REVIEW

Diagnostic significance of urinary enzymes in nephrology

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Abstract

The study of urinary excretion of some enzymes is a sensitive test for the detection of early stages of renal disease. The first part of this review describe the sources of urinary enzymes and discuss the preanalytical and analytical problems with estimation of enzymatic activities in the urine, mainly the problem of quantitative expression of excretion of urinary enzymes. After the description of main characteristics of diagnostically most important enzymes excreted in the urine the diagnostic validity of the measurement of urinary enzyme activities in patients with inflammatory renal diseases, kidney damage due to nephrotoxic substances, diabetic nephropathy and in early detection of the complications after kidney transplantation is discussed. *(Ref. 25.)*

Key words: enzymuria, urinary enzymes, alanine aminopeptidase, N-acetyl-ß-glucosaminidase, review.

The estimation of enzyme activities in biological fluids is a common method used in laboratory diagnostics. The most frequent used biological fluid for enzyme activities estimation is blood plasma or serum. But enzyme activities could be estimated also in other biological fluids such as urine, cerebrospinal fluid, amniotic fluid, etc. Measurement of activities of urinary enzymes is considered to be a useful non-invasive test in detecting the deterioration of renal function in the early stage.

With the description of increased activities of enzymes in the urine of patients with kidney disease by Rosalki and Wilkinson (1959) the clinical era was introduced to the use of urinary enzymes for diagnostic purposes. While in the early fifthies and in the beginning of sixties especially the lactate dehydrogenase and alkaline phosphatase but also leucin aminopeptidase and beta-glucuronidase were clinically applied, broader research attempts with more enzymes in urine had been started. At least 40 enzymes for the diagnosis of urorenal diseases have been analysed so far.

Sources of urinary enzymes

Several sources contribute to the enzyme activities in urine. The greatest part of the urinary enzymes derives from kidney tissue. Renal tubular cells contain high activities of many enzymes in order to fulfill their numerous biochemical functions. Normal turnover rate of the tubular cells as well as changes of their cellular permeability account for the renal enzyme excretion in urine.

Under normal conditions, only few enzymatic activities of the urine derive from the serum, entering the urine by glomerular filtration. Enzymes exceeding a molecular weight of 70000 are not excreted into the urine, at least not in significant amounts. Genital secretions, blood cells and bacteria contribute only in a minor extent to the urinary enzyme activity if at all.

Thus, the urinary enzymes originate, except for low molecular enzymes like amylase and lysozyme, primarily from renal tubular cells. The enzyme activities measured in urine are the result of the amount of enzymes released from tubular cells, of the influence of inhibitors and/or activators on activity determination and of the stability of enzymes in urine after release from the cells.

Preanalytical and analytical problems in the measurement of enzymuria

In comparison to estimation of plasma enzymes activities, there are several problems in the measurement of enzymuria. In

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contrast to the release of enzymes into blood or other body fluids, the release of enzymes into urine represents an excretion into open system (Guder and Heidland, 1986).

Important factor in the estimation of urinary enzyme activities is the elimination of inhibitors present in the urine. A lot of identified inhibitors exist, such as low molecular weight peptides or inorganic phosphates. We also know unidentified inhibitors of gamma-glutamyltransferase, phosphatase and N-acetyl-ß-D-glucosaminidase (Horpacsy, 1983). Urinary enzyme assays are also affected by drugs, like penicillin, salicylate and sulfonamides. According to Werner et al (1969) it is possible to eliminate most of the above-mentioned interferences by a very simple gel filtration procedure. The stability of enzymes in the eluate after filtration is excellent. At 4 °C the eluate can be stored up to a week without any loss of enzyme activities (Horpacsy, 1983).

The stability of urinary enzymes is influenced particularly by pH. At low pH values in urine (about pH 5.0) alanine aminopeptidase, alkaline phosphatase, gamma-glutamyltransferase and lactate dehydrogenase rapidly lose a considerable part of their activity, whereas N-acetyl-ß-D-glucosaminidase is inactivated at higher pH values in urine (about at pH 8.0). This inactivation effect is also time-dependent and can be modified by urinary substances such as creatinine, urea and electrolytes (Jung et al, 1983). To avoid misinterpretation of enzyme activity determinations in urine, the simultaneous measurement of urinary pH should be performed and the samples with low or high pH values are best excluded from assay, or interpreted with great caution.

