low. After the patient has fasted for 12 to 14 hours overnight, the osmolality of the initial morning urine and of subsequent hourly samples is measured. Pediatric patients with polyuria with no evidence of dehydration and normal serum sodium are kept off fluids for 6-8 hr, until weight loss exceeds 3% or until 3 consecutive hourly urine osmolality values are within 10% of each other. Urine osmolality more than 750 mOsm/kg at the end of the evaluation is

suggestive of primary polydipsia. Osmolality less than 750 mOsm/kg after water deprivation should be further evaluated after administration of vasopressin/desmopressin (3, 11, 42, 43).

Urine and plasma osmolalities are observed in response to fluid deprivation and subsequent administration of desmopressin. Careful supervision is essential and the test should be discontinued if 5% of initial body weight is lost; 3% in children. Free access to fluid is permitted until the start of the test. During the test, dry food is permitted but not fluid; at timed hourly intervals for up to 8 hours the following are measured:

- weight
- urine volume and osmolality
- serum osmolality

Desmopressin (2 μg) is administered at the end of 8 hours. Urine is collected for a further 16 hours during which time fluid intake is allowed but restricted to a maximum of 1.5 times the urine volume voided during the dehydration period. Blood is collected at the end of the 16 hours and plasma osmolality measured. Results may be interpreted as:

- urine osmolality less than 300 mOsm/kg after fluid deprivation and greater than 800 mOsmol/kg after desmopressin suggests cranial diabetes insipidus;
- urine osmolality less than 300 mOsm/kg after fluid deprivation and less than 300 mOsmol/kg after desmopressin suggests nephrogenic diabetes insipidus;
- urine osmolality greater than 800 mOsm/kg after fluid deprivation and greater than 800 mosmol/kg after desmopressin suggests primary polydipsia (3, 43).

1.4.1.2. Vasopressin-desmopressin test

Vasopressin-desmopressin test (pituitrin concentration test, pitressin concentration test) is basically similar to the urine concentration test except exogenous ADH (desmopressin) is administered in place of water deprivation. Aqueous synthetic analogue of vasopressin (1-deamino-8-D-arginine vasopressin, dDAVP), is given s.c. or is administered as nasal drops or nasal spray, and the urine osmolality is measured 6 hours afterwards (1,18). The bioavailability of intranasal dDAVP is about 10% and may be modified by nasal congestion (18). However, there is no difference in urine osmolality after intranasal administration (20-40 μg of desmopressin according to body weight) or intravenous application of desmopressin (2-4 μg according to body weight); there is a high correlation in urine osmolality between intranasal and intravenous application (18). Therefore, this test represents a convenient, reliable and simple method for the estimation of renal concentrating capacity in children. The DDAVP-test is as accurate and reproducible as the water deprivation test, irrespective of the degree of

concentrating capacity (1,18). Infants < 1 year age receive 10 μg intranasal dDAVP, children and adolescents 20 μg, and adults up to 40 μg. During the test no fluids or liquid foods should be given to avoid the risk of overhydration, but dry foods are allowed. Urine samples are collected for specific gravity (ward test) and osmolality (laboratory) from 1 to 8 hours after commencement. If patients can void on command, samples should be collected at 4 and 6 hours. The test can be terminated after 6 hours if two urine samples have been obtained, or in any case at 8 hours after encouragement to void. The highest urine osmolality achieved during the test is noted. Maximum urine concentrating ability increases with age, peaking at adult levels at around 3 years of age. From around 20 years of age gradual decline occurs. There are no sex differences. The mean values and range (-2SD to +2SD) are 840 mOsm/kg (525 - 1170) in children below 1 year of age, 1000 mOsm/kg (700 - 1300) in children 1-2 years of age, and 1050 mOsm/kg (825 - 1400) in subjects ≥ 3 years of age (16).

1.4.2.Urinary electrolyte excretion in relation to glomerular filtration rate

Urinary electrolyte excretion in relation to glomerular filtration rate can be assessed by means of the fractional excretion of sodium, potassium, calcium and phosphate, respectively.

1.4.2.1. The fractional excretion of sodium

The rate of sodium excretion is of diagnostic importance in determining the cause of oliguria. With prerenal azotemia, sodium excretion is usually less than 15 mmol/L whereas sodium excretion is usually higher with renal causes (e.g., acute tubular necrosis). However,prerenal factors often coexist with renal disease, thus there is considerable overlap in the urine sodium concentration (UNa) in these two situations (19). Therefore, only values that are clearly high or low are diagnostic. The fractional excretion of sodium (FENa%), obtained by dividing the clearance of sodium by the clearance of creatinine, provides a much better index by which to differentiate renal from prerenal causes. FENa% is calculated by the following formula:

FENa% = (UNa x PCr)/ (PNa x UCr) x100

where: UNa = urine sodium concentration

PNA = plasma sodium concentration

PCr = plasma creatinine concentration

UCr = urine creatinine concentration

A value lower than 1% favors a prerenal etiology while a value above 2% favors a renal cause. Although fairly sensitive and specific, FENa% values less than 1% have been reported in a variety of causes of acute renal failure other than prerenal disease (e.g., myoglobinuria or hemoglobinuria, radiocontrast nephropathy, renal azotemia superimposed on chronic prerenal failure as in hepatic cirrhosis) (19,24)

1.4.2.2. The fractional excretion of potassium

The rate of potassium excretion is of diagnostic importance in determining the cause hyperkalemia. FEK can be calculated with the use of following equation:

FEK% = (UK x PCr) / (PK x UCr)x100

where: UK= urine potassium concentration

PK = plasma potassium concentration

PCr = plasma creatinine concentration

UCr = urine creatinine concentration

FEK values exceeding 40% (normal 10-20%) indicate tubular wasting (3,38).

The action of aldosterone mediated sodium-potassium exchange in the distal renal tubule is evaluated by the transtubular gradient of potassium (TTKG), as given below:

TTKG = (UK/PK) /(Uosm/Posm)

where: UK= urine potassium concentration

PK = plasma potassium concentration

Posm = plasma osmolality

Uosm = urine osmolality concentration

TTKG should be estimated when urinary osmolality exceeds plasma osmolality; values below 5-7 in subjects with hyperkalemia imply impaired potassium secretion due to aldosterone deficiency or resistance. TTKG greater than 10 can be found in patients with appropriate action of aldosterone, with hyperkalemia attributed to an increased potassium load (3).

1.4.2.2. The fractional excretion of calcium

The fractional excretion of calcium has been used to determine the amount of excreted calcium. The FECa is calculated according to the following equation:

FECa% = (UCa x PCr)/(PCa x UCr) x 100

Where:

UCa= urine calcium concentration

PCa = plasma calcium concentration

PCr = plasma creatinine concentration

UCr = urine creatinine concentration

1.4.2.3. The fractional excretion of phosphate

Phosphate homeostasis is chiefly regulated at the level of the proximal renal tubule. Plasma phosphate levels are thus indicators of renal tubular handling. The fractional excretion of phosphate determined on

a timed (6-hr, 12-hr, 24-hr) urine specimen is a widely used investigation for phosphate handling (3). The FEP is calculated according to the following equation:

FEP% = (UP x PCr)/(PP x UCr) x 100

Where:

UP= urine phosphate concentration

PP = plasma phosphate concentration

PCr = plasma creatinine concentration

UCr = urine creatinine concentration

Furthermore, tubular reabsorption of phosphate can be assesed with the use of equation:

Tubular reabsorption of phosphate (%) = 100 – FEP%

Tubular reabsorption of phosphate depends on plasma phosphate and glomerular filtration rate and is not a satisfactory indicator of tubular phosphate handling. This has led to increasing use of tubular maximum for phosphate corrected for GFR (TmP/GFR), a factor independent of plasma phosphate and renal functions for assessment of renal phosphate handling. TmP/GFR (normal 2.8- 4.4 mg/dL) is an index of renal threshold for phosphate which can be determined by a nomogram (3).

1.4.3. Tests for urinary acidification

Tests for urinary acidification include assessment of: plasma anion gap, urine anion gap, urine pH, urine to blood CO_2 difference and fractional excretion of bicarbonate.

1.4.3.1. Plasma anion gap

Anion gap represents the difference of unmeasured anions and cations in the plasma, and is measured as follows:

Anion gap = $Na^+ - (Cl^- + HCO^{3-})$

The normal value of the plasma anion gap is 10-12 mEq/L. Accumulation of organic acids like lactate and acetoacetate, as in diabetic ketoacidosis, and poisoning due to ethylene glycol, are characteristically associated with metabolic acidosis and an increased anion gap. Normal anion gap in the presence of acidosis (hyperchloremic metabolic acidosis) suggests increased urinary (proximal RTA), or gastrointestinal loss (diarrhea) of bicarbonate or impaired excretion of H+ ions (distal RTA) (3).

1.4.3.2. Urine anion gap

Urine anion gap (net charge) (urine $Na^+ + K^+ - Cl^-$) provides an estimate of urinary ammonium (NH_4^+) excretion and is important in the evaluation of hyperchloremic acidosis. Under normal circumstances, urine anion gap is positive due to the presence of dissolved anions *e.g.*, sulfates or phosphates.

Metabolic acidosis is associated with a compensatory rise in NH_4^+ production, resulting in a negative urine anion gap. Patients with RTA typically show impaired renal NH_4^+ excretion and a positive urine anion gap (3).

1.4.3.3. Urine pH

Urine pH is an estimate of the number of free H^+ ions in the urine which are secreted in response to metabolic acidosis. The presence of alkaline urine during metabolic acidosis suggests defective renal acidification, as in distal RTA. However, alkaline urine may also be found in patients with metabolic acidosis due to extrarenal disorders, as in acute or chronic diarrhea. Occasionally, metabolic acidosis may need to be induced by oral administration of ammonium chloride before determining urine pH. This test is, however, cumbersome, and not commonly used (3).

1.4.3.4. Urine to blood CO_2 difference

Based on the observation that urinary CO_2 excretion is an indicator of H+ secretion, urine to blood CO_2 difference is considered a satisfactory index of distal renal acidification. In the presence of normal blood bicarbonate, low urine to blood CO_2 difference (< 10 mm Hg) suggests distal RTA; the levels are normal in proximal RTA (> 20 mm Hg). It is necessary that the difference be determined after adequate alkalinization with oral sodium bicarbonate (2-4 mEq/kg/day) (in order to achieve normal blood pH and bicarbonate, and urine pH > 7.4) (3).

1.4.3.5. Fractional excretion of bicarbonate

Fractional excretion of bicarbonate is a marker of proximal tubular handling of bicarbonate. The proximal tubule normally reabsorbs almost all filtered bicarbonate (fractional excretion below 5%).

$FENaHCO_3\ \% = (U\ NaHCO_3 \times PCr) / (P\ NaHCO_3 \times UCr) \times 100$

where: $U\ NaHCO_3$= urine bicarbonate concentration

$P\ NaHCO_3$ = plasma $NaHCO_3$ concentration

PCr = plasma creatinine concentration

UCr = urine creatinine concentration

A value greater than 15% indicates proximal RTA, while levels are in the normal range in distal RTA. The fractional excretion of bicarbonate should be calculated only after adequate alkalinization (3,19) .

1.4.4. Tubular proteins

Tubular proteins can be detected in cases of renal tubular impairment. The most frequently used renal tubular proteins are alpha-1-microglobulin, beta-2-microglobulin and cystatin C.

1.4.4.1. Alpha-1-microglobulin

Alpha-1-microglobulin (A1M), also known as protein HC (for Heterogeneous Charge), is a low molecular weight (26 000 – 33 000 Da) protein component of plasma first discovered in pathological human urine. It is a member of the lipocalin superfamily. Although much is now known of its structure and properties, the function and physiological role of A1M remains unclear, although evidence suggests that it plays a role in the regulation of the immune system. A1M is known to exist in both a free form and complexed to other macromolecules: immunoglobulin A (IgA) in humans and alpha-1-inhibitor-3 in the rat (44). A1M levels in urine may indicate kidney tubular damage and can occur (25):

- During the course of nephritis or advanced diabetic nephropathy .
- After heavy metal exposure or treatment with nephrotoxic medications.
- In urinary tract infections, where elevated alpha-1 microglobulin levels signal renal involvement.

1.4.4.2. Beta-2-microglobulin

Beta-2- microglobulin (B2M) is a component of major histocompatibility complex class I molecules, which are present on almost all cells of the body. Beta-2- microglobulin lies lateral to the α_3 chain on the cell surface. Unlike alpha 3, beta 2 has no transmembrane region. Directly above beta 2 (i.e. away from the cell) lies the alpha 1 chain, which itself is lateral to the alpha 2 (45). Beta-2- microglobulin is a low molecular weight (11 500 Da) protein. Beta-2-microglobulin is filtered out of the body by the kidney's glomeruli and almost completely reabsorbed by the kidney's proximal tubules. Increased levels are seen in patients with glomerular kidney disease, as the B2M is not being filtered out of the blood. Decreased levels are seen in tubular kidney disease, as the tubules can't reabsorb the B2M back into the blood. B2M levels are monitored closely after a kidney transplant as increased levels may be an early indication of rejection. Several diseases are associated with an increased production of B2M, which results in serum B2M levels higher than expected. Increased serum B2M levels in systemic lupus erythematosus, rheumatoid arthritis and Sjögren's syndrome are also found. Additionally, serum B2M concentrations increase in renal allograft rejections. All these clinical data suggest that lymphocyte and macrophage stimulation result in an increased B2M production (29) .

1.4.4.3. Cystatin C

Cystatin C is a novel serum marker of the glomerular filtration rate, a critical measure of normal kidney function. Human cystatin C (CST3), a basic low molecular mass protein with 120 amino acid residues is a strong inhibitor of cysteine proteinases. CST 3 is freely filtered by the kidney glomerulus and is

reabsorbed by the tubules, where it is almost totally catabolized by the proximal tubular cells, with the remainder then eliminated in urine. Cystatin C has been recently proposed as a new sensitive endogenous serum marker for the early assessment of changes in the glomerular filtration rate. Studies indicate that it is at least as good as serum creatinine for detecting renal dysfunction (46). In tubular diseases, CST3 degradation is reduced and consequently an increase in its urinary elimination would be observed. Increased urinary CST3 concentrations allow the accurate detection of tubular dysfunction among pure and mixed nephropathies (10).

1.4.5.Tubular enzymes

The assessment of urinary enzymes is a relatively simple, cheap, fast and non-invasive method in the detection and follow-up of renal disorders. Brush border enzymes, cytosolic enzymes and lysosomal enzymes can be detected in urine However, the activities of enzymes in urine are, even under physiological conditions, affected by a large number of parameters, such as hormonal influences, volume depletion, diuresis, urine concentration, age and sex (14,15). Furthermore, in normal urinary enzyme activity there is a wide variation which makes the interpretation of a laboratory result rather difficult (14,15). For these reasons, enzymuria is not being used as routine method in comparison with the use of certain established serum enzymes. However, it is important to select a few urinary enzymes that can be used as reliable markers of renal damage. In addition, it is important to point out that enzymuria is associated with the acute and not chronic effects of toxins, because urine is an open system (8). Elevated levels of urinary enzymes are considered more sensitive methods of detecting renal damage than changes in serum creatinine or a drop in creatinine clearance (8,14,15).

The enzymes such as alkaline phosphatase, aminopeptidases, beta-galactosidase, beta-glucosidase, lactic dehydrogenase, glutathione-S-transferase and N-acetyl-beta-D-glucosaminidase have been at various times assessed as markers of nephrotoxicity, however only the last two of them (6,15,27) are currently used for this purpose. **It should be clearly pointed out that the evaluation of enzymuria is really scarce in routine clinical practice when compared to the omnipresent evaluation of enzymes in serum or plasma.**

1.4.5.1. Ligandin (glutathione-S-transferase)

Ligandin is a cytosolic enzyme, originating predominantly from the proximal tubule, and has been considered as a marker of proximal tubular injury of various origin (6).

1.4.5.2. N-acetyl-beta-D-glucosaminidase

N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme which is present in high concentrations in renal proximal tubules. Its high molecular weight of 130 000 - 140 000 daltons does not permit its

filtration through the glomerular basal membrane, and its urinary excretion is relatively constant with minimal diurnal changes. Increased urinary excretion of NAG has been observed to correlate well with tubular dysfunction or damage in patients with a variety of diseases (diabetes mellitus, nephrotic syndrome, vesicoureteric reflux, urinary tract infection, hypercalciuria, urolithiasis, perinatal asphyxia, heavy metals poisoning, treatment with aminoglycosides or valproate). Urinary NAG (U-NAG) activity is the most frequently used method in the assessment of renal tubular disorders when it comes to the assessment of enzymuria. Furthermore, detection of U-NAG is considered a better sensitive method of detecting renal damage than changes in serum creatinine or a drop in creatinine clearance, as changes in U-NAG clearly precede changes in other parameters of kidney function (27).

The assessment of some urinary enzymes is a relatively simple, cheap, fast and non-invasive method in the detection and follow-up of renal disorders. Out of several promising methods, only the evaluation of two urinary enzymes (U-NAG and U- glutathione-S-transferase) should be currently considered as a helpful tool in the diagnosis of renal disorders.

As the evaluation and monitoring of urinary enzymes is not a generally used method in the detection of renal tubular impairment, this thesis is focused on the assessment of U-NAG in various disease states, in order to highlight the important role of this enzyme in patients with kidney disorders. The aims of the thesis are delineated in Chapter 2.

1.5. References

1. Åbyholm G, Monn E. Intranasal DDAVP-test in the study of renal concentrating capacity in children with recurrent urinary tract infections Eur J Pediatr 1979;130:149-154.
2. Andreoli TE, Carpenter CCJ, Plum F, Smith LH Jr. Cecil Essentials of Medicine. Philadelphia, WB Saunders Co; 1986:185.
3. Bagga A, Bajpai A, Menon S. Approach to renal tubular disorders. Indian J Pediatr 2005; 72:771 – 776.
4. Bhandari S. The pathophysiological and molecular basis of Bartter's and Gitelman's syndromes. Postgrad Med J 1999;75:391-396.
5. Blumsohn A. What have we learnt about the regulation of phosphate metabolism? Curr Opin Nephrol Hypertens 2004;13:397-401.
6. Branten AJ, Mulder TP, Peters WH, Assmann KJ, Wetzels JF. Urinary excretion of glutathione S transferases alpha and pi in patients with proteinuria: reflection of the site of tubular injury. Nephron 2000;85:120-126.
7. Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. Physiol Rev 2001;81:239-297.
8. Chiu JSP. Models used to assess renal function. Drug Devel Res 1994;32:247-255.
9. Chan JCM, Scheinman JI, Roth KS. Renal tubular acidosis. Pediatric Rev 2001;22:277-286.
10. Conti M, Moutereau S, Zater M, Lallali K, Durrbach A, Manivet P, Eschwege P, Loric S. Urinary cystatin C as a specific marker of tubular dysfunction.Clin Chem Lab Med. 2006;44:288-291.
11. Cutler RE. Clinical evaluation of genitourinary disorders. In:Berkow R, Fletcher AJ, eds. The Merck Manual of Diagnosis and Therapy. 16 th edn. Rahway: Merck and Co, Inc;1992:1646-1661.
12. Davis ID, MacRae Dell K. Are aquaporin water channels the "holy grail" of primary nocturnal enuresis? J Urol 2002;167:1451-1452.
13. Davis ID, Avner ED. Glomerular disease. In: Behrman RE, Kliegman RM, Jenson HB, eds. Nelson Textbook of Pediatrics. 17th edn. Philadelphia: WB Saunders and Co; 2004:1731-1735.
14. Dubach UC, Le Hir M. Critical evaluation pf the diagnostic use of urinary enzymes. Contr Nephrol 1984;42: 74-80.

15. Dubach UC, Le Hir M, Gandhi R: Use of urinary enzymes as markers of nephrotoxicity. Toxicol Lett 1988;46: 193-196.
16. Endocrinology and Metabolism Testing Protocols, The Ray Williams Institute of Paediatric Endocrinology, Diabetes and Metabolism, The New Children's Hospital, Royal Alexandra Hospital for Children 1996, http://www.seep.es/privado/protocolos/RWTests.doc
17. Favus MJ, Bushinsky DA, Lehman J. Regulation of calcium, magnesium and phosphate metabolism. In: Favus MJ, ed. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism 6th Edition. Washington DC, ASBMR 2006: 76-83.
18. Feber J, Cochat P, Hadj-Aissa A, Dubourg L, Wright C, Pozet N. Renal concentrating capacity test by desmopressin in children: intranasal or intravenous route? Am J Nephrol. 1993;13:129-131.
19. Gilbert BR, Leslie BR, Vaughan ED. Normal Renal Physiology. In: Walsh PC, Retik AB, Starney TA, Vaughan ED, eds. Cambell's Urology. 6th edn. Philadelphia; WB Saunders and Co; 1992:70-90.
20. Hoopes RR, Raja KM, Koich A, Hueber P, Reid R, Knohl SJ, Scheinman SJ. Evidence for genetic heterogeneity in Dent's disease. Kidney International 2004; 65: 1615–1620.
21. Jones DP, Chesney RW. Tubular function. In: Barrat TM, Avner ED, Harmon WE, eds. Pediatric Nephrology, 4th ed, Baltimore, Williams and Wilkins; 1999:59-82.
22. Lewy JE. Nephrology: Fluids and Electrolytes. In: Behrman RE, Kliegman R, eds. Nelson Essentials of Pediatrics. Philadelphia, WB Saunders Co. 1990: 545-580.
23. Liu S, Guo R, Simpson LG, Xiao Z, Quarles LD. Regulation of fibroblastic growth factor 23 expression but not degradation by PHEX. J Biol Chem 2003;278:419–426.
24. MacRae Dell K, Avner ED. Tubular function. In: Behrman RE, Kliegman RM, Jenson HB, eds. Nelson Textbook of Pediatrics. 17th edn. Philadelphia: WB Saunders and Co; 2004:1758-1766.
25. Penders,J., Delanghe,J.R. Alpha 1-microglobulin: clinical laboratory aspects and applications. Clin Chim Acta. 2004;346:107-118.
26. Pitts RF. Physiology of the Kidney and Body Fluids. 3rd Ed, Chicago, Year Book Medical Publishers 1974.
27. Price RG. The role of NAG (N-acetyl-β-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. Clin Nephrol 1992;38:14-19.
28. Radetti G, Paganini C, Rigon F, Gentili L, Gebert U, Ishikawa S. Urinary aquaporin-2 excretion in nocturnal enuresis. Eur J Endocrinol 2001;145:435-438.
29. Revillard JP, Vincent C. Structure and metabolism of beta-2-microglobulin (review). Contrib Nephrol 1988;62:44-53.
30. Rodriguez-Soriano J. Tubular disorders of electrolyte regulation. In Avner ED, Harmon WE, Niaudet P, eds. Pediatric Nephrology. 5th edn. Baltimore; Lippincot Williams & Wilkins; 2004: 729-756.
31. Rowe PS.The wrickkened pathways of FGF23, MEPE and PHEX. Crit Rev Oral Biol Med 2004; 15:264-281.
32. Saladin KS. Anatomy and Physiology: The unity of form and function. McGraw-Hill Inc 1998.
33. Yamamoto T, Sasaki S. Aquaporins in the kidneys: Emerging new aspects. Kidney Int 1998;54:1041-1051.
34. http://www.nephrologychannel.com
35. http://www.ivy-rose.co.uk/Topics/Urinary_System_Kidney_Diagram.php
36. http://en.wikipedia.org/wiki/Renal_tubule#Renal_tubule
37. http://compepid.tuskegee.edu/syllabi/pathobiology/pathology/clinpath/chapter7.html
38. http://www.fpnotebook.com/REN77.htm
39. http://www.emedicine.com/med/topic498.htm
40. http://www.emedicine.com/med/byname/nephritis-interstitial.htm
41. http://www.orpha.net/static/GB/dent_disease.html
42. http://www.diabetesinsipidus.org/water_deprivation_protocol_pdf.pdf
43. http://www.gpnotebook.co.uk/cache/-1435828200.htm
44. http://www.ebi.ac.uk/interpro/IEntry?ac=IPR002968.
45. http://en.wikipedia.org/wiki/Beta-2_microglobulin
46. http://www.thedoctorsdoctor.com/labtests/cystatin_c.htm
47. http://www.ndif.org/facts.html

Figure 1

Gross Anatomy of the Kidney

(adapted from http://www.ivy-rose.co.uk/Topics/Urinary_System_Kidney_Diagram.php)

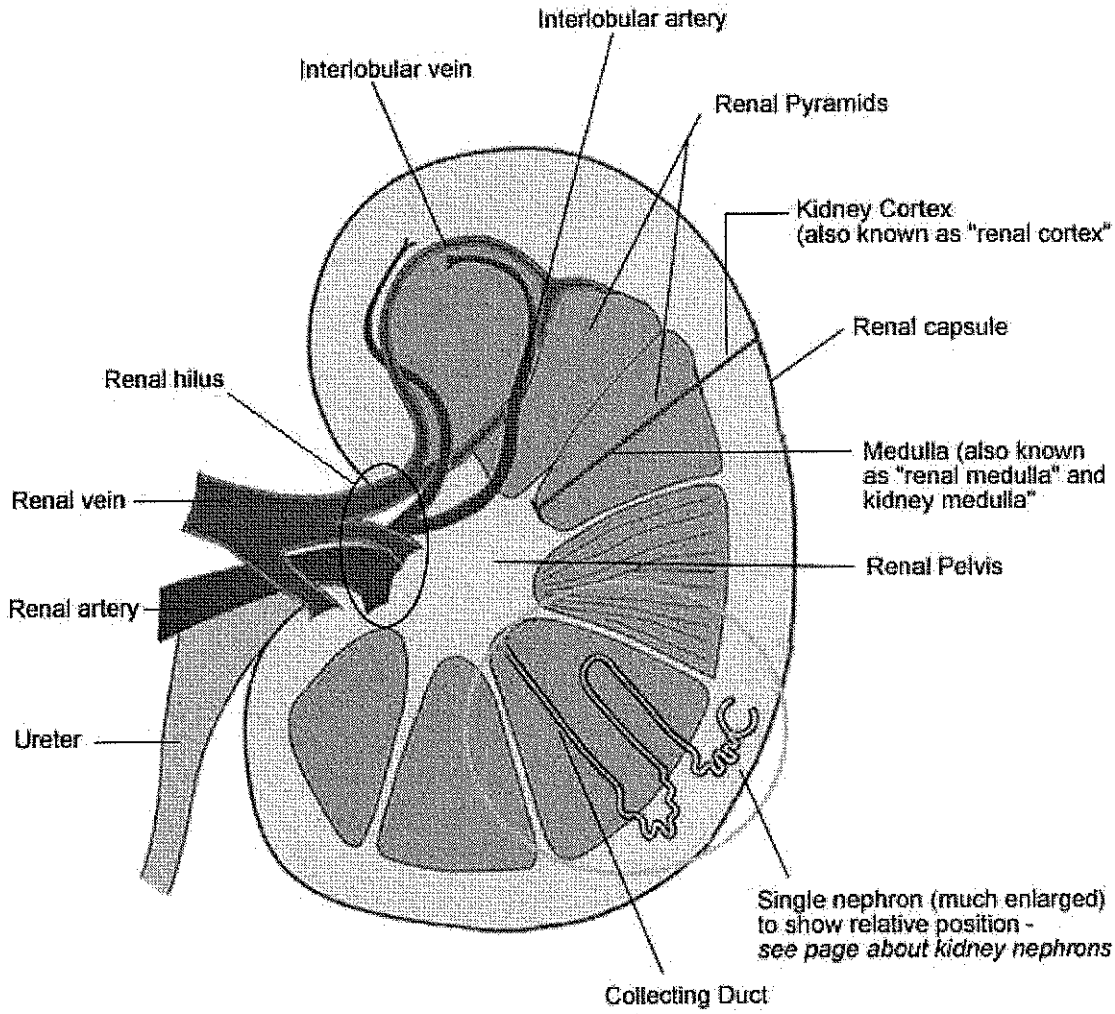


Figure 2

Simple Diagram of a Kidney Nephron

(adapted from http://www.ivy-rose.co.uk/Topics/Urinary_System_Kidney_Diagram.php)

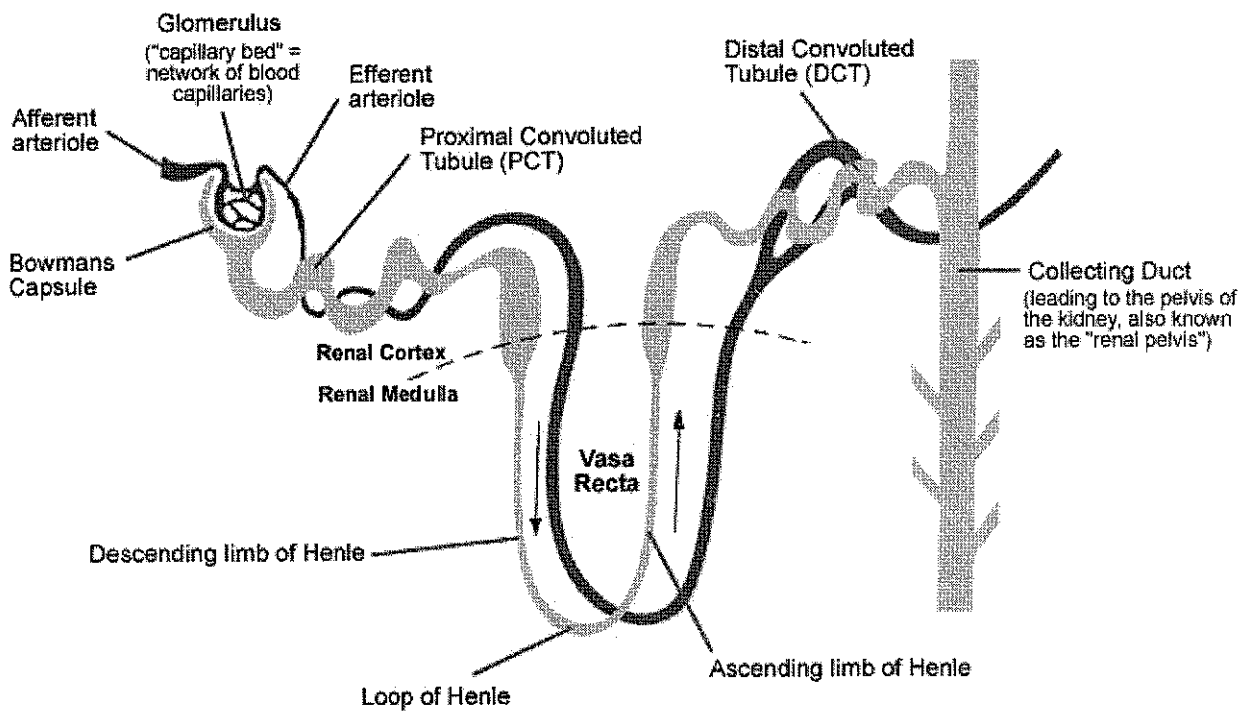


Figure 3

Schematic representation of renal fluid and mineral handling

(adapted from Saladin KS. Anatomy and Physiology: The unity of form and function. McGraw-Hill Inc 1998)

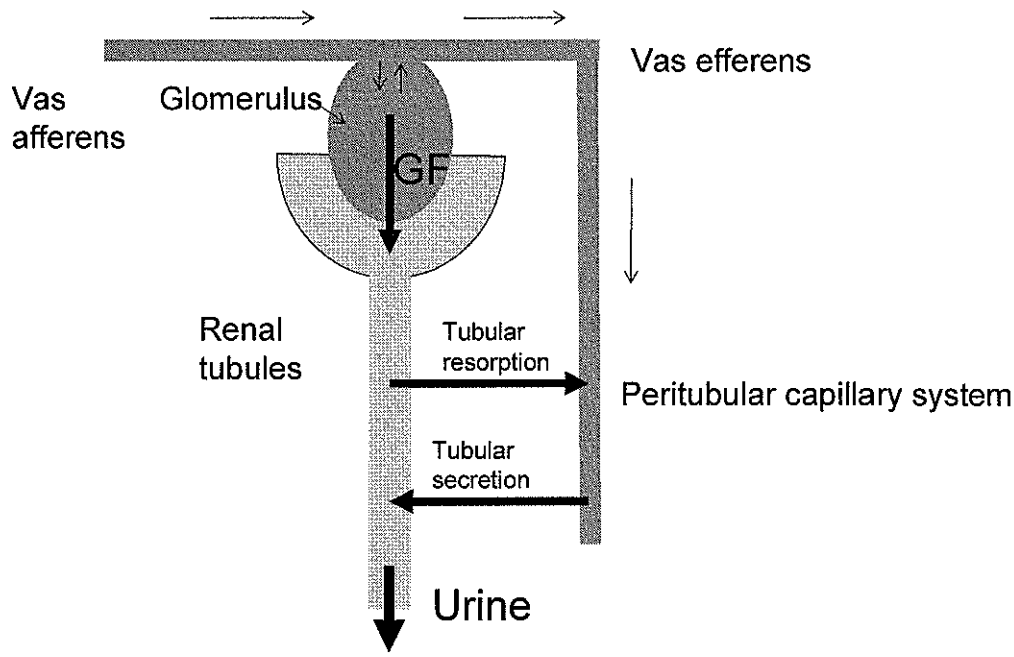
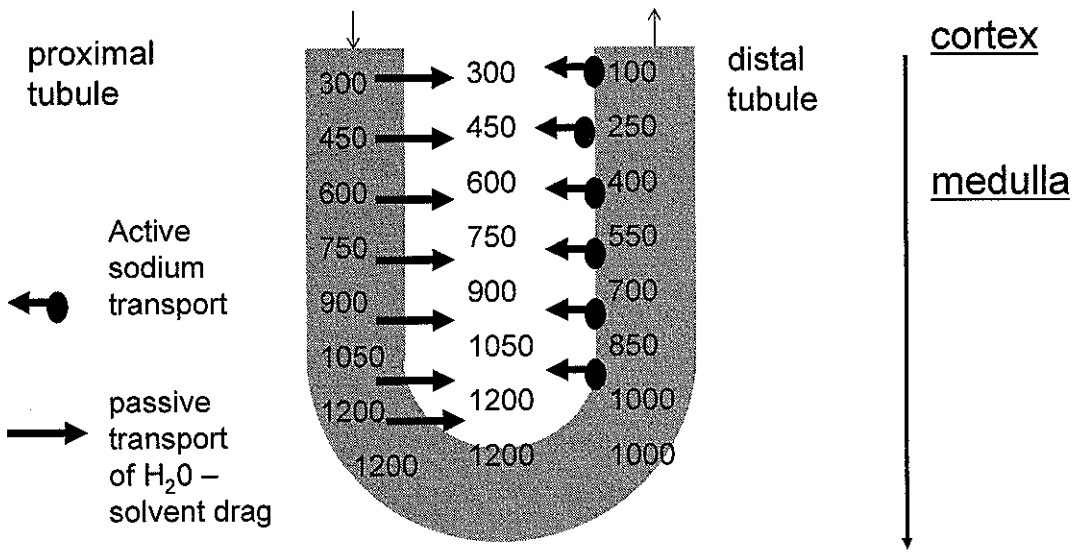


Figure 4

Osmotic gradient in the kidney

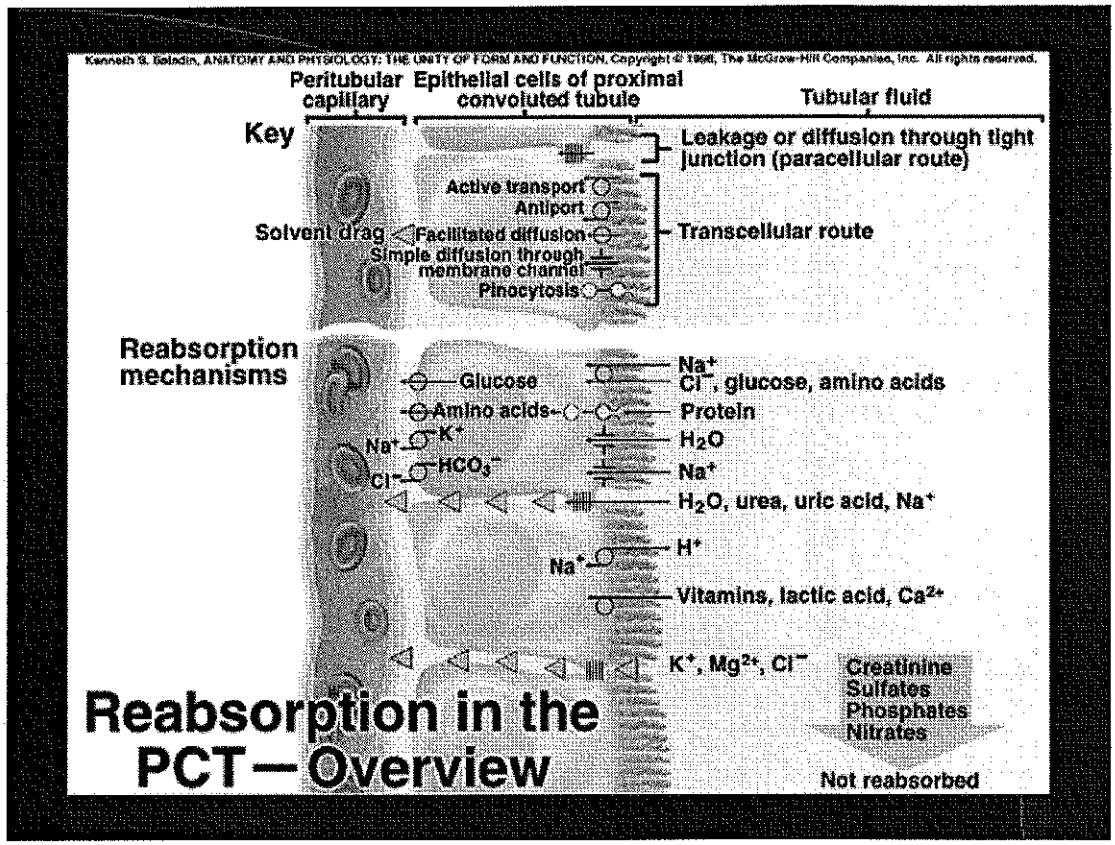
(adapted from Saladin KS. Anatomy and Physiology: The unity of form and function. McGraw-Hill Inc 1998)



Loop of Henle

Figure 5

Reabsorption in the proximal convoluted tubule



(adapted from Saladin KS. Anatomy and Physiology: The unity of form and function. McGraw-Hill Inc 1998)

CHAPTER 2

AIMS OF THE STUDIES AND OUTLINE OF THE THESIS

CHAPTER 2

AIMS OF THE STUDIES AND OUTLINE OF THE THESIS

2.1. Aims

The ***first aim*** of the thesis was to assess reference data for urinary U-NAG activity for all paediatric age groups in order to expand our reference values, because knowledge of normal physiological variation is necessary to identify pathological changes.

The ***second aim*** was to study the changes in U-NAG activity in various disease states and to look for relationship between U-NAG activity and relevant clinical and laboratory data, this by means of original results and primary findings.

The specific research questions were:

- What are the values of U-NAG activity in healthy children and how do these values relate to age?
- Is there any relationship between U-NAG activity and degree of calciuria and skeletal status in children with idiopathic hypercalciuria?
- Are there any changes in U-NAG and calciuria in children with nocturnal enuresis?
- What are the U-NAG levels in children with vesicoureteral reflux and is there any relationship between U-NAG and the grading of vesicoureteral reflux (VUR)?
- Is there any increase of U-NAG in children with hydronephrosis, and if yes, are these changes related to the grading of hydronephrosis and to the surgical prognosis ?

2.2. Outline of the thesis

This thesis gives a detailed account of various studies, not necessarily in the sequence in which these were carried out.

- **Chapter 3** is a review article concerning the diagnostic role of U-NAG activity in the detection of renal tubular impairment, summarizing current knowledge on NAG in renal disease.
- **Chapter 4** is an original article presenting reference data of U-NAG activity for all paediatric age groups. The reference data are based on statistical analysis of U-NAG/Cr evaluation in 262 healthy children aged 0-18 years.
- **Chapter 5** is an original article dealing with U-NAG values in 20 paediatric patients with idiopathic hypercalciuria (IH) and U-NAG relationship to urinary calcium (U-Ca).
- **Chapter 6** is an original article evaluating bone mineral density (BMD) and U-NAG values in 15 children with IH, and looking for mutual relationship among U-Ca, U-NAG and BMD, respectively.

- **Chapter 7** is a Letter to the Editor concerning the values of U-Ca and U-NAG in 14 children with nocturnal enuresis (NE).
- **Chapter 8** is an extended abstract presenting U-NAG in 22 children with various grades of VUR and seeking relationship betwen U-NAG and clinical parameters.
- **Chapter 9** is an original article on U-NAG in 31 paediatric patients with hydronephrosis. Relationship between grading of hydronephrosis and U-NAG is sought.
- **Chapter 10** is a general discussion.
- **Chapter 11** summarizes this thesis.

CHAPTER 3

THE DIAGNOSTIC ROLE OF URINARY N-ACETYL-β-D-GLUCOSAMINIDASE (NAG) ACTIVITY IN THE DETECTION OF RENAL TUBULAR IMPAIRMENT

Acta Medica 2005;48:75-80

CHAPTER 3

THE DIAGNOSTIC ROLE OF URINARY N-ACETYL-β-D-GLUCOSAMINIDASE (NAG) ACTIVITY IN THE DETECTION OF RENAL TUBULAR IMPAIRMENT

Sylva Skálová

Charles University in Prague, Faculty of Medicine and University Hospital in Hradec Králové, Czech Republic; Department of Paediatrics

Summary: The kidney function can be assessed by a number of methods. The urinary excretion of enzymes, in particular N-acetyl-β-D-glucosaminidase (NAG), is considered a relatively simple, cheap, fast and non-invasive method in the detection and follow-up of renal tubular function under various conditions. The determination of urinary NAG provides a very sensitive and reliable indicator of renal damage, such as injury or dysfunction due to diabetes mellitus, nephrotic syndrome, inflammation, vesicoureteral reflux, urinary tract infection, hypercalciuria, urolithiasis, nephrocalcinosis, perinatal asphyxia, hypoxia, hypertension, heavy metals poisoning, treatment with aminoglycosides, valproate, or other nephrotoxic drugs. This paper gives an overview of the current use of urinary NAG in the detection of renal injury.

Key words: *Urinary N-acetyl-β-D-glucosaminidase; Renal tubular function*

Introduction

The kidney plays a major role in maintaining constant volume and composition of the extracellular fluid. In this aspect, the kidney performs three basic functions: glomerular filtration, tubular reabsorption, and tubular secretion. The kidney function can be evaluated by a number of methods, including the assessment of urinary enzymes. Enzyme activity is normally low in urine and may increase when renal tubular cells are injured (15). Urinary enzymes, especially N-acetyl-β-D-glucosaminidase (NAG), alanin-aminopeptidase (AAP), alkaline phosphatase (ALP) are very sensitive indicators of kidney parenchymal damage when compared to functional measurements, such as glomerular filtration rate (GFR) by creatinine or inulin clearance. The relatively low sensitivity of GFR can be attributed to the great functional reserve of the kidney due to its ability to compensate for the

damage (15). The assessment of urinary enzymes is considered a relatively simple, cheap, fast and non-invasive method in the detection and follow-up of renal disorders. The urinary activity of NAG is one of the most frequently evaluated urinary enzymes as it is a very sensitive marker of renal tubular impairment (17,58,59,80). Furthermore, the increased urinary activity of NAG precedes changes in the serum creatinine or endogenous creatinine clearance; and the urinary NAG activity has been reported to correlate with the activity of the disease (15,17,44,58,59,80). This article aims to give an overview of the diagnostic role of urinary NAG.

The characteristics of N-acetyl-β-D-glucosaminidase

NAG is a lysosomal enzyme which is abundantly present in cells of the proximal kidney tubule. The NAG has a relative high molecular weight of 130 000 to 140 000 daltons which does not permit its filtration through the glomerular basal membrane. Therefore, its urinary excretion is relatively constant with minimal diurnal changes. NAG is stable against changes in pH and temperature. The NAG consists of several isoenzymes. The two principal izoenzymes which are present in the kidney and liver, respectively, are the acidic form A and basic form B, together with small amount of intermediate forms I_1 and I_2. In the serum, NAG is represented predominantly by the A^s form which is also the only NAG form in the cerebrospinal and synovial fluid. Serum of the pregnant women contains P form of NAG which is similar to the I_2 form. C form of NAG is present in the nervous tissue. The urine of healthy human subjects contains small amount of NAG, with the A isoenzyme:B isoenzyme ratio of 4:1 to 10:1, while the intermediary forms are not detectable. In patients with tubular and interstitial renal impairment, the total activity of urinary NAG is elevated, in particular its B form, resulting in changes of the A:B ratio (58,59,80). The intermediary forms of NAG are increased as well, but their activity seldom exceeds 5% of the total urinary NAG. In diseases affecting the glomerular membrane, the A^s isoenzyme is usually detectable in the urine (17,58,59,80).