Another problem is the expression of enzyme activities in the urine. One of the main physiological functions of kidneys is their participation in the maintanance of appropriate water balance. One of the remarkable properties of the human kidney is its ability to elaborate urine that is either more concentrated or more dilute than the plasma from which it is derived. When the human body needs to conserve water, as in dehydration, the concentrating mechanism operates maximally, and kidney produces decreased volume of concentrated urine. Conversely, when there is excess water in the body, urine flow increases and kidney produces large volume of diluted urine. These changes of diuresis in connection to the renal regulation of body water are responsible for relatively broad inter- and intra-individual variabilities in concentration of substances excreted in urine. Therefore, the expression of activity of urinary enzymes as „enzyme activity per volume of excreted urine“ is influenced by the amount of excreted water.

The disadvantage of the estimation of enzyme activity in untimed urine samples could be eliminated by the measurement of excretion rates of urinary enzymes in timed urine samples. The urinary excretion of substances is generally characterized in nephrology by a time-related excretion rate, especially by the rate of excretion per 24 hours. This traditional procedure is selected in order to reduce the variability of the excreted analyte caused by diurnal rhythms. Similar recommendations have also been made for urinary enzymes (Maruhn, 1983). Recently, shorter sampling periods (e.g. 3 hours) were proposed, since extended collection periods may be accompanied by inactivation of urinary enzymes either in vivo (in the bladder) or in vitro (Jung, 1991).

Accurately timed collection of urine samples is often unreliable and especially impracticable for screening purposes and for the investigation of outpatients. Thus, single urine samples have been suggested for the determination of urinary analytes (Krieg et al, 1986). In such case, adjustment to reference parameter is considered necessary, in order to reduce the variation of the respective analyte concentration due to the inconstant dilution/concentration of urine samples. Urinary creatinine is proposed parameter for such an adjustment procedure (Alessio et al, 1985). Studies on the use of the different reference bases, comparing the relation of urinary enzyme activities to volume, time and creatinine, showed that the variabilities of enzyme excretion were least when the results were expressed as enzyme activity/creatinine ratios. These results are supported by clinical data (Jung et al, 1985).

The use of the second morning urine (after voiding the night urine) as a random urine sample and the expression of enzyme activity in relation to urinary creatinine seems to be an adequate compromise for characterizing urinary enzyme excretion (Jung, 1991). It is a practical approach to clinical and outpatient situations and has also been recommended for the analysis of other urinary analytes (Krieg et al, 1986).

Clinically important enzymes

N-acetyl-ß-D-glucosaminidase (EC 3.2.1.30, NAG)

NAG is kidney tubular enzyme, which is known to be excreted into urine. It is distributed along the whole nephron with the highest activity in proximal tubules (Le Hir et al, 1979). It is localized within the lysosomes of tubular cells (Price and Dance, 1967) and contributes to the intracellular degradation of carbohydrate-containing macromolecules. The excretion of NAG in normal individuals varies with age and the activity found in pathological samples should always be compared with age-matched controls. There is little diurnal variation in the excretion of NAG and the activity in a single urine sample can be used to distinguish normal and abnormal activities. No significant difference was found between the males and females in each decade, but NAG activity increased with age (Price, 1979). Lowest urinary NAG activities were found in males aged between ten and nineteen years, and in females in the twenty to twenty-nine years age group. Children were found to excrete higher levels than adults (Price, 1979).

Alanine aminopeptidase (EC 3.4.11.2, AAP)

Human alanine aminopeptidase has been found to be present in virtually all tissues studied, with relatively high specific activities in the kidney. AAP seems to be a principal enzyme of the proximal tubule and an integral constituent of the brush-border membrane in human kidney. Analysis of secretion of AAP showed diurnal variation, with morning (8 a.m. – 12 noon) excretion being highest. Enzyme excretion related to urinary creatinine (enzyme/creatinine ratio, U/mmol creatinine) significantly decreases with increasing age. Urinary activity of AAP is a sensitive indicator of tubular function.

Alkaline phosphatase (EC 3.1.3.1, ALP)

ALP is kidney tubular enzyme which is known to be excreted into urine. In the kidney, alkaline phosphatase is essentially found within the proximal part of nephron (Brunette et al, 1981), constituting an integral part of luminal brush-border membranes. The function of this enzyme is not yet known. Urinary alkaline phosphatase activities in healthy subjects are highest in the evening and lowest in the morning.

Gamma-glutamyltransferase (EC 2.3.2.2, GMT)

GMT is present in serum and in all cells except those in muscle. It is predominantly located in the cell membrane and may act to transport amino acids and peptides into the cell in the form of gamma-glutamyl peptides. It may also be involved in some aspects of glutathione metabolism. GMT in the kidney is a typical brush-border membrane enzyme. Reference values of urinary GMT expressed as enzyme/creatinine ratio are decreasing with increasing age. The activity of urinary GMT increases in patients with renal tubular disorders.