Methods of assaying NAG catalytic activity in urine

For the evaluation of NAG, spot urine, collected after the first morning void, should be used. As mentioned before, NAG is stable against changes in pH and temperature and its endogenous inhibitors in the urine specimens, such as urea and ascorbic acid, can be easily eliminated by appropriate sample dilution in the reaction mixture and by keeping the rest of the experimental conditions (pH, substrate concentration and incubation time) close to their optima (17,57). Currently, there are several methods of assaying the urinary NAG catalytic activity. The fluorimetric assay based on the fluorescent 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide substrate was introduced in the late sixties and has been followed by more user-friendly colorimetric and spectrophotometric methods (17,57). The fluorescent method is sensitive enough to determine very low enzyme activities in urinary specimen diluted 20 – 50-fold to eliminate the influence of endogenous low molecular weight effectors (17). Moreover, each laboratory had to establish its own normal reference intervals as a consequence of the

activity arisen in the interlaboratory standardization of the procedure (17). However, fluorimetry still remains a useful method in most routine laboratories, as it is cheap, simple and relatively user-friendly. The spectrophotometric method is based on highly soluble and stable 4-nitrophenyl-N-acetyl-β-D-glucosaminide as substrate. However, the sensitivity of the assay could only be kept at an acceptable level by the addition of large aliquots of the urine samples to the reaction mixtures (17).

Highly sophisticated and powerful colorimetric procedures are based on the use of 2-methoxy-4-(2'-nitrovinyl)-phenyl-N-acetyl-β-D-glucosaminide and m-cresolsulphonphthaleinyl-N-acetyl-β-D-glucosaminide as substrate, respectively. In both these cases the colour of the urine does not disturb the assays (17).

The urinary NAG values should be expressed as a ratio to urinary creatinine concentration, as this relationship shows less variability than the urinary enzyme excretions related to volume or time (17). When evaluating the urinary NAG activity in various disease states, most authors used control groups for comparison. However, especially in children, it seems more appropriate to obtain and use age-dependent urinary NAG reference values from sufficiently large healthy population for the proper evaluation of kidney function (16,17,57,69). Due to the fact that the urinary NAG/creatinine ratio tends do decrease with age in children as a result of a concomitant rise in the urinary creatinine concentration (17,57), and as there is a great interindividual variability of those values in children, as reflected by the standard deviation (17,69), the use of proper pediatric reference values is quite reasonable (16,17,52,57,69).

The most representative articles on urinary NAG reference ranges consisted of values obtained from 528 healthy schoolchildren (69), 123 healthy children aged 1-14 years (16), and 262 healthy children (141 boys and 121 girls) aged 0 month through 18 years (68), respectively, where strong age-dependency has been always observed.

Urinary NAG activity in various disease states

The determination of urinary NAG is a non-invasive test and provides a very sensitive and reliable indicator of renal damage, such as injury or dysfunction due to diabetes mellitus, nephrotic syndrome, inflammation, urinary tract infection, hypercalciuria, urolithiasis, nephrocalcinosis, perinatal asphyxia, heavy metals poisoning, treatment with aminoglycosides, valproate or other nephrotoxic drugs, vesicoureteral reflux, hypoxia, hypertension. Urinary NAG is used as a routine marker of renal tubular impairment in the above mentioned disease states. As of November 20, 2004, there were 1165 publications on urinary NAG indexed in the Medline/PubMed. Concerning each disease state, the most relevant publications are listed and briefly discussed below.

Developmental kidney abnormalities

Increased urinary NAG was found in 18 pediatric patients with multicystic kidney (56) and in 16 patients aged 1-26 years with unilateral renal agenesis or history of nephrectomy (73).

Vesicoureteral reflux

Evidence of tubular dysfunction is common in children with vesicoureteral reflux (VUR) and renal scarring. The urinary NAG excretion was examined in 84 children with VUR grade III to V, and was elevated in children with grades IV and V, especially in patients with associated renal scarring (48). This was further confirmed in another study involving 40 children with history of VUR without evidence of scarring, 93 children with history of VUR and scarring and 10 children with previous urinary tract infection without VUR (76). However, in another study comparing 90 urinary samples from refluxing patients and 142 samples from nonrefluxers, only VUR grade V had a significant elevation of NAG (88). In adults with reflux nephropathy (n=55) with normal blood pressure, normal renal function and ureteric reimplantation in childhood, the urinary NAG was significantly higher in comparison to the control group and the NAG correlated with plasma renin activity. Such a relationship supports the concept of segmental perfusion and filtration as an important mechanism (24). In yet another study, 27 pediatric patients (mean age 1.73 ± 1.43years) with at least 2 episodes of urinary tract infection in the previous 2 months, received prophylactic regimen with cefixime. In the patients with VUR (n=7), high urinary values of NAG were observed in comparison to those children with recurrent urinary tract infection without VUR (n=20), this in spite of cefixime prophylaxis (22).

Obstructive uropathy

High urinary NAG was reported in 40 children aged 3 weeks to 16 years with unilateral ureteropelvic obstruction (n=30) or primary obstructive megaureter (n=10) in comparison to controls (13).

Urinary tract infection (UTI)

Increased urinary NAG was found in children aged 1 to 8 years with upper urinary tract infection (n= 96) compared to control group (n=72) (10), and in 24 febrile infants with urinary tract infection, regardless of the level of infection (30). Urinary NAG may be therefore an infomative indicator of UTI (30). In children with fever of non-renal origin (n =68) and those with pyelonephritis (n=25), there was an increase in urinary NAG with only moderately significant differences between the two groups. This may indicate that proximal tubular dysfunction may additionally be due to fever-associated function processes (92).

Nephrotic syndrome

Urinary NAG is higher in primary nephrotic children, and especially in those in the relapse phase than in those in remission. A positive correlation between proteinuria and urinary NAG was apparent (11,19,23,77). Furthermore, in a study involving 14 children with cortico-sensitive nephrotic syndrome, 5 with cortico-resistant nephrotic syndrome and 30 healthy controls, the urinary excretion of NAG was higher in nephrotic children, especially in those with cortico-resistant nephrotic syndrome (23). There were correlations between urinary NAG and serum cholesterol and negative correlations between urinary NAG and serum total proteins and albumin (23). In 23 pediatric patients with steroid-sensitive and 21 with steroid-resistant nephrotic syndrome the urinary NAG was correlated with

albumin excretion (77). Similar results were reported by Valles et al (83), with higher values of urinary NAG in steroid-resistant nephrotic syndrome. These results suggest tubular impairment in nephrotic syndrome, especially in cortico-resistant patients.

Nephrotoxic drugs

Increased urinary NAG was observed due to application of various drugs. Both paediatric and adult patients treated with antibiotics, such as aminoglycosides, applied either parenterally or locally, had high urinary NAG (36,53,54,60,85,86). The application of aminoglycosides thus leads to transient tubular dysfunction. Urinary NAG is therefore frequently used to monitor the nephrotoxic effects of gentamycin (53,60), tobramycin (54) and their various dosing regimen (54,60).

In patients treated with anticonvulsants, in particular valproate, high urinary NAG was repeatedly confirmed (5,18,84). Treatment of both pediatric and adult patients with antineoplastic drugs, such as methotrexate or cisplatin, is also accompanied by tubular dysfunction, as reflected by increased urinary NAG (9,25,29,47,55,75,90). Mild-to-moderate subclinical glomerular and tubular damage can be identified in many childhood cancer survivors, most probably as a result of drug toxicity (90). However, most patients experience some spontaneous recovery from acute nephrotoxicity. Out of 115 childhood cancer survivors, pathologically elevated urinary NAG was noted in 38% of leukemia/lymphoma, 54% of solid tumor and 20% of Wilms tumor survivors (9).

Increased urinary NAG was observed in patients with low-flow and high-flow sevoflurane anesthesia (26). However, no synergistic effect of low-flow sevoflurane and amikacin was noticed in surgical patients (27).

Urinary NAG is known to be elevated in patients with rheumatoid arthritis treated with methotrexate, and has been used to monitor and compare nephrotoxicity due methotrexate or infliximab. The introduction of infliximab during methotrexate therapy demonstrated no early or delayed nephrotoxicity of the drug in patients with rheumatoid arthritis (87). In patients with amyloid deposits, the NAG activity exceeded twice the upper normal limit (87).

In renal transplant recipients a substantial dependence of the activity of urinary NAG on cyclosporine doses and period after transplantation was observed (43).

Heavy metals poisoning

There is an extensive amount of evidence concerning tubular dysfunction as reflected by the increase in urinary NAG in heavy metals poisoning, or exposure to mercury (42,72) lead (14,21) or cadmium (64,78,82) due to environmental pollution. For example, as a result of an extreme pollution in the region of Central Asia, the renal tubular function of children around the Aral Sea is profoundly impaired, as indicated by increases in urinary NAG and urinary beta-2-microglobulin (31). Furthermore, high concentrations of cadmium in placenta, amniotic fluid and milk was revealed in pregnant smokers,

together with increase in NAG activity in urine, amniotic fluid and milk (46). Cigarette smoking has a nephrotoxic effect and also is synergistic to lead nephrotoxicity on urinary excretion of NAG (21).

Kidney transplants

Low urinary excretion of NAG is helpful in the diagnosis of kidney transplant rejection As the amount of excreted NAG depends on the graft mass and the amount of urinary creatinine depends on the recipient body mass, a low NAG excretion (related to urinary creatinine) could be a surrogate marker of an unfavorable low graft to body weight ration, which in turn might be associated with a reduced graft survival (37).

Hypercalciuria, urolithiasis, nephrocalcinosis

In patiens with hypercalciuria and/or urolithiasis, the urinary NAG was evaluated (8,33,67,71,74). The excretion of urinary NAG has been reported as either increased (8,67,71,74), or normal (33), in children with idiopathic hypercalciuria. Recently, urinary NAG was reported as significantly higher in children with urolithiasis and nephrocalcinosis, but not in children with isolated idiopathic hypercalciuria alone, and did not correlate with the urinary excretion of oxalate or calcium (67). Therefore, the increased urinary NAG in patients with idiopathic hypercalciuria might be a result of local tubular damage due to cell-crystal interactions rather than a manifestation of impaired tubular re-absorption (67).

Hypertension

The urinary NAG excretion was reported as increased in patients with hypertension (2,61,65,66,81), and significant correlations were found between NAG excretion and systolic and diastolic blood pressures (2). Changes in urinary NAG may evidence early hypertensive disease. High urinary NAG was observed in patients with untreated essential hypertension (61). Tubular injury (as reflected by high urinary NAG) is present in the early stages of hypertensive nephropathy and may precede glomerular damage. Ischemia due to changes in small vessels may not be the only factor responsible for this injury (81). The urinary NAG was increased in women with pre-eclampsia, but there were no correlations between urinary NAG and blood pressure (65,66). Therefore, high urinary NAG activity in women with pre-eclampsia seems to be a sign of proximal tubular damage (65,66).

Cardiac surgery

Increased urinary NAG were observed during cardiac surgery, suggesting transient perioperative renal dysfunction (3,40).

Neonatal disorders

High levels of urinary NAG have been reported in neonates with perinatal asphyxia (89) and in premature infants where it tended to be higher with the degree of prematurity, and in term and preterm neonates with renal tubular injury (34,79). The level of inflammatory cytokines in urine was elevated

together with NAG (20). The neonatal asphyxia may induce systemic inflammatory response syndrome, which results in postasphyxial renal injury (20).

Vasculitis (Henoch-Schonlein purpura)

Increased U-NAG has been observed in 12 out of 20 children with Henoch-Schoenlein purpura and correlated well with the extent of early and late renal involvement (49). In pediatric patients with Henoch-Schoenlein purpura, the urinary NAG is considered as a possible prognostic marker for the development of nephritis (49). Urinary NAG was higher in 82 children with treated Henoch-Schoenlein purpura and served as a marker of tubular dysfunction in the course of the disease (91).

Diabetes mellitus

The urinary excretion of NAG is helpful in the diagnosis of diabetic nephropathy (12,28,32,35,41,63), where urinary NAG may be increased in the early stages of diabetes mellitus even before there is any clinical evidence of renal involvement (17). Furthermore, urinary NAG may reflect glycemic control in insulin-dependent diabetic patients (28,35). Young (age 7.4-25 years) insulin-dependent diabetic patients with microangiopathic complications (n= 50) had an increased rate of urinary NAG excretion (41). The urinary NAG was considered as a predictive marker for the development of microalbuminuria in adolescents with diabetes, as urinary NAG excretion preceded the increase of albumin excretion (35). In yet another study, urinary NAG levels in the children with diabetes were significantly higher than those of controls. In 42 children with type 1 diabetes, there were positive correlations between urinary NAG levels and microalbuminuria, Hb A1c and systolic and diastolic blood pressure values. 59.5% of diabetic children were positive for urinary NAG, while 38.1% of them were positive for microalbuminuria (1). In patients with type 1 diabetes mellitus, proximal tubular dysfunction (manifested by high urinary NAG) may occur independently of glomerular alteration (62). Another study found significant correlations between high urinary NAG values and disease duration ($P < 0.05$), HbA1c ($P < 0.05$), diastolic blood pressure ($P < 0.05$) and puberty ($P < 0.05$) (50).

In 27 patients with non-insulin-dependent diabetes mellitus (NIDDM), a significant correlation was found between urinary NAG and creatinine clearance. Elevation of urinary NAG may indicate decreased renal function during early stage NIDDM nephropathy (32). Urinary NAG excretion was elevated in patients with type 2 diabetes mellitus compared with healthy individuals and increased as nephropathy progressed. Pentoxifylline administration was effective in reducing proteinuria and urinary NAG excretion in these patients (51).

Various diseases

High urinary NAG was found in patients with glycogen storage disease (39) and in children with iron overload in beta-thalassaemia major (45), in patients with Wilson's disease (70), in children with liver cirrhosis (7), with Lowe's syndrome (38) and in children with familial Mediterranean fever (4) .

Asymptomatic primary hyper-N-acetyl-beta-D-glucosaminidaseuria

A rare diagnosis of asymptomatic primary hyper-N-acetyl-beta-D-glucosaminidaseuria was proposed in two patients with high urinary NAG excretion and no renal abnormalities, including normal findings on renal biopsy. This is a probaly new clinical entity of renal tubular disorders (6).

Conclusions

The assessment of urinary NAG should be considered as a useful marker of renal tubular impairment in various disease states. It is extensively used both in routine practice as well as for research purposes, when it comes to the evaluation of tubular function. Other urinary enzymes (such as alanin aminopeptidase, alkaline phosphatase) are also sensitive indicators of kidney parenchymal damage compared to functional measurements. However, urinary NAG remains the most widely used marker of renal tubular impairment.

References

1. Abdel Shakour S, El-Hefnawy H, El-Yamani MY, Azmi Y.Urinary N-acetyl-beta-D-glucosaminidase in children with diabetes as an early marker of diabetic nephropathy. East Mediterr Health J 2002;8:24-30.
2. 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;9:157-61.
3. Aho PS, Niemi T, Lindgren L, Lepantalo M. Endovascular vs open AAA repair: similar effects on renal proximal tubular function. Scand J Surg 2004;93:52-6.
4. Akkus S, Caliskan S, Kasapcopur O. Tubular functions in familial Mediterranean fever. Turk J Pediatr 2002;44:317-20.
5. Altunbasak S, Yildizas D, Anarat A, Burgut HR. Renal tubular dysfunction in epileptic children on valproic acid therapy. Pediatr Nephrol 2001;16:256-9.
6. Asami T, Soichiro O, Kasahara T, Uchiyama M. Asymptomatic primary hyper-N-acetyl-beta-D-glucosaminidaseuria: a new clinical entity? Pediatr Nephrol 2002;17:560-5.
7. Aydogdu S, Akil I, Akil T, Kabasakal C, Killi R, Mir S, Yagci R.
Renal resistive indexes and some renal functions in liver cirrhotic children. Pediatr Int 2004;46:67-71.
8. Balla AA, Salah AM, Abdolmotaal E, Hoppe B, Bongartz D, Kessler T, Hesse A. N-acetyl-beta-D-glucosaminidase excretion in healthy children and in pediatric patients with urolithiasis. World J Urol 1998;16:413-6.
9. Bardi E, Olah AV, Bartyik K, Endreffy E, Jenei C, Kappelmayer J, Kiss C. Late effects on renal glomerular and tubular function in childhood cancer survivors. Pediatr Blood Cancer 2004;43:668-73.
10. Belli A, Scarlecio F, Martino F, Guido F, Aprigliano D, Lorusso G, Mannarino O, Ruberto U. Evaluation of N-acetyl-beta-D-glucosaminidase in upper and lower urinary tract infections in childhood. Clinical study of 168 children. Minerva Pediatr 1996;48:503-7.
11. Caliskan S, Hacibekiroglu M, Sever I, Ozbay G, Arisoy N. Urinary N-acetyl-beta-D-glucosaminidase and beta-2-microglobulin excretion in primary nephrotic children. Nephron 1996;74:401-4.
12. Caliskan S, Ficicioglu C, Hacibekiroglu M, Mikla S, Kasapcopur O, Sever L, Aydin A, Arisoy N. Tubular markers in children with insulin dependent diabetes mellitus. Turk J Pediatr 1997;39:213-8.
13. Carr MC, Peters CA, Retik AB, Mandell J. Urinary levels of the renal tubular enzyme N-acetyl-beta-D-glucosaminidase in unilateral obstructive uropathy. J Urol 1994;151:442-5.
14. Chia KS, Jeyaratnam J, Lee J, Tan C, Ong HY, Ong CN, Lee E. Lead-induced nephropathy: relationship between various biological exposure indices and early markers of nephrotoxicity. Am J Ind Med 1995;27:883-95.
15. Chiu JSP. Models used to assess renal function. Drug Devel Res 1994;32:247-55.
16. Csathy L, Olah AV, Pocsi I, Varga J, Balla G, Price RG. Age-dependent urinary N-acetyl-beta-D-glucosaminidase activity in healthy children. Orv Hetil 1994;135:1301-3.
17. Csáthy L, Pócsi I. Urinary N-Acetyl-β-*D*-glucosaminidase determination in newborns and children: methods and diagnostic applications. Eur J Clin Chem Clin Bioch 1995;33:575-87.

18. Csathy L, Olah AV, Clemens B, Gyorgy I, Varga J. Urinary N-acetyl-beta-D-glucosaminidase in epileptic children treated with antiepileptic drugs. Arch Dis Child 2000;83:420-2.
19. Dillon SC, Taylor GM, Shah V. Diagnostic value of urinary retinol-binding protein in childhood nephrotic syndrome. Pediatr Nephrol 1998;12:643-7.
20. Dong WB, Tang ZH, Chen HY, Chen SQ, Wang XY, Wang SH, Zhai XS, Xiao DC.Change in the level of inflammatory cytokines and their relationship to the indicator in evaluating renal tubules injury of urinary in neonates with asphyxia. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2003;15:94-6.
21. EL-Safty IA, Afifi AM, Shouman AE, EL-Sady AK. Effects of smoking and lead exposure on proximal tubular integrity among Egyptian industrial workers. Arch Med Res 2004;35:59-65.
22. Fanos V, Mussap M, Osio D, Pizzini C, Plebani M. Urinary excretion of N-acetyl-beta-D-glucosaminidase and epidermal gfrowth factor in paediatric patients receiving cefixime prophylaxis for recurrent urinary tract infections. Clin Drug Invest 2001; 21:511-18.
23. Fede C, Conti G, Chimenz R, Ricca M. N-acetyl-beta-D-glucosaminidase and beta-2-microglobulin: prognostic markers in idiopathic nephrotic syndrome. J Nephrol 1999;12:51-5.
24. Goonasekera CD, Shah V, Dillon MJ. Tubular proteinuria in reflux nephropathy: post ureteric re-implanatation. Pediatr Nephrol 1996;10:559-63.
25. Hempel L, Misselwitz J, Fleck C, Kentouche K, Leder C, Appenroth D, Rost M, Zintl F.Influence of high-dose methotrexate therapy (HD-MTX) on glomerular and tubular kidney function. Med Pediatr Oncol 2003;40:348-54.
26. Higuchi H, Sumita S, Wada H, Ura T, Ikemoto T, Nakai T, Kanno M, Satoh T. Effects of sevoflurane and isoflurane on renal function and on possible markers of nephrotoxicity. Anesthesiology 1998;89:307-22.
27. Higuchi H, Adachi Y. Renal function in surgical patients after administration of low-flow sevoflurane and amikacin. J Anesth 2002;16:17-22.
28. Hsiao PH, Tsai WS, Tsai WY, Lee JS, Tsau YK, Chen CH. Urinary N-acetyl-beta-D-glucosaminidase activity in children with insulin-dependent diabetes mellitus. Am J Nephrol 1996;16:300-3.
29. Ikarashi Y, Kakihara T, Imai C, Tanaka A, Watanabe A, Uchiyama M. Glomerular dysfunction, independent of tubular dysfunction, induced by antineoplastic chemotherapy in children. Pediatr Int 2004;46:570-5.
30. Jantausch BA, Rifai N, Getson P, Akram S, Majd M, Wiedermann BL. Urinary N-acetyl-beta-D-glucosaminidase and beta-2-microglobulin in the diagnosis of urinary tract infection in febrile infants. Pediatr Infect Dis J 1994;13:294-9.
31. Kaneko K, Chiba M, Hashizume M, Kunii O, Sasaki S, Shimoda T, Yamashiro Y, Caypil W, Dauletbaev D.Renal tubular dysfunction in children living in the Aral Sea Region. Arch Dis Child 2003;88:966-8.
32. Kato H, Takashima T, Kishikawa H, Emura S, Ohmori K. The significance of urinary N-acetyl-beta-D-glucosaminidase for predicting early stage diabetic nephropathy. Int J Clin Pract 1997;51:489-90.
33. Kavukcu S, Aydin A, Turkmen M, Akhunlar H, Fadiloglu M, Tavli V. Investigation of relationship between idiopathic hypercalciuria and urinary enzyme activities. J Assoc Physicians India 1998;46:784-5.
34. Kojima T, Sasai-Takedatsu M, Hirata Y, Kobayashi Y. Characterization of renal tubular damage in preterm infants with renal failure. Acta Paediatr Jpn 1994;36:392-5.
35. Kordonouri O, Hartman R, Muller C, Danne T, Weber B. Predictive value of tubular markers for the development of microalbuminaurie in adolescents with diabetes. Horm Res 1998;50:Suppl 1:23-7.
36. Kos M, Jazwinska-Tarnawska E, Hurkacz M, Orzechowska-Juzwenko K, Pilecki W, Klempous J.The influence of locally implanted high doses of gentamicin on hearing and renal function of newborns treated for acute hematogenous osteomyelitis. Int J Clin Pharmacol Ther 2003;41:281-6.
37. Kotanko P, Margreiter R, Pfaller W. Reduced renal allograft survival is related to low urinary N-acetyl-beta-D-glucosaminidase excretion during the first posttransplant month. Transplantation 1996;61:388-92.
38. Laube GF, Russell-Eggitt IM, van't Hoff WG.Early proximal tubular dysfunction in Lowe's syndrome. Arch Dis Child 2004;89:479-80.
39. Lee PJ, Dalton RN, Shah V, Hindmarsh PC, Leonard JV. Glomerular and tubular function in glycogen storage disease. Pediatr Nephrol 1993;9:705-10.
40. Loef BG, Henning RH, Epema AH, Rietman GW, Van Oeveren W, Navis GJ, Ebels T. Effect of dexamethasone on perioperative renal function impairment during cardiac surgery with cardiopulmonary bypass. Br J Anaesth. 2004;.....
41. Lorini R, Scaramuzza A, Cortona L, Valenti G, d'Annunzio G, Melzi d Éril GV. Increased urinary N-acetyl-beta-D-glucosaminidase excretion in young insulin-dependent diabetic patients. Diabetes Res Clin Pract 1995;29:99-105.
42. Mandic L, Radmila M, Jelena A, Dubravka D. Change in the iso-enzyme profiles of urinary N-acetyl-beta-D-glucosoaminidase in workers exposed to mercury. Toxicol Ind Health 2002;18:207-14.
43. Marchewka Z, Kuzniar J, Dlugosz A. Enzymatic markers of cyclosporine nephrotoxicity in patients after renal transplantation. Int Urol Nephrol 1999;31:727-34.

44. McAuley FT, Whiting PH, Thomson AW, Simpson JG. The influence of enalapril or spironolactone on experimental cyclosporin nephrotoxicity. Biochem Pharmacol 1987;36:699-703.
45. Michelakakis H, Dimitriou E, Georgakis H, Karabatsos F, Fragodimitri C, Saraphidou J, Premetis E, Karagiorgia-Lagana M. Iron overload and urinary lysosomal enzyme levles in beta-thalassaemia major. Eur J Pediatr 1997;156:602-4.
46. Milnerowicz H, Slowinska M. Concentration of metal, ceruloplasmin, metallothionein and the activity of N-acetyl-beta-D-glucosaminidase and gamma-glutamyltransferase in pregnant women who smoke and in those environmentally exposed to tobacco smoke and in their infants. Int J Occup Med Environ Health 1997;10:187-202.
47. Mishra J, Mori K, Ma Q, Kelly C, Barasch J, Devarajan P.Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity. Am J Nephrol 2004;24:307-15.
48. Miyakita H, Puri P. Urinary levels of N-acetyl-beta-D-glucosaminidase: a simple marker for predicting tubular damage in higher grades of vesicoureteric reflux. Eur Urol 1994;25:135-7.
49. Muller D, Greve D, Eggert P. Early tubular proteinuria and the development of nephritis in Henoch-Schonlein purpura. Pediatr Nephrol 2000; 15:85-9.
50. Mungan N, Yuksel B, Bakman M, Topaloglu AK, Ozer G.Urinary N-acetyl-beta-D-glucosaminidase activity in type I diabetes mellitus. Indian Pediatr 2003;40:410-4.
51. Navarro JF, Mora C, Muros M, Maca M, Garca J.
Effects of pentoxifylline administration on urinary N-acetyl-beta-glucosaminidase excretion in type 2 diabetic patients: a short-term, prospective, randomized study. Am J Kidney Dis 2003;42:264-70.
52. 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-betaD-glucosaminidase in normal healthy infants. Acta Paediatr Jpn 1998;40:424-6.
53. Nix DE, Thomas JK, Symonds WT, Spivey JM, Wilton JH, Gagliardi NC, Schentag JJ. Assessment of the enzymuria resulting from gentamicin alone and combinations of gentamicin with various beta-lactam antibiotics. Ann Pharmacother 1997;31:696-70.
54. Olsen KM, Rudis MI, Rebuck JA, Hara J, Gelmont D, Mehdian R, Nelson C, Rupp ME.Effect of once-daily dosing vs. multiple daily dosing of tobramycin on enzyme markers of nephrotoxicity. Crit Care Med 2004;32:1678-82.
55. Ollah VA, Bekesi A, Toth A, Kovacs I, Balla G. Urinary N-acetyl-beta-D-glucosaminidase activity in leukaemic children during high-dose methotrexate therapy. Haematologia 1995;26:151-7.
56. Orikasa K, Konda R, Saka K, Ota S, Orikasa S. Eighteen cases of multicystic kidney: natural history and renal function of the contralateral kidney. Nippon Hinyokika Gakkai Zasshi 1996;87:780-8.
57. Pócsi I, Berki AC, Taylor SA, Price RG. Urea inhibition of urinary N-acetyl-β-D-glucosaminidase is mixed-type and is sensitive to changes of pH away from the optimum. Clin Chem 1993;39:1918-9.
58. Price RG. The role of NAG (N-acetyl-β-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. Clin Nephrol 1992;38:14-19.
59. Price RG. Measurement of N-acetyl-β-D-glucosaminidase and its isoenzymes in urine. methods and clinical applications. Eur J Clin Chem Clin Biochem 1992;30:693-705.
60. Ring E, Eber E, Erwa W, Zach MS. Urinary N-acetyl-beta-D-glucosaminidase activity in patients with cystic fibrosis on long-term gentamicin inhalation. Arch Dis Child 1998;78:540-3.
61. Rutkowski B, Tylicki L, Manitius J, Lysiak-Szydłowska W. Hypertensive nephropathy - an increasing clinical problem. Miner Electrolyte Metab 1999;25:65-8.
62. Salem MA, el-Habashy SA, Saeid OM, el-Tawil MM, Tawfik PH.
Urinary excretion of n-acetyl-beta-D-glucosaminidase and retinol binding protein as alternative indicators of nephropathy in patients with type 1 diabetes mellitus. Pediatr Diabetes 2002;3:37-41.
63. Sanchez-Hueso MC, Mateo-Caas J, Zamora-Madaria E. Influence of glycemic blood glucose control and incipient diabetic nephropathy on the urinary excretion of N-acetyl-beta-D-glucosaminidase in diabetes mellitus. An Med Interna 1995;12:216-20.
64. Satarug S, Nishijo M, Ujjin P, Vanavanitkun Y, Baker JR, Moore MR.
Effects of chronic exposure to low-level cadmium on renal tubular function and CYP2A6-mediated coumarin metabolism in healthy human subjects. Toxicol Lett 2004;148:187-97.
65. Semczuk-Sikora A, Sikora P, Semczuk M. N-acetyl-beta-D-glucosaminidase activity in women with preeclampsia. Ginekol Pol 2000;71:141-5.
66. Semczuk-Sikora A, Sikora P, Biadun U, Semczuk M. [Urinary N-acetyl-beta-D-glucosaminidase (NAG) excretion in women with pregnancy complicated with hypertension] Ginekol Pol 2003;74:1269-75.
67. Sikora P, Glatz S, Beck BB, Stapenhorst L, Zajaczkowska M, Hesse A. Urinary NAG in children with urolithiasis, nephrocalcinosis, or risk of urolithiasis. Pediatr Nephrol 2003;18:996-9.

68. Skálová S, Chládek J. Urinary N-acetyl-beta-D-glucosaminidase activity in healthy children. Nephrology 2004;9:19-21.
69. 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;155:596-602.
70. Sozeri E, Feist D, Ruder H, Scharer K.Proteinuria and other renal functions in Wilson's disease. Pediatr Nephrol 1997;11:307-11.
71. Stapleton FB, Chesney RW, Behrmann AT, Miller LA. Increased urinary excretion of renal N-acetyl-beta-glucosaminidase in hypercalciuria. Am J Dis Child 1985;139:950-2.
72. Tassi C, Mancuso F, Lomurno G, Lotito M, Feligioni L, De Bellis F, Eramo S, Capodicasa E.N-acetyl-beta-D-glucosaminidase activity in urine of dental personnel. Clin Chim Acta 2004;344:211-3.
73. Tekin N, Kural N, Uslu S. N-acetyl-beta-D-glucosaminidase excretion in patients with unilateral renal agenesis or nephrectomy in childhood. Turk J Pediatr 1996;38:485-90.
74. Tekin N, Kural N, Torun M. Renal function in children with hypercalciuria. Turk J Pediatr 1997;39:335-9.
75. Tomaszewska B, Zoch-Zwierz WM. Nephrotoxicity evaluation of cytostatic agents in children with acute lymphoblastic leukemia. Pediatr Pol 1995;70:915-20.
76. Tomlinson PA, Smellie JM, Prescod N, Dalton RN, Chantler C. Differential excretion of urinary proteins in children with vesicoureteric reflux and reflux nephropathy. Pediatr Nephrol 1994;8:21-5.
77. Tomlinson PA, Dalton RN, Hartley B, Haycock GB, Chantler C. Low molecular weight protein excretion in glomerular disease: a comparative analysis. Pediatr Nephrol 1997;11:285-90.
78. Trzcinka-Ochocka M, Jakubowski M, Razniewska G, Halatek T, Gazewski A.The effects of environmental cadmium exposure on kidney function: the possible influence of age. Environ Res 2004;95:143-150.
79. Tsukahara H, Hori C, Tsuchida S, Hiraoka M, Sudo M, Haruki S, Suehiro F. Urinary N-acetyl-beta-D-glucosaminidase excretion in term and preterm neonates. J Paediatr Child Health 1994;30:536-8.
80. Tucker SM, Pierce RJ, Price RG. Characterization of human N-acetyl-β-D-glucosaminidase isoenzymes as an indicator of tissue damage in disease. Clin Chim Acta 1980;102:29-40.
81. Tylicki L, Manitius J, Lysiak-Szydlowska W, Rutkowski B. Tubular injury: the first symptom of hypertensive kidney involvement? Med Sci Monit 2003;9:135-41.
82. Uchida M, Teranishi H, Aoshima K, Katoh T, Kasuya M, Inadera H.Reduction of erythrocyte catalase and superoxide dismutase activities in male inhabitants of a cadmium-polluted area in Jinzu river basin, Japan. Toxicol Lett 2004;151:451-7.
83. Valles P, Peralta M, Carrizo L, Martin L, Principi I, Gonzalez A, Manucha W. Follow-up of steroid-resistant nephrotic syndrome: tubular proteinuria and enzymuria. Pediatr Nephrol 2000;15:252-8.
84. Verrotti A, Greco R, Pascarella R, Matera V, Morgese G, Chiarelli F. Renal tubular function in patients receiving anticonvulsant therapy: a long-term study. Epilepsia 2000;41:1432-5.
85. Watanabe A, Nagai J, Adachi Y, Katsube T, Kitahara Y, Murakami T, Takano M.Targeted prevention of renal accumulation and toxicity of gentamicin by aminoglycoside binding receptor antagonists. J Control Release 2004;95:423-33.
86. Wiland P, Szechcinski J. Proximal tubule damage in patients treated with gentamicin or amikacin. Pol J Pharmacol 2003;55:631-7.
87. Wiland P, Wiela-Hojenska A, Glowska A, Chlebicki A, Hurkacz M, Orzechowska-Juzwenko K, Szechinski J.Renal function in rheumatoid arthritis patients treated with methotrexate and infliximab. Clin Exp Rheumatol 2004;22:469-72.
88. Williams MA, Jones D, Noe HN. Urinary N-acetyl-beta-D-glucosaminidase as a screening technique for vesioureteral reflux. Urology 1994;43:528-30.
89. Willis F, Summers J, Minutillo C, Hewitt I. Indices of renal tubular function in perinatal asphyxia. Arch Dis Child Fetal Neonatal Ed 1997;77:F57-60.
90. Zajaczkowska M, Stefaniak J, Sikora P, Choma-Smaga M, Filiks-Litwin B, Szajner-Milart I, Borzecka H. Urinary excretion of N-acetyl-beta-D-glucosaminidase and alpha1-microglobulin in children with proliferative blood diseases. Ann Univ Mariae Curie Sklodowska [Med] 2003;58:348-53.
91. Zhou JH, Huang AX, Liu TL.Clinical study on treatment of childhood Henoch-Schonlein purpura nephritis with colquhounia root tablet. Zhongguo Zhong Xi Yi Jie He Za Zhi 2004;24:418-21.
92. Zoller G, Wiedemann G, Kallerhoff M, Zappel H, Weber MH, Ringert RH. Microproteinuria and enzymuria in fever and pyelonephritis in childhood. A prospective study of 180 children. Urologe A 1997;36:68-76.

CHAPTER 4

URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE ACTIVITY IN HEALTHY CHILDREN

Nephrology 2004;9:19-21

CHAPTER 4

URINARY N-ACETYL-β-D-GLUCOSAMINIDASE (NAG) ACTIVITY IN HEALTHY CHILDREN

S. Skálová[1], J. Chládek[2]

Departments of Pediatrics[1] and Clinical Pharmacology[2], Faculty of Medicine, Charles University, Hradec Králové 500 05, Czech Republic

Summary:

Aim: The principal aim was to establish pediatric reference data for the urinary N-acetyl-β-D-glucosaminidase (U-NAG) activity.

Method and Results: 262 healthy children aged 0-18 years (0-1 month, n=38; 1 mo-1 year, n=50; 1-3 y, n=50; 3-6 y, n=46; 6-10 y, n=29; 10-18 y, n=49) had a urine sample collected and the U-NAG activity was evaluated by using fluorimetry and related to urinary creatinine as nkat/mmol ratio. A strong age dependence of the U-NAG/creatinine ratio and its high interindividual variability in children was observed; the highest values of upper reference range being in the 0-1 mo and 1 mo-1y group (134.8 and 50, respectively), which dropped gradually to 7.25 in the oldest age group (10-18 years).

Conclusion: The establishment of urinary NAG reference pediatric values is a potentially useful tool for proper evaluation of renal tubular function in childhood.

Key words: *Urinary N-acetyl-β-D-glucosaminidase; Reference values; Children*

Introduction

The assessment of urinary enzymes is considered to be a relatively simple, cheap, fast and non-invasive method in the detection and follow-up of renal disorders. The urinary activity of N-acetyl-β-D-glucosaminidase (NAG) is one of the most frequently evaluated urinary enzymes as it is a very sensitive marker of renal tubular impairment (1). Furthermore, the increased urinary activity of NAG precedes changes in the renal functions detectable by other routinely established methods; the urinary NAG activity correlates with the activity of the disease (2). N-acetyl-β-D-glucosaminidase is a lysosomal enzyme that is present in high concentrations in renal proximal tubules. Its relative high molecular weight of 130 000 - 140 000 daltons does not permit the filtration of NAG through the glomerular basal membrane and its urinary excretion is relatively constant with minimal diurnal changes (2,3). The urine of healthy humans contains small amount of NAG. Increased excretion of

NAG in urine has been as sociatedwith tubular dysfunction or damage in patients wiith diabetes mellitus, nephrotic syndrome, vesicoureteral reflux, urinary tract infection, hypercalciuria, urolithiasis, perinatal asphyxia or heavy metals poisoning and in patients treated with aminoglycosides or valproate(2, 4 -16). Currently, there are several methods of assaying the urinary NAG catalytic activity. The fluorimetric assay, which is based on the fluorescent 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide substrate was introduced in the late sixties and has been followed by more user-friendly colorimetric and spectrophotometric methods (2). The urinary NAG values should be expressed as a ratio to urinary creatinine concentration, as this relationship shows less variability than the urinary enzyme excretions related to volume or time (2). Because of the fact that the urinary NAG/creatinine ratio tends do decrease with age in children as a result of a concomitant rise in the urinary creatinine concentration, establishment of proper pediatric reference values is necessary. Regarding the previously published urinary NAG values in sick children, these have been evaluated by comparison to control subjects which have ranged in number from 14 to 183 (4-16). The most relevant articles on urinary NAG reference ranges consisted of values obtained from 528 healthy schoolchildren (17) and 123 healthy children aged 1-14 years (18), where strong age-dependency has been observed. The aim of our study was to establish physiologic reference values of the urinary NAG/creatinine ratio in healthy children aged 0-18 years.

Materials and Methods

262 healthy children (141 boys and 121 girls) aged 0 month through 18 years were enrolled. All children had a negative personal history of kidney diseases. At the time of the urinary collection none of the children suffered from fever or acute infection, or had received any medication. After the first morning void, a urine example was collected for evaluation. The influence of endogenous enzyme inhibitors was eliminated by diluting the urine specimens 20-fold. The urinary catalytic activity of NAG was determined by fluorimetric assay. The urinary creatinine was assayed by using the autoanalyser-modified Jaffe's kinetic method. The urinary NAG values were expressed as the urinary NAG/creatinine ratio. The statistical evaluation was performed with the use of SOLO programme, version 4.0 (BMDP Statistical Software, USA). The Kolmogorov-Smirnov test was used for the normality testing. Both parametric and non-parametric methods were used to establish the upper reference ranges. The age-dependency was evaluated with the use of non-parametric ANOVA (Kruskal-Wallis test). The relationship between gender and urinary NAG was assessed both for the entire group and for each age-group by using the Mann-Whitney and Kolmogorov-Smirnov test.

Results

The reference values are expressed in Table 1. The urinary NAG/creatinine values were highest in the youngest age groups (neonates and infants). The data were not distributed homogenously. Therefore, the normal distribution of the values was obtained by logarithmic transformation. Regarding the

upper reference range (95th percentile), the results of both parametric and non-parametric methods were similar. Age-dependency of the urinary NAG/creatinine ratio was statistically significant only between the two youngest age-groups (neonates and infants) and the rest of the age-groups. The urinary NAG/creatinine values were not gender-dependent.

Discussion

Previously published papers regarding the urinary NAG excretion in childhood scarcely dealt with reference data obtained from larger pediatric population or did not have enough reference data pertinent to neonates and infants (5, 17, 18). Our results represent data from sufficiently large healthy paediatric population inclusive of neonates and infants, and shows strong age-dependence of the urinary NAG (Table 1) as a result of a concomitant rise in the urinary creatinine concentration. There is a great interindividual variability of those values in children, as reflected by the standard deviation. Such an observation is further strongly in favour of establishing reference paediatric data, as the reproducibility of results obtained from relatively small patient and control groups should be considered as difficult, especially when taking into consideration the large interindividual variability of the urinary NAG values in healthy children. As non-Gaussian distribution of the urinary NAG values has been previously reported in some age cohorts (2), we used a statistical analysis including the Kolmogorov-Smirnov test and the non-parametric Kruskal Wallis test to estimate the age dependence of the urinary NAG. Our results are in accordance with previously published data (18), especially in terms of the strong age-dependency of urinary NAG. As mentioned previously, fluorimetry, which was the method used, was established in the 1960s, and since then more sophisticated methods have been introduced (2). However, fluorimetry still remains the method of choice in most routine laboratories, because it is cheap, simple, user-friendly and easily reproducible.

In conclusion, we present the urinary NAG values of healthy children aged 0-18 years, obtained by using the method of fluorimetry. We consider the establishment of urinary NAG reference paediatric values as a potentially useful tool for proper evaluation of renal tubular impairment in childhood.

References

1. Price RG. Measurement of N-acetyl-β-D-glucosaminidase and its isoenzymes in urine. methods and clinical applications. Eur. J. Clin. Chem. Clin. Biochem. 1992; 30:693-705.
2. Csáthy L, Pócsi I Urinary N-Acetyl-β-*D*-glucosaminidase determination in newborns and children: methods and diagnostic applications. Eur. J. Clin. Chem. Clin. Bioch. 1995;33:575-587.
3. Pócsi I, Berki AC, Taylor SA, Price RG. Urea inhibition of urinary N-acetyl-β-D-glucosaminidase is mixed-type and is sensitive to changes of pH away from the optimum. Clin. Chem. 1993;39:1918-1919.
4. Caliskan S, Ficicioglu C, Hacibekiroglu M, Mikla S, Kasapcopur O, Sever L, Aydin A, Arisoy N. Tubular markers in children with insulin dependent diabetes mellitus. Turk. J. Pediatr. 1997;39:213-218.
5. Dillon SC, Taylor GM, Shah V. Diagnostic value of urinary retinol-binding protein in childhood nephrotic syndrome. Pediatr. Nephrol. 1998;12:643-647.
6. Nix DE, Thomas JK, Symonds WT, Spivey JM, Wilton JH, Gagliardi NC, Schentag JJ. Assessment of the enzymuria resulting from gentamicin alone and combinations of gentamicin with various beta-lactam antibiotics. Ann. Pharmacother. 1997;31:696-703.

7. Csathy L, Olah AV, Clemens B, Gyorgy I, Varga J. Urinary N-acetyl-beta-D-glucosaminidase in epileptic children treated with antiepileptic drugs. Arch. Dis. Child. 2000;83:420-422.
8. Miyakita H, Puri P. Urinary levels of N-acetyl-beta-D-glucosaminidase: a simple marker for predicting tubular damage in higher grades of vesicoureteric reflux. Eur. Urol. 1994;25:135-137.
9. Williams MA, Jones D, Noe HN. Urinary N-acetyl-beta-D-glucosaminidase as a screening technique for vesioureteral reflux. Urology. 1994; 43:528-530.
10. Belli A, Scarlecio F, Martino F, Guido F, Aprigliano D, Lorusso G, Mannarino O, Ruberto U. Evaluation of N-acetyl-beta-D-glucosaminidase in upper and lower urinary tract infections in childhood. Clinical study of 168 children. Minerva Pediatr. 1996;48:503-507.
11. Jantausch BA, Rifai N, Getson P, Akram S, Majd M, Wiedermann BL. Urinary N-acetyl-beta-D-glucosaminidase and beta-2-microglobulin in the diagnosis of urinary tract infection in febrile infants. Pediatr. Infect. Dis. J. 1994;13:294-299.
12. Caliskan S, Hacibekiroglu M, Sever I, Ozbay G, Arisoy N. Urinary N-acetyl-beta-D-glucosaminidase and beta-2-microglobulin excretion in primary nephrotic children. Nephron. 1996;74:401-404.
13. Fede C, Conti G, Chimenz R, Ricca M. N-acetyl-beta-D-glucosaminidase and beta-2-microglobulin: prognostic markers in idiopathic nephrotic syndrome. J. Nephrol. 1999;12:51-55.
14. Tekin N, Kural N, Torun M. Renal function in children with hypercalciuria. Turk. J. Pediatr. 1997;39:335-339.
15. Balla AA, Salah AM, Abdolmotaal E, Hoppe B, Bongartz D, Kessler T, Hesse A. N-acetyl-beta-D-glucosaminidase excretion in healthy children and in pediatric patients with urolithiasis. World J. Urol. 1998;16:413-416.
16. Willis F, Summers J, Minutillo C, Hewitt I. Indices of renal tubular function in perinatal asphyxia. Arch. Dis. Child. Fetal Neonatal Ed. 1997;77:F57-60.
17. 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;155:596-602.
18. Csathy L, Olah AV, Pocsi I, Varga J, Balla G, Price RG. Age-dependent urinary N-acetyl-beta-D-glucosaminidase activity in healthy children. Orv. Hetil. 1994;135:1301-1303.