Arylsulphatase A (EC 3.1.6.1, ASA)

ASA catalyzes the hydrolysis of the ester bond in various aromatic sulfates. Three arylsulfatases are known: two of them, A and B, are lysosomal enzymes which are easily extracted the third one, C, is insoluble and localized in microsomes (Ishibashi et al, 1980). ASA activity in human urine was first reported by Huggins and Smith (1947). The activity of this enzyme is markedly reduced in the urine of patients with metachromatic leukodystrophy. Although an increased activity of ASA has been reported in the urine of patients with urinary tract infections and carcinomas of the bladder and other sites, the clinical significance of this findings has not yet been determined (Mitsuhashi et al, 1984).

Acid α– glucosidase (EC 3.2.1.3)

Acid glucosidase is a lysosomal enzyme present in a wide variety of tissues (liver, kidney, gut). This enzyme is normally present at low levels in human urine as a result of normal turnover and lysis of tubular cells since it is practically absent from plasma, its increase in urine appears to be a sensitive indicator for tubular damage (Ceriotti et al, 1985).

Lysozyme (EC 3.2.1.17)

Lysozyme, also known as muramidase, occurs in lysosomes intracellularly and in most extracellular fluids, especially in exocrine secretion. It is bactericidal and is produced by granulocytes and monocytes but not by lymphocytes. The enzyme, as low molecular weight protein, is filtered by the glomerulus, but almost all of it is reabsorbed and catabolized by the tubules hence, its measurement in urine may be employed to test renal tubular function.

Changes of urinary enzymes in various renal diseases

The ability to assess kidney function and to detect renal damage caused by disease or toxic materials is of great interest to clinicians and toxicologists. Urinary enzymes have been recommended as useful markers for the detection of small changes in the proximal tubule (Piscator, 1991). Since excretions of these markers usually change long before elevations of other markers, such as glucose, occurence of amino acids or even proteinuria, and a rise in serum creatinine, the laboratory diagnostic program for detecting renal diseases should include tubular marker (Jung, 1994).

Acute renal failure

An important field of application of urinary enzymology are the different types of acute renal failure. In contrast to other renal diseases, in acute renal failure not only excessive increases of brush border enzymes, but also very high excretion rates of lysosomal enzymes were found (Burchardt et al, 1979).

Inflammatory renal diseases

Glomerulonephritis (GN) is often complicated by chronic renal failure leading to end-stage renal disease. The histological abnormalities observed in patients with GN and progressive renal failure mostly consist of global glomerular sclerosis and tubulointerstitial damage. Tubular damage results from increased glomerular capillary permeability for macromolecules and obliteration of peritubular capillaries with consecutive interstitial fibroblast proliferation and infiltration of inflammatory cells. These tubulointerstitial changes determine the progression of GN. Urinary activities of AAP and NAG were significantly elevated in GN-patients (Holdt-Lehmann et al, 2000). Urinary AAP and NAG reflected histologically proven tubulus alteration in GN, although in most cases, the renal function is still intact. The authors suggested that the enzyme activities are useful in diagnostics of early stages of the disease.

Urinary excretions of NAG and AAP were significantly higher in patients with acute pyelonephritis (PN) with or without bacteremia than in those with acute cystitis. Patients with acute PN and bacteremia had significantly higher urinary excretion of NAG and glucuronidase than the non-bacteremic patients, while there were no significant differences in the urinary excretion of AAP and acid-glucosidase (Sandberg et al, 1986). In chronic PN, the activities of urinary enzymes (NAG, AAP) were only slightly increased (Burchardt et al, 1979, Vlaskou et al, 2000). Burchardt et al (1979) showed, that in these patients the administration of lysosomotropic substances, such as diatrizoat or mannitol, is followed by a significant increase of the output of brush border enzymes in the urine. The clinical significance of this test lies in the possibility to detect patients suffering from latent PN without bacteremia and without an increased enzymuria under basal conditions.

Toxic renal damage

A large number of compounds, which are in common usage in industry and medicine, are potentially nephrotoxic. Renal damage and disease resulting from toxic exposure is progressive and will, if unarrested, culminate in irreversible renal disease. The role of urinary enzymes in the detection of kidney damage due to toxins is generally accepted, but the full potential for the use of urinary enzymes to detect and monitor kidney damage has not been realized. Several authors described the increased levels of NAG, AAP

and lysozyme in urine of patients undergoing intravenous urography and arteriography (Hartmann et al, 1984, Severini and Aliberti, 1987). The study of urinary NAG isoenzymes in different nephrotoxic states shows that each antibiotic is characterized by a specific isoenzyme profile which probably reflects the very nature of its toxic mechanism. Those aminoglycosides which are responsible for important elimination of B- and I-forms would directly induce the synthesis of these isoenzymes in the endoplasmic reticulum. Cephalosporins, which are less nephrotoxic, present profiles which are closer to those for normal urine (Gibey et al, 1984). Estimation of urinary enzymes activities can be also used as early indicators of renal dysfunction in chronic exposure to heavy metals, such as lead or cadmium (Jung et al, 1993).