Table 1 Reference values of the urinary NAG/creatinine excretion

Age (years)	Number of subjects	Mean value (nkat/mmol)	Standard deviation	95 centile (non-parametric)	95 centile (parametric)
0-0.08	38	53.44	35.69	134.80	148.55
0.08-1	50	20.28	13.06	50.0	51.71
1-3	50	6.19	3.75	14.02	16.63
3-6	46	4.98	3.33	13.21	15.41
6-10	29	4.53	2.22	7.5	11.5
10-18	49	3.32	1.96	7.25	8.82

CHAPTER 5

RENAL TUBULAR IMPAIRMENT IN CHILDREN WITH IDIOPATHIC HYPERCALCIURIA

Acta Medica 2006;49: 109-111

CHAPTER 5

RENAL TUBULAR IMPAIRMENT IN CHILDREN WITH IDIOPATHIC HYPERCALCIURIA

Sylva Skálová[1], Štěpán Kutílek[2]

Charles University in Prague, Faculty of Medicine and University Hospital in Hradec Králové, Czech Republic; Department of Paediatrics[1], Centre for Clinical and Basic Resarch (CCBR), Pardubice, Czech Republic[2]

Abstract:

Idiopathic hypercalciuria (IH) is defined as hypercalciuria that persists after correction of dietary inbalances and has no detectable cause. Renal tubular dysfunction has been described in patients with IH. The excretion of urinary N-acetyl-beta-D-glucosaminidase (U-NAG), a marker of proximal tubular damage, has been previously reported as either increased or normal in children with IH. We evaluated U-NAG in 20 children (13 boys and 7 girls, mean age 10.3 years ± 5.7 SD) with IH (urinary calcium excretion above 0.1 mmol/kg/24 hours, with no detectable cause) and with otherwise normal renal function tests. Ultrasound examination revealed urolithiasis (n = 4) and nephrocalcinosis (n = 1). The U-NAG values were evaluated in the spot urine collected from the second morning void and calculated as the urinary NAG/creatinine ratio (U-NAG/Cr) and expressed in nkat/mmol. The 24-hour urinary calcium excretion (U-Ca/24h) was assessed in a urinary sample from 24 – hour collected urine and calculated in mmol/kg. The obtained results of U-Ca/24h and U-NAG/Cr were expressed as Z-scores. When compared to the reference data, the U-Ca/24h and U-NAG/Cr were significantly higher ($p<0.0004$ and $p<0.006$, respectively). There was no correlation between the U-NAG/Cr and U-Ca/24h ($r = 0.18$, $p = 0.20$). The U-NAG/Cr values were significantly higher in the 5 patients with urolithiasis/nephrocalcinosis, whether compared to the rest of the group ($p<0.02$), or to the reference data ($p<0.01$). The U-NAG/Cr activity was higher in 15 children without urolithiasis/nephrocalcinosis when compared to reference data ($p < 0.01$). There was no difference in U-Ca/24h between the children with and without urolithiasis/nephrocalcinosis ($p = 0.58$). These findings suggest that tubular impairment, as reflected by U-NAG/Cr, might occur in children with IH, especially in patients with urolithiasis/nephrocalcinosis. There doesn't seem to be a direct relationship between the U-NAG/Cr activity and the degree of calcium leakage.

Key words: *Urinary NAG; Idiopathic hypercalciuria*

Introduction

Idiopathic hypercalciuria (IH) is defined as hypercalciuria in the presence of normocalcemia, that persists after correction of dietary inbalances with no detectable cause, and whose clinical manifestation varies with age (3). The renal tubular dysfunction seems as less likely the primary cause of IH (3,6,9). However, renal tubular impairment can be encountered in patients with urolithiasis or nephrocalcinosis, as cell-crystal interactions may lead to tubular damage and/or dysfunction (1,6,10). In children with IH there is an age-dependent risk of formation of microcalculi or stones, and development of osteoporosis (4,5,8). The excretion of urinary N-acetyl-beta-D-glucosaminidase (U-NAG), a marker of proximal tubular damage, has been reported as either increased (8-10), or normal (1), in children with IH. Recently, U-NAG was reported as significantly higher in children with urolithiasis and nephrocalcinosis, but not in children with isolated IH alone, and did not correlate with the urinary excretion of oxalate or calcium (6). Therefore, we looked for relationship between calciuria and U-NAG in children with IH.

Patients, Materials, Methods

Patients

We enrolled 20 children (13 boys and 7 girls, mean age 10.3 years ± 5.7 SD) with IH (urinary calcium excretion above 0.1 mmol/kg/24 hours, with no detectable cause) and with otherwise normal renal function tests and normal values of serum calcium, phosphate, alkaline phosphatase and parathyroid hormone. These children were referred because of hematuria and abdominal pain. Ultrasound examination revealed urolithiasis (n = 4) and nephrocalcinosis (n = 1).

Materials and Methods

For the evaluation of U-NAG and calciuria, the urine was collected on the same day. The 24-hour urinary calcium excretion (U-Ca/24h) was assessed in a urinary sample from 24 – hour collected urine by means of photometry and calculated in mmol/kg. The catalytic activity of NAG was measured by fluorimetric assay in spot urine collected from the second morning void. The influence of endogenous enzyme inhibitors was eliminated by diluting the urine specimens 20-fold. The urinary creatinine concentration was estimated by Jaffe's kinetic method on Modular Analyser (Roche Diagnostics GmbH, Sandhofer Strasse 116, Mannheim, Germany). The U-NAG values were calculated as the urinary NAG/creatinine ratio (U-NAG/Cr) and expressed in nkat/mmol. To eliminate the influence of age, the obtained results of U-Ca/24h and U-NAG/Cr were calculated as Z-scores by the equation SDS = (actual individual value – mean value for age) /standard deviation (SD) for age. The reference values for U-NAG/Cr and U-

Ca/24h were represented by the previously published data of healthy European paediatric populations (8,9).

Statistical Evaluation

The statistical evaluation was performed by t-test, ANOVA and linear regression analysis. For all results, $p < 0.05$ was required for statistical significance.

Results

U-Ca/24h

When compared to the reference data, the U-Ca/24h was significantly higher (Fig. 1). The U-Ca/24h was significantly higher in comparison to the reference data either in patients without urolithiasis/nephrocalcinosis (n=15) or with urolithiasis/nephrocalcinosis (n=5) (Fig.2). However, there was no difference in U-Ca/24h between the children without urolithiasis/nephrocalcinosis and with urolithiasis/nephrocalcinosis (Fig. 2).

U-NAG/Cr

In comparison to the reference data, the U-NAG/Cr was significantly higher (Fig. 1). In 7 patients (35 %), the U-NAG/Cr values exceeded the 95th percentile of the age-related reference range. Furthermore, regarding children with urolithiasis/nephrocalcinosis (n=5), 3 patients had the U-NAG/Cr values above the 95th percentile. The U-NAG/Cr values were significantly higher in the 5 patients with urolithiasis/nephrocalcinosis, whether compared to the rest of the group, or to the reference data. The U-NAG/Cr activity was still higher in 15 children without urolithiasis/nephrocalcinosis when compared to reference data (Fig. 3).

Relationship between U-Ca/24h and U-NAG/Cr

There was no correlation between the U-NAG/Cr and U-Ca/24h ($r = 0.18$, $p = 0.20$).

Discussion

Our findings suggest that tubular impairment, as reflected by U-NAG/Cr, might occur in children with IH, especially in patients with urolithiasis/nephrocalcinosis. The absence of correlation between U-NAG/Cr and U-Ca/24h also suggests that renal tubular impairment really seems to be less likely the primary cause of IH (3,6,8,9). However, in contrast to the findings of Sikora et al (6), we found increased U-NAG/Cr even in patients with IH and without urolithiasis/nephrocalcinosis. Therefore, increased urinary concentration of calcium might lead to damage of tubular cells, even in the absence of lithiasis. Furthermore, the findings of higher U-NAG/Cr in patients with urolithiasis/nephrocalcinosis in comparison to the values in children with IH but without urolithiasis/nephrocalcinosis further support the hypothesis that cell-crystal interactions lead to tubular impairment. In conclusion, children with IH have some degree of secondary renal tubular impairment. The tubular impairment is most probably aggravated by the increased urinary concentration of calcium, and, in particular, by the cell-crystal interactions.

However, there doesn't seem to be a direct relationship between this tubular impairment and the degree of calcium leakage.

References

1. Kavukcu S, Aydin A, Turkmen M, Akhunlar H, Fadiloglu M, Tavli V. Investigation of relationship between idiopathic hypercalciuria and urinary enzyme activities. J Assoc Physicians India 1998;46:784-5
2. Manz F, Keprt R, Larsen B, Merkem A. Urinary calcium excretion in healthy children and adolescents. Pediatr Nephrol 1999;13:894-9
3. Penido MG, Diniz JS, Moreira ML, Tupinamba AL, Franca A, Andrade BH, et al. Idiopathic hypercalciuria: presentation of 471 cases. J Pediatr (Rio J). 2001;77:101-4.
4. Penido MG, Lima EM, Marino VS, Tupinamba AL, Franca A, Souto MF. Bone alterations in children with idiopathic hypercalciuria at the time of diagnosis. Pediatr Nephrol 2003;18:133-9.
5. Polito C, La Manna A, Cioce F, Villani J, Nappi B, Di Toro R Clinical presentation and natural course of idiopathic hypercalciuria in children. Pediatr Nephrol 2000;15:211-4.
6. Sikora P, Glatz S, Beck BB, Stapenhorst L, Zajaczkowska M, Hesse A, et al. Urinary NAG in children with urolithiasis, nephrocalcinosis, or risk of urolithiasis. Pediatr Nephrol 2003;18:996-9
7. Skálová S, Chládek J. Urinary N-acetyl-beta-D-glucosaminidase activity in healthy children. Nephrology 2004;9:19-21.
8. Skálová S, Palicka V, Kutilek S. Bone mineral density and urinary N-acetyl-β-D-glucosaminidase activity in paediatric patients with idiopathic hypercalciuria. Nephrology 2005;10:99-102.
9. Stapleton FB, Chesney RW, Behrmann AT, Miller LA. Increased urinary excretion of renal N-acetyl-beta-glucosaminidase in hypercalciuria. Am J Dis Child 1985;139:950-2
10. Tekin N, Kural N, Torun M. Renal function in children with hypercalciuria. Turk J Pediatr 1997;39:335-9

Figures 1-3

Figure 1
24-hour urinary calcium excretion (U-Ca/24h) and urinary N-acetyl-beta-D-glucosaminidase activity/creatinine (U-NAG/Cr) in 20 children with idiopathic hypercalciuria (expressed as Z-scores ± SD, compared to reference data).

Figure 2
The U-Ca/24h values in children without urolithiasis/nephrocalcinosis (n=15) and with urolithiasis/nephrocalcinosis (N/L/C) (n=5) (expressed as Z-scores ± SD, compared to reference data). There was no difference in U-Ca/24h between the children without urolithiasis/nephrocalcinosis and with urolithiasis/nephrocalcinosis (p = 0.58).

Figure 3
The U-NAG/Cr values in patients without urolithiasis/nephrocalcinosis (n=15) and with urolithiasis/nephrocalcinosis (N/L/C) (n=5) (expressed as Z-scores ± SD, compared to reference data). The U-NAG/Cr values were significantly higher in the 5 patients with urolithiasis/nephrocalcinosis, when compared to the rest of the group (p = 0.02).

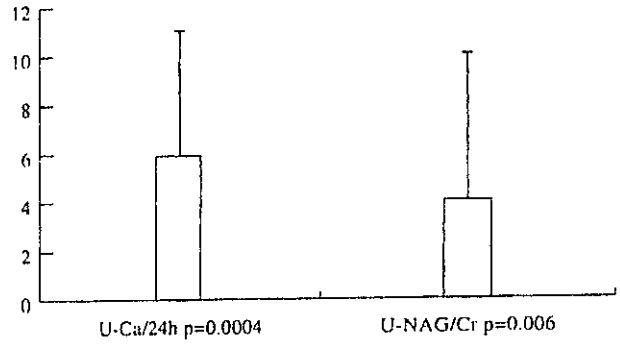


Fig. 1: 24-hour urinary calcium excretion (U-Ca/24h) and urinary N-acetyl-beta-D-glucosaminidase activity/creatinine (U-NAG/Cr) in 20 children with idiopathic hypercalciuria (expressed as Z-scores ± SD, compared to reference data).

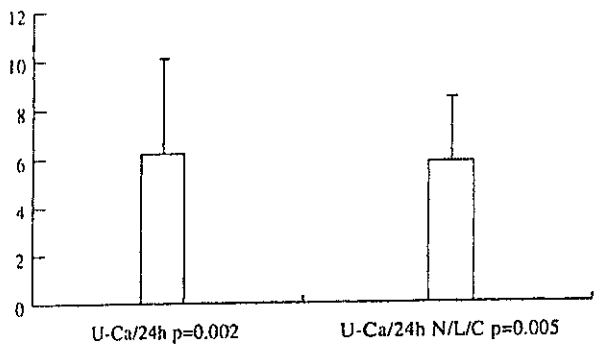


Fig. 2: The U-Ca/24h values in children without urolithiasis/nephrocalcinosis (n = 15) and with urolithiasis/nephrocalcinosis (N/L/C) (n = 5) (expressed as Z-scores ± SD, compared to reference data). There was no diference in U-Ca/24h between the children with and without urolithiasis/nephrocalcinosis (p = 0.58).

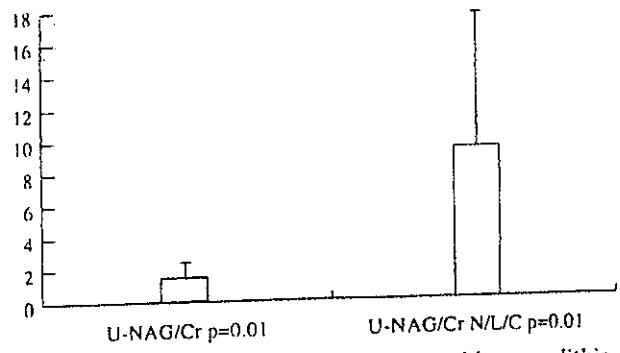


Fig. 3: The U-NAG/Cr values in patients without urolithiasis/nephrocalcinosis (n = 15) and with urolithiasis/nephrocalcinosis (N/L/C) (n = 5) (expressed as Z-scores ± SD, compared to reference data). The U-NAG/Cr values were significantly higher in the 5 patients with urolithiasis/nephrocalcinosis when compared to patients without urolithiasis/nephrocalcinosis (p = 0.02).

CHAPTER 6

BONE MINERAL DENSITY AND URINARY *N*-ACETYL-β-D-GLUCOSAMINIDASE ACTIVITY IN PAEDIATRIC PATIENTS WITH IDIOPATHIC HYPERCALCIURIA

Nephrology 2005;10:99-102

CHAPTER 6

BONE MINERAL DENSITY AND URINARY *N*-ACETYL-β-D-GLUCOSAMINIDASE ACTIVITY IN PAEDIATRIC PATIENTS WITH IDIOPATHIC HYPERCALCIURIA

S. Skalova[1], V. Palicka[2], S. Kutilek[3]

[1]Department of Paediatrics, [2]Institute for Clinical Biochemistry and Diagnostics, Faculty of Medicine, Charles University, Hradec Králové and [3]Bone Disease Centre, Pilsen Faculty Hospital, Charles University, Pilsen, Czech Republic

Summary:

Background: Idiopathic hypercalciuria (IH) is defined as hypercalciuria that persists after correction of dietary inbalances and has no detectable causes. Patients with IH have a higher prevalence of osteoporosis. Defective reabsorption of calcium by the renal tubule is considered a likely mechanism of IH. *N*-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme that is a very sensitive marker of renal tubular impairment.

Methods: Fifteen patients (nine boys and six girls, mean age 12.4 ± 4.0 years) with IH (urinary calcium excretion >0.1 mmol/kg per 24 h) had their bodyweight, height, body mass index (BMI), urinary NAG/creatinine ratio (U-NAG/Cr) and 24-h urinary calcium excretion (U-Ca/24 h) assessed. L1–L4 bone mineral density (BMD) was measured by dual energy X-ray absorptiometry and volumetric BMD (BMDvol) was calculated. The obtained results were expressed as Z-scores.

Results: The values of basic anthropometric parameters did not differ significantly from the values of the reference population and there was a tendency to short stature, which did not reach statistical significance ($P = 0.08$). The values of calciuria and U-NAG/Cr were significantly higher while BMD was significantly lower when compared to the reference values ($P < 0.0006$, $P < 0.006$ and $P < 0.001$, respectively). Inverse and significant correlations were found between U-Ca/24 h and BMD, U-Ca/24 h and body height, and U-Ca/24 h and BMDvol ($r = -0.64$ and -0.70, respectively, $P < 0.01$; $r = -0.55$, $P < 0.05$), while there was no correlation between U-NAG/Cr and U-Ca/24 h, nor between BMD and weight or BMD and BMI.

Conclusion: Tubular impairment is highly probable in children with IH, but there is a poor relationship with the degree of calcium leakage. Idiopathic hypercalciuria should be considered as a risk factor for stunted growth and low bone mass.

Key words: *Bone mineral density; Children; Idiopathic hypercalciuria; Urinary N-acetyl-beta-D-*

glucosaminidase.

Introduction

Idiopathic hypercalciuria (IH) is defined as hypercalciuria that persists after correction of dietary inbalances and has no detectable cause (1-3). Defective reabsorption of calcium by the renal tubule is considered a likely mechanism of IH. Furthermore, crystals containing calcium are believed to damage the cells of the renal tubule, thus leading to impaired tubular function (4-6). *N*-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme that is abundantly present in the cells of the proximal tubule and is considered to be a very sensitive marker of renal tubular impairment (7). Currently available reports give conflicting results regarding the urinary excretion of NAG in patients with IH (4,8-10). In addition, several papers have documented that both children and adults with IH have lower values of bone mineral density (BMD) and are prone to the development of osteoporosis (11-22). Our objectives were to assess the tubular function by means of urinary NAG (U-NAG) evaluation and BMD by dual energy X-ray absorptiometry (DXA) in paediatric patients with IH, and to look for a possible relationship between calciuria, BMD and U-NAG.

Methods

Patients

Fifteen patients (nine boys and six girls, mean age 12.4 ± 4.0 years) with IH were enrolled in the study after they and their parents had given their consent. These children were referred to the Paediatrics Department at Hradec Králové Hospital because of haematuria and abdominal pain. Their urinary calcium excretion exceeded 0.1 mmol/kg per 24 h, while their values of serum calcium, phosphate, magnesium, urea, creatinine, alkaline phosphatase and blood and urine pH were within laboratory reference ranges. Ultrasound examination revealed urolithiasis in two children and nephrocalcinosis in one child. The children were not receiving any medication known to influence bone and mineral metabolism prior to the enrolment.

Procedure

Basic anthropometric parameters (i.e. body height and weight) were taken from all participating children at the day of the urinary collection by trained paediatric nurses. Body height was recorded to the nearest ±0.5 cm using a stadiometer; weight was measured on a calibrated scale to ±0.5 kg. The body mass index (BMI) was calculated using the equation BMI = weight (kg)/height^2 (m).

For the evaluation of U-NAG and calciuria, the urine was collected on the same day. The 24-h urinary calcium excretion (U-Ca/24 h) was assessed by photometry in a urinary sample from 24-h collected urine and calculated in mmol/kg bodyweight/24 h.

The U-NAG was evaluated in a sample of urine collected from the second morning void. The

influence of endogenous enzyme inhibitors was eliminated by diluting the urine specimens 20-fold. The urinary catalytic activity of NAG was determined by fluorimetric assay. The urinary creatinine concentration was estimated by Jaffe's kinetic method on Modular Analyser (Roche Diagnostics GmbH, Mannheim, Germany). The U-NAG values were expressed as the urinary NAG/creatinine (U-NAG/Cr) ratio in nkat/L : mmol/L.

Spinal (L1–L4) BMD (g/cm^2) was measured by DXA (Hologic QDR 4500, Bedford, MA , USA) at the day of the urinary collection. Measurement precision, expressed as coefficient of variation, was 1.0%. Volumetric bone density (BMDvol; g/cm^3) was then calculated as follows:

$$BMDvol = BMC/Volume = areal\ BMD\ [4/(\pi\ Width)]$$

where Width = mean width of vertebral body(23).

To eliminate the influence of age, the obtained results of anthropometric parameters, U-Ca/24 h, U-NAG/Cr, spinal BMD and BMDvol were expressed as standard deviation scores (SDS) or Z-scores by the equation SDS = actual individual value – mean value for age/standard deviation for age. The reference values were represented by anthropometric, U-Ca/24 h and U-NAG/Cr data of healthy European paediatric populations (24-26). The BMD reference data (concerning European paediatric population) were supplied by the manufacturer within the DXA software package. The volumetric reference values were calculated from previously recorded results of DXA measurements of 100 age- and sex-matched individuals with normal body height and normal areal BMD Z-scores within ±1 SD.

Data analysis

The results of anthropometric parameters, U-Ca/24 h, U-NAG/Cr and BMD measurements are reported as Z-scores (mean ± SD). The statistical analysis was performed by *t*-test. The linear regression analysis was performed to compare the relationship among respective parameters. For all results, $p < 0.05$ was required for statistical significance.

Results

The values of basic anthropometric parameters did not differ significantly from the values of the reference population. There was a tendency to short stature, which did not reach statistical significance (Table 1). In only one child (6.6%) the body height was below –2 SD. In the three children with urolithiasis/nephrocalcinosis, the body height, weight and BMI were within ±2 SD.

In the entire group of 15 children, the values of U-Ca/24 h were significantly increased in comparison to reference data (Table 1).

The U-NAG/Cr values exceeded the 95th percentile in five children (33%), including one with urolithiasis and one with nephrocalcinosis. For the entire group of 15 children, the U-NAG/Cr values were significantly higher in comparison to the reference data (Table 1).

Out of 15 patients, six (40%) had BMD between –1 and –2 SD and three (20%) had BMD below –

2 SD. The children with urolithiasis had BMD values of –1.8 and –2.4 SD, while in the child with nephrocalcinosis the BMD was –3.3 SD, respectively. In the entire group of 15 children, BMD and BMDvol were significantly decreased when compared to the reference values (Table 1). There was a high and significant correlation between Z-scores of BMD and BMDvol ($r = 0.97$, $p < 0.001$).

We found inverse and significant correlation between BMD and U-Ca/24 h, BMDvol and U-Ca/24 h and a similar correlation between U-Ca/24 h and body height (Table 2).

There was no correlation between U-NAG/Cr and U-Ca/24 h. Furthermore, with the exception of relationship between U-Ca/24 h and body height, there were poor or no correlations between anthropometric parameters and U-NAG/Cr, U-Ca/24 h and BMD, respectively. Neither was there any correlation between U-NAG/Cr and BMD or U-NAG/Cr and BMDvol (Table 2).

Discussion

The present study demonstrates increased U-NAG/Cr values in children with IH, suggesting renal tubular impairment in this group of patients. These results are in accordance with previous reports (8,9). Idiopathic hypercalciuria might be a result of primary tubular defect, as reflected by increased U-NAG/Cr. The development of urolithiasis or nephrocalcinosis most probably leads to brush border injury by the calcium-oxalate or calcium-phosphate crystals, with enzymuria as a sign of further deterioration of renal tubular function (4-6). However, poor correlation between U-NAG/Cr and U-Ca/24 h suggests that there is no clear-cut relationship between the impairment of tubular function and the degree of calciuria. Similarly, low correlation between U-NAG/Cr and BMD or U-NAG/Cr and BMDvol implies a poor relationship between the impairment of tubular function and skeletal status in patients with IH.

Our results confirm the previously reported low BMD in patients with IH (11-22). Forty per cent of the present patients had BMD Z-score between –1 and –2 SD and 20% had BMD Z-score below –2 SD. This is somewhat higher than described in previous reports, where BMD below –1 SD was observed in 20–35% patients with IH (13-15, 20-22). Furthermore, it should be noted that the nomenclature and World Health Organization (WHO) classification of osteoporosis (osteopenia –1.0 to –2.5 T-score, and osteoporosis below –2.5 T-score) and consequent fracture risk applies only to postmenopausal caucasian women (27,28), and that the risk of fracture for children whose BMD decline –1 or more SD below the mean for age, bone age or body size has not been established (28-31). Bone mineral density is a normally distributed variable in the population (28), therefore, when evaluating the BMD in children, Z-score values below –2 SD imply decreased bone mass (31). The estimation of BMD is influenced by bone size and can be underestimated in smaller individuals, and vice versa, overestimated in larger bones (29,32,33). The poor correlations between anthropometric parameters and BMD in the present patients suggest low bone mass in children with IH, because body size is a better correlate of spinal BMD than the chronological age (29, 32-34). This was further confirmed by low BMDvol because

volumetric bone density gives more reliable information than the areal data (23,35). In addition, the inverse and significant correlations between U-Ca/24 h and spinal BMD or between U-Ca/24 h and BMDvol suggest that the degree of bone demineralization is related to the degree of urinary calcium leakage. This further supports the hypothesis that patients with IH are more prone to decreased bone mass and consequently to osteoporosis (20-22). This is of particular importance because optimal peak bone mass is a significant determinant of the risk of sustaining osteoporotic fractures during adult life (36).

The inverse relationship between body height and U-Ca/24 h, together with the presence of stunted growth in one child (6.6%), is in accordance with some previous reports in which growth delay was recorded in 2.5–8% of IH children (13,37). However, normal body height has been reported by other authors in children with IH (20,38). A surprisingly high prevalence of growth delay (32–41%) was previously noticed in children with hypercalciuria and nephrocalcinosis (39). The interstitial and tubular damage induced by renal calcifications might explain the growth delay of children with nephrocalcinosis, possibly due to the excessive urinary loss of water and solutes(20,39). Apparently, growth retardation is known to be more severe in complex hereditary tubular disorders than in isolated ones, such as IH (40). Growth failure and low bone mass are considered closely related entities (20). Hypercalciuria can contribute to the skeletal demineralization and to the development of nephrocalcinosis, both resulting in stunted growth. Therefore, growth velocity and BMD should be given special attention in children with IH. In conclusion, tubular impairment is highly probable in children with IH, but there seems to be a poor relationship with the degree of calcium leakage. Idiopathic hypercalciuria should be considered as a risk factor for stunted growth and low bone mass. Further studies are necessary for more detailed clarification of these issues.

References

1. Audran M, Legrand P. Hypercalciuria. Joint Bone Spine 2000; 67: 509–15.
2. Reuss G. Idiopathic hypercalciuria in childhood. Orv. Hetil. 1998; 139: 2957–62.
3. Polito C, La Manna A, Cioce F, Villani J, Nappi B, Di Toro R. Clinical presentation and natural course of idiopathic hypercalciuria in children. Pediatr. Nephrol. 2000; 15: 211–14.
4. Sikora P, Glatz S, Beck BB et al. Urinary NAG in children with urolithiasis, nephrocalcinosis, or risk of urolithiasis. Pediatr. Nephrol. 2003; 18: 996–9.
5. Turkmen M, Kavukcu S, Islekel H et al. Urinary N-acetyl-beta-D-glucosaminidase activity in rabbits with experimental hypercalciuria. Pediatr. Nephrol. 1997; 11: 481–4.
6. Balla AA, Salah AM, Abdolmotaal E et al. N-acetyl-beta-D-glucosaminidase excretion in healthy children and in pediatric patients with urolithiasis. World J. Urol. 1998; 16: 413–16.
7. Csáthy L, Pócsi I. Urinary N-acetyl-□-D-glucosaminidase determination in newborns and children: Methods and diagnostic applications. Eur. J. Clin. Chem. Clin. Biochem. 1995; 33: 575–87.
8. Stapleton FB, Chesney RW, Behrmann AT, Miller LA. Increased urinary excretion of renal N-acetyl-beta-glucosaminidase in hypercalciuria. Am. J. Dis. Child. 1985; 139: 950–2.
9. Tekin N, Kural N, Torun M. Renal function in children with hypercalciuria. Turk. J. Pediatr. 1997; 39: 335–9.
10. Kavukcu S, Aydin A, Turkmen M, Akhunlar H, Fadiloglu M, Tavli V. Investigation of relationship between idiopathic hypercalciuria and urinary enzyme activities. J. Assoc. Physicians India 1998; 46: 784–55.
11. Zanchetta JR, Rodriguez G, Negri AL, del Valle E, Spivacow FR. Bone mineral density in patients with hypercalciuric nephrolithiasis. Nephron 1996; 73: 557–60.
12. Ghazali A, Fuentes V, Desaint C et al. Low bone mineral density and peripheral blood monocyte activation profile in calcium stone formers with idiopathic hypercalciuria. J. Clin. Endocrinol. Metab. 1997; 82: 32–8.

13. Garcia-Nieto V, Ferrandez C, Monge M, de Sequera M, Rodrigo MD. Bone mineral density in pediatric patients with idiopathic hypercalciuria. Pediatr. Nephrol. 1997; 11: 578–83.
14. Garcia-Nieto V, Navarro JF, Ferrandez C. Bone loss in children with idiopathic hypercalciuria. Nephron 1998; 78: 341–2.
15. Freundlich M, Alonzo E, Bellorin-Font E, Weisinger JR. Reduced bone mass in children with idiopathic hypercalciuria and in their asymptomatic mothers. Nephrol. Dial. Transplant. 2002; 17: 1396–401.
16. Tasca A, Cacciola A, Ferrarese P et al. Bone alterations in patients with idiopathic hypercalciuria and calcium nephrolithiasis. Urology 2002; 59: 865–9.
17. Misael da Silva AM, dos Reis LM, Pereira RC et al. Bone involvement in idiopathic hypercalciuria. Clin. Nephrol. 2002; 57: 183–91.
18. Heilberg IP, Teixeira SH, Martini LA, Boim MA. Vitamin D receptor gene polymorphism and bone mineral density in hypercalciuric calcium-stone-forming patients. Nephron 2002; 90: 51–7.
19. Giannini S, Nobile M, Dalle Carbonare L et al. Hypercalciuria is a common and important finding in postmenopausal women with osteoporosis. Eur. J. Endocrinol. 2003; 149: 209–13.
20. Polito C, Iolascon G, Nappi B, Andreoli S, La Manna A. Growth and bone mineral density in long-lasting idiopathic hypercalciuria. Pediatr. Nephrol. 2003; 18: 545–7.
21. Garcia-Nieto V, Navarro JF, Monge M, Garcia-Rodriguez VE. Bone mineral density in girls and their mothers with idiopathic hypercalciuria. Nephron. Clin. Pract. 2003; 94: 89–93.
22. Penido MG, Lima EM, Marino VS, Tupinamba AL, Franca A, Souto MF. Bone alterations in children with idiopathic hypercalciuria at the time of diagnosis. Pediatr. Nephrol. 2003; 18: 133–9.
23. Kroger HPJ. Measurement of bone mass and density in children. In: Schonau E (ed.) Paediatric Osteology: New Developments in Diagnostics and Therapy. Amsterdam: Elsevier Science, 1996; 103–8.
24. Lhotská L, Bláha P, Vignerová J, Roth Z, Prokopec M. Vth Nation-Wide Anthropological Survey of Children and Adolescents 1991 (Czech Republic). Prague: National Institute of Public Health, 1993.
25. Manz F, Keprt R, Larsen B, Merkem A. Urinary calcium excretion in healthy children and adolescents. Pediatr. Nephrol. 1999; 13: 894–9.
26. Skálová S, Chládek J. Urinary N-acetyl-beta-D-glucosaminidase activity in healthy children. Nephrology 2004; 9: 19–21.
27. World Health Organization. Assessment of Fracture Risk and its Application to Screening for Postmenopausal Osteoporosis. WHO Technical Report Series. Geneva: WHO, 1994.
28. Arden N, Cooper C. Present and future of osteoporosis: Epidemiology. In: Meunier PJ (ed). Osteoporosis: Diagnosis and Management. London: Martin Dunitz, 1998; 1–16.
29. Mora S, Bachrach L, Gilsanz V. Noninvasive techniques for bone mass measurements. In: Glorieux FH, Pettifor JM, Juppner H (eds). Pediatric Bone. Biology and Diseases. San Diego, CA: Academic Press, 2003; 303–24.
30. Leib ES, Lewiecki EM, Binkley N et al. Official positions of the International Society for Clinical Densitometry. J. Clin. Densitom. 2004; 7: 1–6.
31. Writing Group for the ISCD Position Development Conference. Diagnosis of osteoporosis in men, premenopausal women, and children. J. Clin. Densitom. 2004; 7: 17–26.
32. Carter DR, Bouxsein ML, Marcus R. New approaches for interpreting projected bone densitometry data. J. Bone Miner. Res. 1992; 7: 137–45.
33. Specker BL, Johannsen N, Binkley T, Finn K. Total body bone mineral content and tibial cortical bone measures in school children. J. Bone Miner. Res. 2001; 16: 2298–305.
34. Plotkin H, Nunez M, Alvarez Filgueira ML, Zanchetta JR. Lumbar spine bone density in Argentine children. Calcif. Tissue Int. 1996; 58: 144–9.
35. Lu PW, Cowell CT, Lloyd-Jones SA et al. Volumetric bone mineral density in normal subjects, aged 5–27 years. J. Clin. Endocrinol. Metab. 1996; 81: 1586–90.
36. Carrie Fassler AL, Bonjour JP. Osteoporosis as a pediatric problem. Pediatr. Clin. North Am. 1995; 42: 811–24.
37. Cervera A, Corral MJ, Gómez Campdera FJ, De Lecea BM, Luque A, López Gómez JM. Idiopathic hypercalciuria in children. Acta Paediatr. Scand. 1987; 76: 271–8.
38. Harangi F, Szelid Z. Normal body height in hypercalciuric children. Orv. Hetil. 1991; 132: 2497–8.
39. Ronnefarth G, Misselwitz J. Nephrocalcinosis in children: A retrospective survey. Members of the Arbeitsgemeinschaft fur padiatrische Nephrologie. Pediatr. Nephrol. 2000; 14: 1016–21.
40. Haffner D, Weinfurth A, Manz F et al. Long-term outcome of paediatric patients with hereditary tubular disorders. Nephron 1999; 83: 250–60.

Table 1 Patient data expressed as Z-scores ± SD

Parameter	Mean	SD	p †
U-Ca/24 h	5.24	3.55	**0.0006**
U-NAG/Cr	2.43	3.75	**0.006**
BMDarea	–1.41	0.97	**0.001**
BMDvol	–1.22	0.98	**0.003**
Height	–0.53	0.99	0.08
Weight	–0.54	0.98	0.10
BMI –0.30	0.95	0.24	

BMD, bone mineral density; BMDvol, volumetric BMD; BMI, body mass index; U-Ca/24 h, 24-h urinary calcium excretion; U-NAG/Cr, urinary *N*-acetyl-beta-D-glucosaminidase/creatinine ratio.

†Compared to reference data.

Table 2 Correlations between observed parameters (parameters expressed as Z-scores)

	U-Ca/24 h		U-NAG/Cr		BMDarea		BMDvol		
Variables	r	p	r	p	r	p	r	p	
U-Ca/24 h	--	--	0.15	NS	**–0.64**	**0.01**	**–0.55**		**0.05**
U-NAG/Cr	--	--	--	--	–0.20	NS	–0.07		NS
Height	**–0.70**	**0.01**	–0.14	NS	0.46	NS	–0.24		NS
Weight	–0.30	NS	–0.41	NS	0.30	NS	0.10	NS	
BMI –0.12	NS	–0.33	NS	0.42	NS	0.30	NS		

BMDarea, areal bone mineral density; BMDvol, volumetric BMD; BMI, body mass index; U-Ca/24 h, 24-h urinary calcium excretion; U-NAG/Cr, urinary *N*-acetyl-beta-D-glucosaminidase/creatinine ratio.

CHAPTER 7

HIGH URINARY N-ACETYL-β-D-GLUCOSAMINIDASE (NAG) ACTIVITY AND NORMAL CALCIURIA IN CHILDREN WITH NOCTURNAL ENURESIS

Indian Pediatrics, 2006;43:655-656

CHAPTER 7

HIGH URINARY N-ACETYL-β-D-GLUCOSAMINIDASE (NAG) ACTIVITY AND NORMAL CALCIURIA IN CHILDREN WITH NOCTURNAL ENURESIS

Sylva Skalova[1] , Stepan Kutilek[2]

[1]*Department of Paediatrics, Faculty of Medicine Teaching Hospital, Charles University, Hradec Králové,* [2]*Centre for Clinical and Basic Resarch (CCBR), Pardubice Czech Republic*

Letter to the Editor:

Nocturnal enuresis (NE) is the occurrence of involuntary voiding at night at 5 years, the age when volitional control of micturition is expected. NE may be primary or secondary (1). The role of hypercalciuria in the ethiopathogenesis of primary NE has been discussed (2). N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme abundantly present in the cells of proximal tubule and is considered a very sensitive marker of renal tubular impairment (3). As increased urinary NAG activity has been reported in patients with hypercalciuria (3), our objective was to evaluate the urinary NAG and calciuria in patients with NE. Fourteen patients (11 boys and 3 girls, mean age 6.8 yr ± 1.6 SD, range 5-10 yr) with primary NE were enrolled on basis of the inclusion criteria: age 5 – 15 yr; absence of urinary tract anomalies; absence of diabetes insipidus and diabetes mellitus; urine osmolality (morning void > 400 mOsm/kg); absence of urinary tract infection; no previous treatment for NE; >4 bedwetting episodes within the last 14 days. The blood levels of creatinine, urea, glucose, calcium, sodium, potassium and magnesium and urinary beta-microglobuline were within the normal range. Urinary calcium/creatinine (UCa/Cr; mmol/L:mmol/L) and urinary NAG/creatinine ratios (UNAG/Cr; nkat/L:mmol/L) were assessed in urine collected after the first morning void. To eliminate the influence of age, the obtained results of UCa/Cr and UNAG/Cr were expressed as Z-scores by the equation Z-score = (actual of individual value – mean reference value for age)/standard deviation for age. The reference values were based on previously published data on healthy Czech children (4,5). For statistical evaluation, t-test and linear regression were performed. UCa/Cr values were within the reference range in 13

children, and in only 1 patient the value exceeded the 95th percentile. The values of UCa/Cr did not differ significantly from the reference data (Table I). In 4 patients the UNAG/Cr values exceeded the age-related 95th percentile range In the entire group of 14 patients, the UNAG/Cr values were significantly higher compared to reference values (Table I). There was no correlation between UNAG/Cr and UCa/Cr ($r = 0.13$, $P = 0.55$). In conclusion, hypercalciuria was not found in children with NE. The presence of elevated urinary levels of UNAG/Cr suggest that tubular dysfunction might be important in patients with enuresis.

References

1. Elder JS. Voiding dysfunction. In: Behrman RE, Kliegman RM, Jenson HB, es. Nelson Textbook of Pediatrics. 17th edn. Philadelphia: WB Saunders and Co; 2004. p.1808-1812.
2. Aceto G, Penza R, Coccioli MS, Palumbo F, Cresta L, Cimador M et al. Enuresis subtypes based on nocturnal hypercalciuria: a multicenter study. J Urol 2003;170:1670-1673.
3. Skalova S, Palicka V, Kutilek S. Bone mineral density and urinary N-acetyl-beta-D-glucosaminidase activity in paediatric patients with idiopathic hypercalciuria. Nephrology 2005;10:99-102
4. Skalova S, Chladek J. Urinary N-acetyl-beta-D-glucosaminidase activity in healthy children. Nephrology 2004;9:19-21.
5. Janda J, Feber J, Sikut M, Carkova S, Smisková R. Assessment of the urinary calcium/creatinine index in healthy neonates, infants and children. Cs Pediat 1992;47:353-356.

Table 1

Results of U-NAG/Cr and U-Ca/Cr (expressed as Z-scores)

Parameter	Results (mean ± SD)	Reference values (mean ± SD)	p*
U-Ca/Cr (Z-scores)	0.19 ± 0.92	0 ± 1	0.77*
U-NAG/Cr (Z-scores)	1.64 ± 1.65	0 ± 1	**0.003***

*P compared to reference data (4,5)

CHAPTER 8

INCREASED URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE ACTIVITY IN CHILDREN WITH VESICOURETERAL REFLUX

Pediatric Nephrology 2006;21:1528

Presented at 40th European Society for Pediatric Nephrology (ESPN) Meeting, 7-10 October 2006, Palermo, Italy

CHAPTER 8

INCREASED URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE ACTIVITY IN CHILDREN WITH VESICOURETERAL REFLUX

Sylva Skalova[1], Pavel Rejtar[2], Stepan Kutilek[3]

Departments of [1]Paediatrics and [2]Radiology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Czech Republic

[3]Center for Clinical and Basic Research (CCBR), Pardubice, Czech Republic

Abstract

Aim: The aim was to measure U-NAG in children with vesicoureteral reflux (VUR) and look for relationship among selected clinical parameters.

Methods: 22 children. (10 boys and 12 girls, mean age 2.83 ± 2.42 years) with VUR had the U-NAG/creatinine ratio measured in the spot urine. In 8 patients the VUR was unilateral, grade I-IV, and in 14 patients, the VUR was bilateral, grade I-V. In patients with bilateral reflux and different VUR grade on each side, the highest grade of VUR was taken into consideration.

Results: The U-NAG/Cr values were significantly higher in the VUR patients in comparison to the reference data (p= 0.0001). There was no difference in U-NAG/Cr between children with unilateral (n=8) and bilateral (n=14) VUR (p= 0.66). There was no difference in U-NAG/Cr between patients with VUR grade I-III and VUR grade IV-V (p=0.67). The U-NAG/Cr activity was high in patients with reflux nephropathy (RN; n=9) when compared to reference data (p= 0.0001), however there was no difference in comparison to children without RN (p= 0.84).

Conclusions: U-NAG/Cr is increased in children with VUR grade I-V and there is a very weak relationship with the grade of VUR. U-NAG/Cr is a useful marker of renal tubular impairment, however there is poor relationship with the degree of kidney damage in patients with VUR .

Key words:

N-acetyl-β-D-glucosaminidase; Vesicureteral reflux

Introduction

Vesicoureteral reflux (VUR) is defined as an abnormal backflow of urine from the bladder to ureter or kidney (1). International classification of VUR based on the appearance of the urinary tract during voiding cystourethrography (VCUG) distinguishes five grades of VUR, according to its severity (1). VUR is potentially harmful because of the exposure of the kidney to increased hydrodynamic pressure during voiding. Furthermore, the incomplete emptying of the ureter and bladder on voiding predisposes the patient to urinary tract infection and consequent developement of renal scarring, termed as reflux nephropathy (RN)(2). Grades I-III VUR tend to resolve faster than grades IV-V, and association between grades IV-V VUR and the presence of renal damage has been observed (2,3). N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme which is abundantly present in the cells of the proximal tubule and is considered as a very sensitive marker of renal tubular impairment (4,5). Furthermore, increased urinary NAG activity (U-NAG) has been repeatedly reported in patients with VUR grade III to V, suggesting tubular dysfunction (6,9). Our principal aim was to measure urinary NAG activity in patients with VUR grade I through V and relate the obtained results to the grade of VUR and several clinical parameters.

Patients, methods

Patients

22 children (10 boys and 12 girls) aged 0.33 to 11.8 years (mean age 2.83 ± 2.42 years) with VUR were enrolled. Informed consent was obtained from each parent/guardian (and patient if applicable) prior to any procedures described in this paper. VUR was initially diagnosed on the basis of prenatal ultrasound screening in 14 children. In 8 patients, the VUR was diagnosed postnatally due to pyelonephritis. In all patients, the VUR was confirmed by VCUG. In 8 patients the VUR was unilateral, grade I-IV, and in 14 patients, the VUR was bilateral, grade I-V. In patients with bilateral reflux and different VUR grade on each side, the highest grade of VUR was taken into consideration. Therefore, the diagnostic distribution was as follows: VUR grade I, n = 2; VUR grade II, n = 3; VUR grade III, n = 4; VUR grade IV, n = 12; VUR grade V, n = 1. Pyelonephritis occurred in 8 patients (36%) who experienced a total of 20 episodes of pyelonephritis (range 1 to 6 per patient). Renal scintigraphy with ^{99m}Tc-dimercaptosuccinic acid (DMSA) was performed in all patients (n=22) in order to reveal renal scarring/RN. In those patients with documented pyelonephritis (n=8), the VCUG was performed at least 6 weeks and DMSA scan 6 months after the initial episode of pyelonephritis, respectively. RN was diagnosed in 9 patients (41%).

Methods

All patients had their U-NAG and urinary concentrations of creatinine (U-Cr) evaluated. None of the patients suffered from pyelonephritis at the time of the U-NAG and U-Cr evaluation. All patients were free from infection at least 5 months prior to the U-NAG and U-Cr evaluation. U-NAG was evaluated in the spot urine, collected after the first morning void. The blood and spot urine were collected either at the time of the DMSA scan or in a time frame of ± 1 month, and within less than 5 months after the VCUG. The influence of endogenous enzyme inhibitors was eliminated by diluting the urine specimens 20-fold. The urinary catalytic activity of NAG was then determined by fluorimetric assay. The U-Cr was estimated by Jaffe's kinetic method on Modular Analyser (Roche Diagnostics GmbH, Mannheim, Germany). The U-Cr values were expressed in mmol/L. The U-NAG values were expressed as the urinary NAG/creatinine (U-NAG/Cr) ratio in nkat/L : mmol/L.

To eliminate the influence of age, the obtained results of U-NAG/Cr were expressed as standard deviation scores (SDS) or Z-scores by the equation SDS = (actual individual value – mean value for age) /standard deviation for age with the use of previously obtained age-related reference data (5). The obtained values were compared to the age-related reference data and correlated with grade of VUR and number of pyelonephritic episodes in the patients'personal history. The presence of RN was also taken into consideration, together with patients'age and VUR grading.