Diabetic nephropathy

Renal damage is a serious complication of diabetes mellitus. Diabetic nephropathy is the most important cause of death in diabetes mellitus type I. Death due to diabetic nephropaty with renal failure is less common in diabetes mellitus type II. When diabetic nephropathy is diagnosed by the classical methods, such as detection of proteinuria or decrease in creatinine clearance, little can be done to prevent the progressive downhill course of renal failure. It is well established that the detection of microalbuminuria in a patient with diabetes mellitus indicates the presence of glomerular involvement in early renal damage. It would be better prognostically, if diabetic nephropathy can be detected at an even earlier stage, before the appearence of microalbuminuria, so that intervention could reverse the process, or even prevent the onset of nephropathy altogether.

Recent studies have demonstrated that there is also a tubular component to renal complications of diabetes, as shown by the detection of renal tubular proteins and enzymes in the urine. In fact, tubular involvement may precede glomerular involvement, as several of these tubular markers are detectable even before the appearance of microalbuminuria (Hong and Chia, 1998). Increased urinary excretion of enzymes that either originate from the proximal renal tubule, like AAP, ALP, GMT and NAG, or that are typical low-molecular-weight proteins, like lysozyme and ribonuclease were estimated in patients with diabetes mellitus type I (Jung et al, 1988, Yaqoob et al, 1994) and diabetes mellitus type II (Ishii et al, 1994, Turecký et al, 2001). When the patients with diabetes were divided into group of diabetics with detectable proteinuria (micro- or macroalbuminuria) and group without proteinuria, the excretion of tubular enzymes was significantly higher in diabetics than in controls and was greater in group with proteinuria than in group without proteinuria. The proportion of subjects with elevated enzyme activities increases significantly with the presence of albumin, poor glycaemic control and increased duration of disease. The existence of patients with increased urinary enzymes activities in the group without microalbuminuria supports the hypothesis about early changes of tubular function in the development of diabetic nephropathy. Diagnostic significance of enzymuria as a marker of early tubular involvement was confirmed by investigation of renal biopsies (Golov et al, 1995).

Kidney transplantation

The clinical practice of kidney transplantation demands specific tests to check the renal function and the immunological status. According to this requirement a large number of variables has been recommended as good indicators of kidney viability and rejection episodes. The monitoring of urinary enzymes can be used in the follow-up of renal transplant patients as diagnostic test of renal injury related to cyclosporine nephrotoxicity or acute rejection. The most used enzymes are AAP, NAG and lysozyme.

In dialysis patients before transplantation the tubular enzymes activities are subnormal, but the excretion of lysozyme is increased. The activities of AAP and NAG are low, which means that the structures of the sources of the enzyme are coming from (the proximal tubuli) are destroyed and only a minimal synthesis of new enzymes takes place. This sign is very important in the differential diagnosis of irreversibility of kidney function. If the increase of urine volume during the first phase of recovery after kidney damage or kidney transplantation is not connected with continuous increase of subnormal activities we can assume that no full recovery is possible.

During the posttransplant oliguria high enzymes excretion means that the enzyme source is graft. Thereafter a continuous normalization of graft function is connected with a continuous decrease of high activities.

The most frequent complication after kidney transplantation is the rejection. Reversible rejection leads to a temporary but marked increase of tubular enzymes output. After successful treatment of rejection episode the enzymes return to a normal level (Horpacsy, 1983). In the case of irreversible rejection continuous high activities are observed. They decrease only during the terminal phase and they reached a subnormal level during the period of chronic renal insufficiency.

In contrast with tubular enzymes the urinary lysozyme activity was high before transplantation. It decreases in parallel with the resumption of function. The disappearance of lysozymuria is a useful and early sign of normalization of physiological renal function. On the other hand, a typical changes in lysozymuria during rejection exists (Horpacsy et al, 1978). A rapid increase of lysozyme concentration follows the appearance of other biochemical signs of rejection. After successful prednisone bolus therapy the decrease in lysozyme activity reflects the regeneration of tubular function. In irreversible damaged grafts with minimal urine output, high lysozyme activity in conjunction with high sodium excretion lasting more than 25 days may be useful supporting evidence of a need for graft removal.

In our short review we try to summarize current knowledge about enzymuria and its diagnostic significance. Urinary enzymology like other diagnostic procedures should aim at finding special signs for diseases or groups of diseases. Like other analytical test methods assays of urinary enzymes have a limited field of indication. A succesful introduction of these analytes into a routine diagnostic program requires that the clinician is provided with reliable reference intervals in order to use the results of tubular markers for screening or monitoring patients.

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