Statistical analysis was performed by t-test. The linear regression analysis was performed to compare the relationship among respective parameters. For all results, a p-value < 0.05 was required for statistical significance.

Results

The U-NAG/Cr values were significantly higher in the VUR patients in comparison to the reference data (Table 1, Fig. 1,2). There was no difference in U-NAG/Cr between children with unilateral and bilateral VUR (Fig. 3). As there were low patient numbers with VUR grade I-III and V, we pooled the U-NAG/Cr data for VUR I-III and VUR IV-V, respectively. In both groups the values were still significantly higher in comparison to the reference data (Table 1, Fig. 2), however there was no significant difference between VUR I-III and VUR IV-V subgroups (Table 1, Fig. 2). The U-NAG/Cr activity was high in patients with RN when compared to reference data (Table 1, Fig. 4), but there was no difference in comparison to children with VUR without RN (Fig. 4). We found almost no correlation between U-NAG/Cr and grade of VUR ($r = 0.38$), which didn't reach statistical significance ($p = 0.08$). In addition, inverse and significant correlation between patients'age and VUR grade was observed ($r = -0.49$, $p = 0.05$). Number of pyelonephritic episodes in patients' personal history was not related to U-NAG/Cr or VUR grade ($r = -0.18$ and 0.21, respectively).

Discussion and conclusions

The high values of U-NAG/Cr in children with VUR suggest renal tubular impairment and correspond, in part, with previously published data (6-10). However, the U-NAG/Cr values were increased in our patients with VUR, regardless of whether the reflux was unilateral or bilateral. Previous reports indicated high U-NAG in patients with VUR, (6-9), especially in children with VUR grade IV and V (6), or grade V only (10), or in patients with VUR and renal scarring (9). It was therefore of particular interest to look for relationship between U-NAG/Cr and the grade of VUR. Our results suggest that high activity of U-NAG/Cr in children with VUR is only poorly related to the degree of renal damage, as there was almost no correlation between U-NAG/Cr and the grade of VUR, with no difference in U-NAG/Cr between VUR grades I-III and IV-V, respectively. Furthermore, there was no difference in U-NAG/Cr between patients with and without RN. This result differs from another observation, where high U-NAG was most prominent in children with renal scarring (9). These results further suggest that U-NAG/Cr is not related to the amount of affected renal tissue, as strong association between grades IV-V VUR and the presence of renal damage has been repeatedly observed (1,3,11-13). However, there is also published evidence of no relationship between severity of VUR, urinary tract infection symptoms and renal scarring (14,15). This shares some features with our findings of no correlation between number of pyelonephritic episodes in patients' personal history and severity of VUR, weak correlation between VUR grade and U-NAG/Cr, and no difference in U-NAG/Cr in patients with and without RN. Furthermore, it has been already reported that, despite good medical management, even mild and moderate VUR can be associated with renal injury (11,16), and that VUR is a weak predictor of renal damage in children with urinary tract infection (17). Furthermore, in yet another systematic analysis, the authors questioned the values of identification of VUR after a symptomatic urinary tract infection on subsequent renal parenchymal damage (18). This corresponds to our observation concerning lack of relationship between severity of VUR and number of pyelonephritic episodes in patients' personal history, and weak correlation between severity of VUR and U-NAG/Cr values.

Therefore, the findings of high U-NAG with poor relationship to severity of VUR might reflect the fact that the grade of VUR is not always associated with degree of renal impairment. Furthermore, we can't rule out that the U-NAG can detect even very mild changes in renal tubular function, which might occur even in low-grade VUR due to low increases of hydrodynamic pressure.

The inverse correlation between age and the grade of VUR further supports well known fact that VUR grade decreases with age (2,11,19). In conclusion, tubular dysfunction is common in children with VUR. U-NAG/Cr should be considered as a useful marker of renal tubular impairment in patients with VUR, however there is a very weak relationship with the grade

of VUR. This might further support more recent observations that severity of VUR doesn't always fully correspond with the degree of kidney damage.

Acknowledgements

We are most grateful to prof. V. Palicka and his team from the Institute of Clinical Biochemistry and Diagnosis at the Faculty of Medicine in Hradec Králové for performing U-NAG/Cr measurements.

References

1. Lebowitz RL, Olbing H, Parkkulainen KV, Smellie JM, Tamminen-Mobius TE: (1985) International system of radiographic grading of vesicoureteric reflux. International Reflux Study in Children. Pediatr Radiol 15, 105-109.
2. Schwab CW, Wu HY, Selman H, Smith GH, Snyder HM, Canning DA: (2002) Spontaneous resolution of vesicoureteral reflux: A 15-year perspective. J Urol 168, 2594-2599.
3. McIlroy PJ, Abbott GD, Anderson, NG, Turner JG, Mogridge N, Wells JE: (2000) Outcome of primary vesicoureteric reflux detected following fetal renal pelvic dilatation. J Paediatr Child Health 36, 569-573.
4. Csáthy L, Pócsi I: (1995) Urinary N-Acetyl-β-D-glucosaminidase determination in newborns and children: methods and diagnostic applications. Eur J Clin Chem Clin Bioch 33, 575-587.
5. Skálová S, Chládek J: (2004) Urinary N-acetyl-beta-D-glucosaminidase activity in healthy children. Nephrology 9, 19-21.
6. Miyakita H, Puri P: (1994) Urinary levels of N-acetyl-beta-D-glucosaminidase: a simple marker for predicting tubular damage in higher grades of vesicoureteric reflux. Eur Urol 25, 135-137.
7. Fanos V, Mussap M, Osio D, Pizzini C, Plebani M: (2001) Urinary excretion of N-acetyl-beta-D-glucosaminidase and epidermal growth factor in paediatric patients receiving cefixime prophylaxis for recurrent urinary tract infections. Clin Drug Invest 21, 511-518.
8. Goonasekera CD, Shah V, Dillon MJ: (1996) Tubular proteinuria in reflux nephropathy: post ureteric re-implanatation. Pediatr Nephrol 10, 559-563.
9. Tomlinson PA, Smellie JM, Prescod N, Dalton RN, Chantler C: (1994) Differential excretion of urinary proteins in children with vesicoureteric reflux and reflux nephropathy. Pediatr Nephrol 8, 21-25.
10. Williams MA, Jones D, Noe HN: (1994) Urinary N-acetyl-beta-D-glucosaminidase as a screening technique for vesioureteral reflux. Urology 43, 528-530.
11. Mattoo TK: (2006) A changing scenario in our understanding of vesicoureteral reflux in children. Indian Pediatrics 43, 111-115.
12. Silva JM, Santos Diniz JS, Marino VS, Lima EM, Cardoso LS, Vasconcelos MA, Oliveira EA: (2006) Clinical course of 735 children and adolescents with primary vesicoureteral reflux. Pediatr Nephrol 21, 981-988.
13. Silva JM, Diniz JS, Silva AC, Azevedo MV, Pimenta MR, Oliveira EA: (2006) Predictive factors of chronic kidney disease in severe vesicoureteral reflux. Pediatr Nephrol 21, 1285-1292.
14. Ozen HA, Whitaker RH: (1987) Does the severity of presentation in children with vesicoureteric reflux relate to the severity of the disease or the need for operation? Br J Urol 60, 110-112.
15. Lee JH, Son CH, Lee MS, Park YS: (2006) Vesicoureteral reflux increases the risk of renal scars: a study of unilateral reflux. Pediatr Nephrol 21,1281-1284
16. Arant BS, Jr: (1992) Medical management of mild and moderate vesicoureteral reflux: follow up studies of infants and young children. A preliminary report of the Southwest Pediatric Nephrology Study Group. J Urol 148,1683-1687.
17. Gordon I, Barkovics M, Pindoria S, Cole TJ, Woolf AS: (2003) Primary vesicoureteric reflux as a predictor of renal damage in children hospitalized with urinary tract infection: A systematic review and meta-analysis. J Am Soc Nephrol 14, 739-744.
18. Wheeler D, Vimalachandra D, Hodson EM, Roy LP, Smith G, Craig JC: (2003) Antibiotics and surgery for vesicoureteric reflux: A meta-analysis of randomised controlled trials. Arch Dis Child 88, 688-694.
19. Bensman A, Ulinski T: (2006) International Vesicoureteral Reflux Study: unsolved questions remaining. Pediatr Nephrol 21,757-758.

Table 1

U-NAG/Cr and S-Cr values expressed as Z-scores ± SD

Parameter	Mean	SD	P†
U-NAG/Cr (VUR I-V)	5.63	4.88	0.0001
U-NAG/Cr (VUR I-III)	5.39	4.76	0.0001
U-NAG/Cr (VUR IV-V)	6.35	5.26	0.0001
U-NAG/Cr (RN)	5.69	4.80	0.0001

VUR I-V, patients with VUR grade I-V (n=22); VUR I-III, pooled data from patients with VUR I-III (n=9); VUR IV-V, pooled data from patients with VUR IV-V (n=13); RN, patients with reflux nephropathy (n=9); †Compared to reference data

Fig. 1
U-NAG/Cr (mean values expressed as Z-scores) in VUR grade I-V

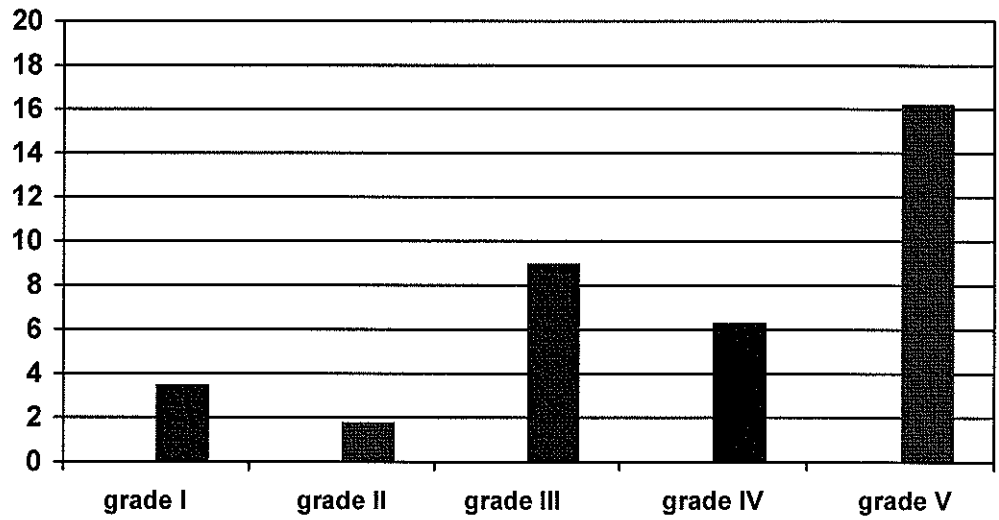


Fig. 2
U-NAG/Cr (Z-scores) related to reference values
Grade I-III (n = 9) versus grade IV-V (n = 13) non-significant (p = 0.67)

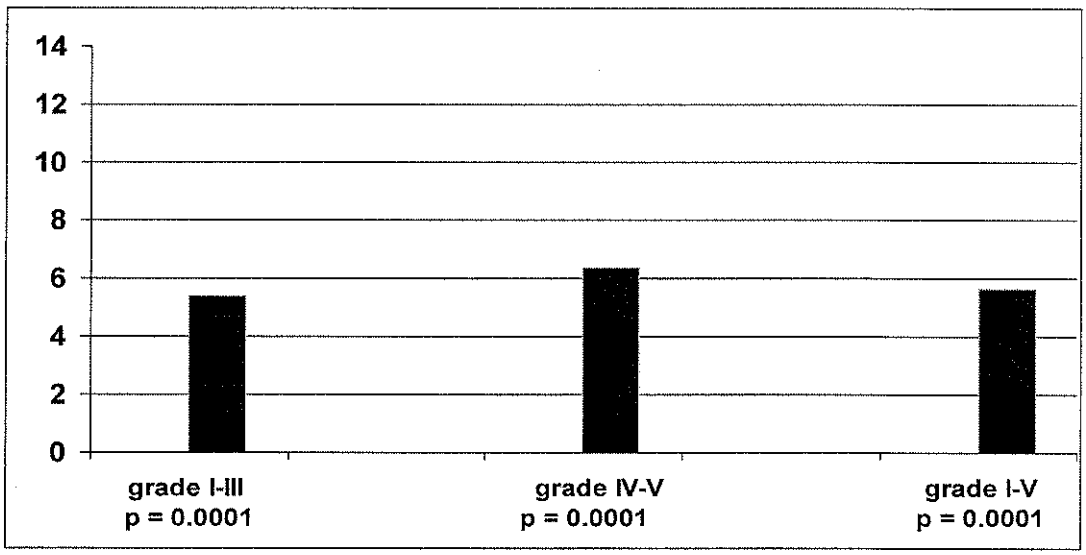


Fig.3
U-NAG/Cr (Z-scores) related to reference values
Unilateral (n=8) vs bilateral VUR (n=14) non-significant
(p = 0.66)

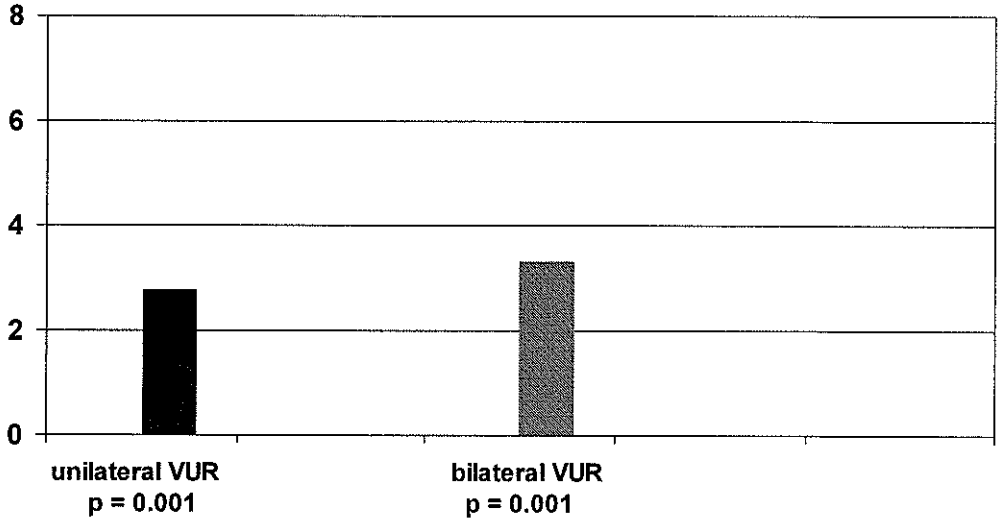
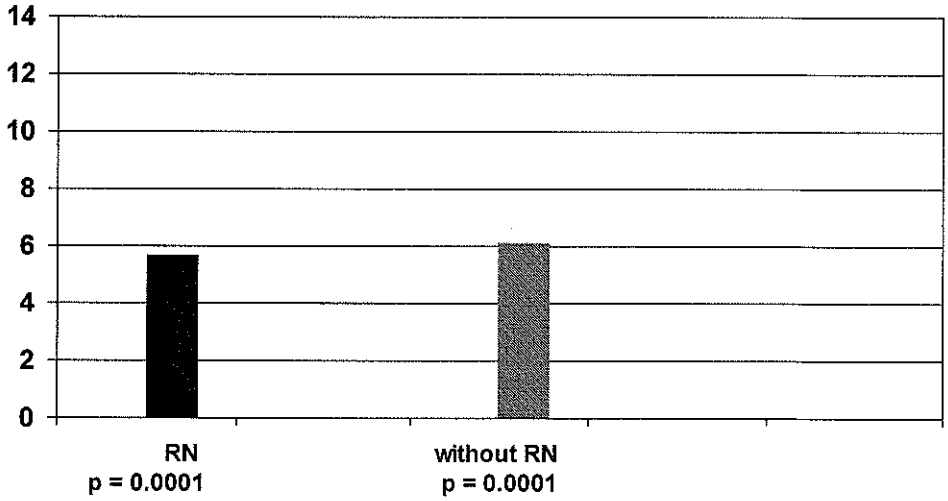


Fig.4
U-NAG/Cr (Z-scores) related to reference values
Reflux nephropaty (RN) patients (n=9) vs patients without RN
(n=13) non-significant
(p = 0.84)



CHAPTER 9

INCREASED URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE ACTIVITY IN CHILDREN WITH HYDRONEPHROSIS

International Brazilian Journal of Urology 2007;33:80-86

CHAPTER 9

INCREASED URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE ACTIVITY IN CHILDREN WITH HYDRONEPHROSIS

Sylva Skálová[1], Pavel Rejtar[2], Stepan Kutilek[3]

Departments of [1]Paediatrics and [2]Radiology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Czech Republic

[3]Center for Clinical and Basic Research (CCBR), Pardubice, Czech Republic

Abstract

Purpose: Hydronephrosis leads to deterioration of renal function. As urinary N-acetyl-beta-D-glucosaminidase (U-NAG) activity is considered a sensitive marker of renal tubular impairment, our aim was to measure U-NAG in children with hydronephrosis and to look for relationship among selected clinical parameters.

Materials and Methods: 31 children (22 boys and 9 girls, mean age 2.3 ± 2.5 years) with hydronephrosis grade 1-4 had U-NAG/creatinine ratio (U-NAG/Cr) measured.

Results: The U-NAG/Cr was significantly higher in the patients with hydronephrosis compared to reference data ($p = 0.002$). There was no difference in U-NAG/Cr between children with unilateral and bilateral hydronephrosis ($p= 0.51$). There was no significant difference in U-NAG/Cr between children with grades 1-3 (pooled data) and grade 4, respectively ($p=0.89$). There was no correlation between U-NAG/Cr and the grade of hydronephrosis ($r = 0.01$).

Conclusions: U-NAG/Cr is increased in children with hydronephrosis grade 1-4, and there is no relationship with the grade of hydronephrosis. U-NAG is a useful marker of renal tubular dysfunction, however its relationship with the degree of kidney damage in patients with hydronephrosis should be considered as doubtful.

Key words: *N-acetyl-β-D-glucosaminidase; hydronephrosis*

Introduction

Hydronephrosis leads to deterioration of renal function (1,2). N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme which is abundantly present in the cells of the proximal tubule and is considered as a very sensitive marker of renal tubular impairment in various disease states (3,4). Our aim was to measure urinary NAG activity (U-NAG) in children with hydronephrosis and to look for possible relationship between patients' clinical data and U-NAG.

Patients, methods

Patients

31 children. (22 boys and 9 girls, mean age 2.25 ± 2.50 years; range 0.08 - 9.08 y) with hydronephrosis were enrolled. Informed consent was obtained from parents of each patient prior to any procedures described in this paper. Hydronephrosis was diagnosed by means of abdominal ultrasonography either prenatally (n= 20) or postnatally (n=11), the latter at the mean age of 6 ± 14 months (range 0.1 - 48 months). In all patients, the hydronephrosis and its grade was further evaluated postnatally by means of ultrasound and ^{99m}Tc mercaptoacetyltriglycine (MAG3) "well tempered" renography (5,6). Hydronephrosis was graded according to the Society for Fetal Urology (SFU) classification (1). Vesicoureteral reflux was ruled out in all patients by voiding cystourethrography. None of the patients had solitary kidney. In 18 patients the hydronephrosis was unilateral, grade 1-4 (mean 3.1 ± 0.8), and in 13 patients, the hydronephrosis was bilateral, grade 1-4 (mean 2.9 ± 0.7). In the patients with bilateral hydronephrosis and different grade on each side, the highest grade was taken into consideration. Therefore, the diagnostic distribution was as follows: grade 1, n = 1; grade 2, n = 2; grade 3, n = 16; grade 4, n = 12. All patients had their kidney functions evaluated by the "well-tempered" diuretic renogram with ^{99m}Tc MAG3 (5-7). The relative renal function, expressed as percentage represented by the contribution of each kidney to the global renal function was evaluated. In only 2 children with unilateral hydronephrosis, the relative function of the affected kidney was 35%. In the remaining 17 children with unilateral hydronephrosis, the relative function of the affected kidney exceeded 40%. The mean value of the relative function of the affected kidney in the 18 patients with unilateral hydronephrosis was 47.3%. In the entire group of 31 children, the mean relative renal function of the right and left kidney was 50.4% : 49.6%. In patients with hydronephrosis grade 1-3 there were no signs of obstruction, while obstruction was present in patients with grade 4. The obstruction was evidenced by several criteria, such as: progressive dilatation of

the calyces and pelvis on ultrasound imaging; >5% decrease per year in the function of hydronephrotic kidney on ^{99m}Tc MAG3 renogram; obstructive pattern of renogram curve after administration of furosemide with a clearance half-life greater than 20 minutes (5-7). None of the patients underwent any surgical procedure due to hydronephrosis prior to the U-NAG measurements. The patients with grade 4 were later confined to surgical treatment.

Materials and Methods

All patients had their U-NAG and serum and urinary concentrations of creatinine (S-Cr, U-Cr) evaluated. None of the patients suffered from pyelonephritis at the time of the U-NAG/Cr and S-Cr evaluation. All patients were free from infection at least 4 months prior to the U-NAG/Cr and S-Cr evaluation. Urinary NAG was evaluated in the spot urine, collected after the first morning void. The blood and spot urine were collected either at the time of the ultrasonographic examination or in a time frame of ± 1 month within abdominal ultrasonography and ^{99m}Tc MAG3 renography. The influence of endogenous enzyme inhibitors was eliminated by diluting the urine specimens 20-fold. The urinary catalytic activity of NAG was then determined by fluorimetric assay. The S-Cr and U-Cr were estimated by Jaffe's kinetic method on Modular Analyser (Roche Diagnostics GmbH, Mannheim, Germany). The S-Cr values were expressed in μmol/L. The U-NAG values were expressed as the urinary NAG/creatinine (U-NAG/Cr) ratio in nkat/L : mmol/L. To eliminate the influence of age, the obtained results of S-Cr and U-NAG/Cr were expressed as standard deviation scores (SDS) or Z-scores by the equation SDS = (actual individual value – mean value for age) /standard deviation for age with the use of age-related laboratory reference data for S-Cr and previously obtained reference data for U-NAG/Cr (4). These reference standards of U-NAG/Cr were obtained from a total of 262 children (aged 0-18 years), and in particular from 213 children aged 0-10 years (4). The obtained values were compared to the age-related reference data and correlated with grade of hydronephrosis. The presence of either unilateral or bilateral hydronephrosis was also taken into consideration.

The statistical analysis was performed by t-test. The linear regression analysis was performed to compare the relationship among respective parameters. For all results, a p-value < 0.05 was required for statistical significance.

Results

The U-NAG/Cr values were significantly higher in the patients with hydronephrosis in comparison to the reference data (Table 1). There was no difference in U-NAG/Cr between children with unilateral and bilateral hydronephrosis ($p = 0.51$).

As there were low patient numbers with hydronephrosis grade 1-2, we pooled the U-NAG/Cr data for this group of children together with hydronephrosis grade 3. When compared to reference data, patients with grade 1-3 ($n = 19$) and those with grade 4 ($n = 12$)

had significantly higher U-NAG/Cr activity (Table 1). However there was no significant difference in U-NAG/Cr between children with grade 1-3 and grade 4, respectively (p=0.89). Neither was there any significant difference in the U-NAG/Cr values between children with unilateral and bilateral hydronephrosis when stratified for grade (grade 1-3 and 4, respectively; p = 0.55 and p = 0.50, respectively). The S-Cr was within ± 2SD range in 30/31 patients, however this was still significantly higher in comparison to reference data (Table 1). There was no difference in S-Cr between children with unilateral and bilateral hydronephrosis (p= 0.82). No correlations were observed between U-NAG/Cr and the grade of hydronephrosis (r = 0.01), or between S-Cr and the grade of hydronephrosis (r = -0.07). We found a positive correlation between U-NAG/Cr and S-Cr, which reached statistical significance (r = 0.40, p = 0.05).

Discussion

The high values of U-NAG/Cr in our patients with hydronephrosis suggest renal tubular impairment and are in accordance with previously reported results, which are only scarce (8-13). Experimental studies revealed high U-NAG in rats with partial ureteral obstruction and hydronephrotic atrophy (8,9). In children with unilateral hydronephrosis, increased U-NAG was detected in urine obtained from renal pelvis (10,11) and bladder (11), with pelvic U-NAG levels higher than bladder U-NAG levels (11). High U-NAG/Cr levels were observed in children with renal pyelectasis (12). Interestingly, post-operative increase in U-NAG levels was reported in patients with hydronephrosis (13).

In our patients, the U-NAG/Cr values, measured in the spontaneously voided urine, were increased, regardless whether there was unilateral or bilateral hydronephrosis. Previously published observations based on evaluation of isotope renal function and imaging procedures gave evidence that children with grade 4, and some with grade 3 of hydronephrosis, have impaired renal functions and should be confined to surgical treatment, which has been proven as beneficial (1,2, 14,15). It was therefore of particular interest to see if U-NAG was somehow related to the grade of hydronephrosis. However, the high levels of U-NAG did not correspond with the ultrasonographic degree of renal damage, as there was no correlation between U-NAG and the grade of hydronephrosis, and there was no difference in U-NAG between grades 1-3 and 4, respectively. Similarly, the renal functions, as assessed by the ^{99m}Tc MAG3 renography, were not severely impaired. There was no difference in U-NAG/Cr between children with unilateral and bilateral hydronephrosis. These results might suggest that the renal function, as assessed by ^{99m}Tc MAG3 renography might not be solely related to the grade of hydronephrosis, and that U-NAG in hydronephrosis is not dependent on the amount of affected renal tissue. Furthermore, we can't rule out that the U-NAG can reflect even very mild changes in renal tubular function, which might occur even in low-

grade non-obstructive hydronephrosis. There was a mild elevation of S-Cr which reached statistical significance and there was also a mild correlation between U-NAG/Cr and S-Cr. However, the changes in S-Cr in our group of patients are strongly obscured by the fact that all but one S-Cr values remained within the ± 2 SD range and that there was no difference between unilateral and bilateral hydronephrosis.

In conclusion, U-NAG/Cr is increased in children with hydronephrosis grade 1-4, however there is no relationship with the grade of hydronephrosis or with the amount of affected renal tissue. U-NAG/Cr is a useful marker of renal tubular impairment, however its relationship with the degree of kidney damage in patients with hydronephrosis should be considered as doubtful.

Acknowledgements

We are most grateful to prof. V. Palicka and his team from the Institute of Clinical Biochemistry and Diagnosis at the Faculty of Medicine in Hradec Králové for performing the S-Cr and U-NAG/Cr measurements.

References

1. Maizels M, Mitchell B, Kass E, Fernbach SK, Conway JJ: Outcome of nonspecific hydronephrosis in the infant: A report from the Registry of the Society for Fetal Urology. J Urol. 1994;152:2324-7.
2. Rodriguez LV, Lock J, Kennedy WA, Shortliffe LM: Evaluation of sonographic renal parenchymal area in the management of hydronephrosis. J Urol. 2001;165:548-51.
3. Csáthy L, Pócsi I: Urinary N-Acetyl-β-*D*-glucosaminidase determination in newborns and children: methods and diagnostic applications. Eur J Clin Chem Clin Bioch. 1995;33:575-87.
4. Skálová S, Chládek J: Urinary N-acetyl-beta-D-glucosaminidase activity in healthy children. Nephrology 2004;9:19-21.
5. Society for Fetal Urology and Pediatric Nuclear Medicine Council. The "well tempered" diuretic renogram: a standard method to examine the asymptomatic neonate with hydronephrosis or hydroureteronephrosis. J Nucl Med 1992;33:2047-51.
6. Rossleigh MA. Renal cortical scintigraphy and diuresis renography in infants and children. J Nucl Med 2001;42:91-5.
7. Saremi F, Jadvar H, Siegel ME. Pharmacologic interventions in nuclear radiology: Indications, imaging protocols and clinical results. Radiographics 2002;2:477-90.
8. Huland H, Gonnermann D, Werner B, Possin U: A new test to predict reversibility of hydronephrotic atrophy after stable partial unilateral ureteral obstruction. J Urol. 1988;140:1591-4.
9. Everaert K, Van Der Wiele C, Delanghe J, Vander Eecken H, Van Haelst JP, Van de Voorde J et al: Urinary excretion of tubular proteins and the technetium-99m-dimercaptosuccinic acid (DMSA) absolute renal uptake in partial ureteral obstruction in rats: a functional evaluation of hydronephrotic kidneys. Urol Res. 1999;27:127-33.
10. Konda R, Orikasa S, Sakai K, Kuji S, Ota S, Kaneda T et al: Evaluation of renal function and prediction of renal functional recovery in children with unilateral hydronephrosis using renal pelvic urine. Nippon Hinyokika Gakkai Zasshi 1992;83:1815-22.
11. Carr MC, Peters CA, Retik AB, Mandell J: Urinary levels of the renal tubular enzyme N-acetyl-beta-D-glucosaminidase in unilateral obstructive uropathy. J Urol. 1994;151:442-5.
12. Leon González J, García Nieto V, Hernández Rodríguez A, Fernández González LM: Estudio de la función renal en niños diagnosticados de ectasia piélica en el primer año de vida. An Esp Pediatr 2001;54:458-62.

13. Ueda K, Kato J, Seki T: Urinary excretion of N-acetyl-beta-D-glucosaminidase in patients with urological disease: with special reference to hydronephrosis. Hinyokika Kiyo 1984;30:877-82.
14. Palmer LS, Maizels M, Cartwright PC, Fernbach SK, Conway JJ: Surgery versus observation for managing obstructive grade 3 to 4 unilateral hydronephrosis: a report from the Society for Renal Urology. J Urol. 1998;159:222-8.
15. Sibai H, Sall JL, Houle AM, Lambert R: Hydronephrosis with diffuse or segmental cortical thinning: impact on renal function. J Urol. 2001;165:2293-5.

Table 1

U-NAG/Cr and S-Cr values expressed as Z-scores ± SD

Parameter	Mean	SD	P†
U-NAG/Cr (grade 1-4)	4.92	5.38	0.002
U-NAG/Cr (grade 1-3)	5.02	5.29	0.0006
U-NAG/Cr (grade 4)	4.76	5.74	0.015
S-Cr (grade 1-4)	0.53	1.09	0.05

U-NAG/Cr (grade 1-4), data from patients with hydronephrosis grade 1-4 (n=31); U-NAG/Cr (grade 1-3), pooled data from patients with grade 1-3 (n=19); U-NAG/Cr (grade 4), data from patients with grade 4 (n=12); S-Cr (grade 1-4), data from patients with hydronephrosis grade 1-4; †Compared to reference data

CHAPTER 10

GENERAL DISCUSSION AND FUTURE RESEARCH

CHAPTER 10

GENERAL DISCUSSION AND FUTURE RESEARCH

10.1. General discussion

In this chapter we will discuss the most important findings of this thesis, and put them into perspective of current knowledge. The discussion will finish with suggestions for future research.

U-NAG was measured by fluorimetric assay in 262 healthy children aged 0-18 years and in 87 children with following disorders: idiopathic hypercalciuria (IH; n=20), nocturnal enuresis (NE; n=14), vesicoureteral reflux (VUR; n=22) and hydronephrosis (HN; n=31). In 15 children with IH bone mineral density (BMD) was also assesed.

The U-NAG/Cr values of 262 healthy children aged 0-18 years, obtained with fluorimetric assay, are strongly age-dependent, i.e. decreasing with age. Previously published papers regarding the urinary NAG excretion in childhood scarcely dealt with reference data obtained from larger pediatric population or did not have enough reference data pertinent to neonates and infants (1-3). Our results represent data from sufficiently large healthy paediatric population inclusive of neonates and infants, and shows strong age-dependence of the urinary NAG as a result of a concomitant rise in the urinary creatinine concentration. There is a great interindividual variability of those values in children, as reflected by the standard deviation. Such an observation is further strongly in favour of establishing reference paediatric data, as the reproducibility of results obtained from relatively small patient and control groups should be considered as difficult, especially when taking into consideration the large interindividual variability of the urinary NAG values in healthy children. Our results are in accordance with previously published data, especially in terms of the strong age-dependency of urinary NAG (1-3). The establishment of U-NAG/Cr reference paediatric values is a potentially useful tool for proper evaluation of renal tubular impairment in childhood, because knowledge of normal physiological variation is necessary to identify pathological changes.

Renal tubular impairment can be encountered in patients with IH and urolithiasis or nephrocalcinosis (4,5). Other authors reported significantly higher U-NAG in children with urolithiasis and nephrocalcinosis, but not in children with isolated IH alone, which did not correlate with the urinary excretion of oxalate or calcium (5). However, we found high U-NAG/Cr in patients with nephrocalcinosis/urolithiasis and also in patients with isolated IH.

This suggests some degree of renal tubular impairment, which is most probably a secondary one. Therefore, increased urinary concentration of calcium might lead to damage of tubular cells, even in the absence of lithiasis (6). Furthermore, the findings of higher U-NAG/Cr in patients with urolithiasis/nephrocalcinosis in comparison to the values in children with IH but without urolithiasis/nephrocalcinosis might support the hypothesis that cell-crystal interactions lead to tubular impairment (6). In conclusion, children with IH have some degree of secondary renal tubular impairment. The tubular impairment is most probably aggravated by the increased urinary concentration of calcium, and, in particular, by the cell-crystal interactions. However, there doesn't seem to be a direct relationship between this tubular impairment and the degree of calcium leakage.

In addition, high U-NAG/Cr, low BMD and a tendency to short stature were observed in children with IH. We found inverse and significant correlation between BMD and U-Ca/24 h, and a similar correlation between U-Ca/24 h and body height. There was no correlation between U-NAG/Cr and U-Ca/24 h. It seems likely that hypercalciuria can contribute to the skeletal demineralization and to the development of nephrocalcinosis, both resulting in stunted growth. Idiopathic hypercalciuria should be considered as a risk factor for stunted growth and low bone mass, however it doesn't correlate with U-NAG/Cr. Therefore, growth velocity and BMD should be given special attention in children with IH (7).

In children with NE, the values of U-Ca/Cr did not differ significantly from the reference data, however U-NAG/Cr values were significantly higher compared to reference values. Therefore, contrary to some other authors (8-10), we did not confirm increased calciuria in these patients. There was no correlation between U-NAG/Cr and U-Ca/Cr. The presence of elevated urinary levels of U-NAG/Cr suggest that tubular dysfunction might be present in patients with NE (11). Therefore, the underlying mechanism of such tubular dysfunction is not clear (11).

In children with VUR, the U-NAG/Cr values were significantly higher in comparison to data from healthy children. Our results might suggest that high activity of U-NAG/Cr in children with VUR is not related to the degree of renal damage, as there was no relationship between U-NAG/Cr and the grade of VUR, with no difference in U-NAG/Cr between VUR grades I-III and IV-V, respectively. Furthermore, there was no difference in U-NAG/Cr between patients with and without RN. This result differs from another observation, where high U-NAG was most prominent in children with renal scarring (12). These results suggest that U-NAG/Cr is not related to the amount of affected renal tissue. Furthermore, it has been already reported that, despite good medical management, even mild and moderate VUR can be associated with renal injury (13,14), and that VUR is a weak predictor of renal damage in children with urinary tract infection. Furthermore, in yet another systematic analysis, the

authors questioned the values of identification of VUR after a symptomatic urinary tract infection on subsequent renal parenchymal damage (15). This corresponds to our observation concerning lack of relationship between severity of VUR and number of pyelonephritic episodes in patients' personal history, and no relationship between severity of VUR and U-NAG/Cr values. Therefore, the findings of high U-NAG with poor relationship to severity of VUR might reflect the fact that the grade of VUR is not always associated with degree of renal impairment. Furthermore, we can't rule out that the U-NAG can detect even very mild changes in renal tubular function, which might occur even in low-grade VUR due to low increases of hydrodynamic pressure.

U-NAG/Cr values were significantly higher in the patients with HN in comparison to the reference data. There was no difference in U-NAG/Cr between children with unilateral and bilateral HN. There was no significant difference in U-NAG/Cr between children with HN grade 1-3 and grade 4, respectively No correlations were observed between U-NAG/Cr and the grade of hydronephrosis. The renal functions, as assessed by the ^{99m}Tc MAG3 renography, were not severely impaired. These results might suggest that the renal function, as assessed by ^{99m}Tc MAG3 renography might not be solely related to the grade of hydronephrosis, and that U-NAG in hydronephrosis is not dependent on the amount of affected renal tissue. We can't rule out that the U-NAG can reflect even very mild changes in renal tubular function, which might occur even in low-grade non-obstructive hydronephrosis. The finding that U-NAG/Cr levels were elevated in all patients with hydronephrosis merits further consideration. It implies that even small degrees of hydronephrosis may adversely affect tubular function beyond our capability to measure. Due to the small numbers of grades 1 and 2, these conclusions are best limited to grades 3 and 4. U-NAG/Cr is increased in children with hydronephrosis grade 1-4, however there is no relationship with the grade of hydronephrosis or with the amount of affected renal tissue. U-NAG/Cr is a useful marker of renal tubular impairment, however there is no relationship with the degree of kidney damage in patients with hydronephrosis.

Patients with IH, NE, VUR, HN all had significantly elevated U-NAG/Cr values. This implies impaired renal tubular function, maybe even beyond our capability to evaluate by involving other markers. When looking for correlations between U-NAG/Cr and clinical data, the changes in U-NAG/Cr are only related to BMD and body height in patients with IH, while in other pathologic conditions (NE, VUR, HN) the U-NAG/Cr had only very poor relationship to other important clinical parameters. However, in the absence of a universal "gold standard" of renal tubular impairment, the U-NAG remains a useful tool.

10.2. Future research

We are still searching for the "holy grail", "gold standard" of tubular impairment — a highly sensitive, highly specific marker of functionally significant tubulopathy, which is detectable before deterioration, or before clinical symptoms develop. The evaluation of U-NAG in paediatric patients with various renal disease may be one the first steps in this direction. Future studies should include prospective follow-up of children with IH, NE, and, especially VUR and HN, with special regard to the changes in U-NAG/Cr and its relationship to the clinical course of the disease. U-NAG/Cr should be also related to some novel markers of tubular function, such as cystatin C.

References

1. Dillon SC, Taylor GM, Shah V. Diagnostic value of urinary retinol-binding protein in childhood nephrotic syndrome. Pediatr. Nephrol. 1998;12:643-647.
2. 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;155:596-602.
3. Csathy L, Olah AV, Pocsi I, Varga J, Balla G, Price RG. Age-dependent urinary N-acetyl-beta-D-glucosaminidase activity in healthy children. Orv. Hetil. 1994;135:1301-1303.
4. Sikora P, Glatz S, Beck BB, Stapenhorst L, Zajaczkowska M, Hesse A, et al. Urinary NAG in children with urolithiasis, nephrocalcinosis, or risk of urolithiasis. Pediatr Nephrol 2003;18:996-9
5. Stapleton FB, Chesney RW, Behrmann AT, Miller LA. Increased urinary excretion of renal N-acetyl-beta-glucosaminidase in hypercalciuria. Am J Dis Child 1985;139:950-952
6. Skálová S, Kutílek Š. Renal tubular impairment in children with idiopathic hypercalciuria. Acta Medica 2006; 49: 109-111
7. Skálová S, Palicka V, Kutilek S. Bone mineral density and urinary N-acetyl-β-D-glucosaminidase activity in pediatric patients with idiopathic hypercalciuria. Nephrology 2005;10:99-102.
8. Vachvanichsanong P, Malagon M, Moore ES. Urinary incontinence due to idiopathic hypercalciuria in children. J Urol 1994;152:1226-1228.
9. Pace G, Aceto G, Cormio L, Traficante A, Tempesta A, Lospalluti ML, Selvaggi FP, Penza R. Nocturnal enuresis can be caused by absorptive hypercalciuria. Scand J Urol Nephrol 1999;33:111-114.
10. Aceto G, Penza R, Coccioli MS, Palumbo F, Cresta L, Cimador M, Chiozza ML, Caione P. Enuresis subtypes based on nocturnal hypercalciuria: a multicenter study. J Urol 2003;170:1670-1673.
11. Skalova S, Kutilek S. High urinary N-acetyl-β-D-glucosaminidase (NAG) activity and normal calciuria in children with nocturnal enuresis. Indian Pediatrics 2006;43:655-656
12. Tomlinson PA, Smellie JM, Prescod N, Dalton RN, Chantler C. Differential excretion of urinary proteins in children with vesicoureteric reflux and reflux nephropathy. Pediatr Nephrol 1994;8:21-25.
13. Gordon I, Barkovics M, Pindoria S, Cole TJ, Woolf AS. Primary vesicoureteric reflux as a predictor of renal damage in children hospitalized with urinary tract infection: A systematic review and meta-analysis. J Am Soc Nephrol 2003;14: 739-744.
14. Wheeler D, Vimalachandra D, Hodson EM, Roy LP, Smith G, Craig JC. Antibiotics and surgery for vesicoureteric reflux: A meta-analysis of randomised controlled trials. Arch Dis Child 2003;88:688-694.
15. Bensman A, Ulinski T. International Vesicoureteral Reflux Study: unsolved questions remaining. Pediatr Nephrol 2006;21:757-758.
16. Skálová S, Rejtar P, Kutílek S. Increased urinary N-acetyl-beta-D-glucosaminidase activity in children with hydronephrosis Int Braz J Urol 2007;33:80-86.

CHAPTER 11

SUMMARY AND CONCLUSIONS, RECOMMENDATIONS

CHAPTER 11

SUMMARY AND CONCLUSIONS, RECOMMENDATIONS

11.1. Summary

Chapter 1

Chapter 1 gives brief information on renal anatomy and physiology, and in particular on anatomy, physiology and pathophysiology of renal tubules, including diagnostic procedures. This introduction is essential for the integrity of the thesis.

Chapter 2

Chapter 2 gives basic information concerning this thesis, its primary and secondary aims.

Chapter 3

Chapter 3 gives a state-of-art overview of the diagnostic role of U-NAG in the detection of renal tubular impairment and its clinical applicability. 92 articles are evaluated in this review in a critical way. U-NAG activity is a useful marker of renal tubular impairment in various disease states. When compared to other urinary enzymes, U-NAG is the most frequently used urinary enzymatic marker when it comes to the evaluation of tubular function. However, the routine evaluation of enzymuria is much less frequently used when compared to the routine evaluation of enzymes in serum or plasma.

Chapter 4

Chapter 4 presents reference data of U-NAG for all paediatric age groups. In conclusion, the U-NAG/Cr values of 262 healthy children aged 0-18 years, obtained with fluorimetric assay, are strongly age-dependent, ie. decreasing with age. The establishment of U-NAG/Cr reference paediatric values is a potentially useful tool for proper evaluation of renal tubular impairment in childhood, because knowledge of normal physiological variation is necessary to identify pathological changes.

Chapter 5

Chapter 5 gives information on U-NAG/Cr values in 20 paediatric patients with idiopathic hypercalciuria (IH). In conclusion, children with IH have some degree of secondary renal tubular impairment. There is not a direct relationship between tubular impairment in IH and the degree of calcium leakage.

Chapter 6

Chapter 6 analyses the relationship of bone mineral density (BMD), body height and U-NAG/Cr values in 15 children with idiopathic hypercalciuria (IH). In conclusion, tubular impairment is highly probable in children with IH, as high U-NAG/Cr was observed, but there is a poor relationship with the degree of calcium leakage. BMD was significantly lower when compared to the reference values. Idiopathic hypercalciuria should be considered as a risk factor for stunted growth and low bone mass.

Chapter 7

Chapter 7 presents data on calciuria and U-NAG/Cr activity in 14 children with NE. In conclusion, hypercalciuria was not found in children with NE. The presence of elevated urinary levels of U-NAG/Cr suggest tubular dysfunction in patients with NE.

Chapter 8

Chapter 8 presents U-NAG activity in 22 children with various grades of vesicoureteral reflux (VUR). In conclusion, U-NAG/Cr should be considered as a marker of renal tubular impairment in patients with VUR, however there is no relationship with the grade of VUR and no relationship to RN.

Chapter 9

Chapter 9 gives data on U-NAG activity in 31 patients with hydronephrosis (HN). In conclusion, U-NAG/Cr is increased in children with hydronephrosis grade 1-4, however there is no relationship with the grade of hydronephrosis or with the amount of affected renal tissue.

11.2.General conclusions and recommendations

1. U-NAG/Cr is an important marker of renal tubular impairment/dysfunction
2. U-NAG/Cr can reflect even very mild changes in renal tubular function.
3. U-NAG/Cr is significantly increased in the following urinary tract disorders: idiopathic hypercalciuria, nocturnal enuresis, vesicoureteral reflux and hydronephrosis.
4. In the above mentioned disease states affecting the uropoetic system, the U-NAG/Cr is elevated, but not correlated with the severity of the disease.

ACKNOWLEDGMENTS

I would like to express my thanks to my tutors Eliška Marklová, MD, PhD, and Alexander Kolský, MD, PhD, as well as to my superiors Assoc. Prof. Eva Pařízková, MD, PhD, and Assoc. Prof. Oldřich Pozler, MD, PhD for their support. Furthermore, I am very grateful to Prof. Vladimír Palička, MD, PhD, and Assoc. Prof. Pavel Živný, MD, PhD, for the evaluation of U-NAG/Cr in their laboratory, and to my co-authors, Jaroslav Chládek, PhD, and Assoc. Prof. Štěpán Kutílek, MD, PhD for their assistance, advice and support in the preparation of the manuscripts. Many thanks to Pavel Rejtar, MD, for ultrasound examinations of the urinary tract in the presented patients.