

Diagnostic and Prognostic Value of Renal Enzymes Estimation in Different Renal Conditions

Thesis

Submitted in Partial Fulfillment
of the Doctorate Degree in
Internal Medicine

BY

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Supervisors

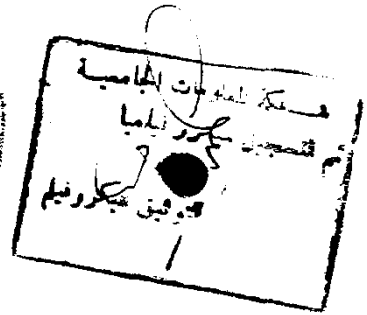
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LIST OF ABBREVIATIONS

NAG	N-acetyl-B-D-glucosaminidase
B.gl.	B-glucuronidase
A.P.	Alkaline phosphatase
B2-m	B ₂ -microglobulin
A.N.S.	Acute Nephritic syndrome
A.P.GN.	Acute poststreptococcal glomerulonephritis
R.P.GN.	Rapidly progressive glomerulonephritis
N.S.	Nephrotic syndrome
N.S.-st.	Nephrotic syndrome not on steroids
N.S. + st/Res.	Nephrotic syndrome resistant to steroids
N.S. + st/Sen.	Nephrotic syndrome sensitive to steroids
Ch.PN.	Chronic Pyelonephritis
A.Ch.PN.	Active chronic Pyelonephritis
I.Ch.PN.	Inactive chronic Pyelonephritis
Ch.gl.	Chronic glomerulopathies
A.Ch.GN.	Active chronic glomerulonephritis
I.Ch.GN.	Inactive chronic glomerulonephritis
E.S.R.D.	End stage renal disease
U.T.I.	Urinary tract infection
L.M.W.	Low molecular weight
H.M.W.	High molecular weight
G.F.R.	Glomerular filtration rate

Forward and Aim of the work

FORWARD AND AIM OF WORK

* History and Background

Until recently, diagnostic enzymology has been confined mainly to the assay of serum enzymes; determinations of these enzymes have been extensively used in the diagnosis and follow up of many diseases for quite a long time (*Saatci et al., 1978*).

Although the presence of active enzymes in urine has been known for an equally considerable time, yet their use as diagnostic indicators has relatively received little attention (*Raab, 1972*).

Two principal reasons are commonly held responsible for the slow development in this important field of investigation; one, is the difficulty involved in assaying enzymes in a fluid (urine) which constantly varies in volume and composition besides being a hostile environment for many enzymes; the other, is the fact that enzymes are present in urine in small amounts hence inducing low activity requiring particularly sensitive methods for their detection (*Price, 1982*).

During the last 30 years or so, urinary enzymes have received special interest; As research in this new field advances, studies are increasingly focusing on the use of enzymuria as an index of cellular injury at a specific site of the renal parenchyma (*Amador et al., 1965*).

Early in 1959, a new era of approaching urinary enzymes as diagnostic tools has started when *Rosalki and Wilkinson* described increasing activities of enzymes in the urine of patients with kidney diseases (*Rosalki and Wilkinson, 1959*).

Later on, in 1962, *Wacker and Co-workers* recommended assaying two urinary enzymes, lactic dehydrogenase (LDH) and alkaline phosphatase (AP), as a screening test for carcinoma of the kidney and urinary bladder (*Wacker et al., 1962*).

Though their observations were hardly validated by others, yet, it was soon realized that other serious renal diseases - besides tumors - might cause increased urinary enzymatic activity (*Raab, 1968*). In 1964, *Hartman* had first made use of enzymuria in detecting renal tubular damage after parenteral injection of contrast media (*Hartman et al., 1965 "cited"*).

Ballentyne, in 1968, reported that urinary enzyme determinations were the most convenient method for detecting early graft rejection in renal homotransplants (*Ballentyne et al., 1968*).

Recently, most researchers in nephrology have agreed that the conventional parameters for renal investigations as routine urinalysis, clearance studies, radiography of the urinary tract and measurements of nitrogenous

metabolites in serum are only capable of revealing moderate to severe renal damage (*Jung et al., 1987*).

On the other hand, *Hartman et al (1985)*, have undergone extensive research-work on enzymuria and have concluded that the quantitative estimation of certain enzymes in urine offers a high degree of diagnostic sensitivity in detecting mild to moderate lesions of the renal parenchyma as well as early deterioration of renal function.

New insights in this field have started with the technological developments in quantitative histochemistry. *Guder and Schmidt, in 1976*, were able to reveal the sites in the nephron from which the various urinary enzymes originate; they reported that the quantitative determinations of specific, structure-related enzymes in urine can provide promising information about the location and severity of tubular pathologies (*Guder and Schmidt, 1976*).

Simultaneously, the determination of low molecular weight proteinuria (tubular proteinuria) has gained similar interest and achieved considerably equal success in the field of urinary investigations. In the last 20 years or so B₂-microglobulin has been broadly investigated and has so far stood the test of time for being a sensitive indicator of renal tubular damage (*Karlsson et al., 1980*).

Recently, there has been an apparent ongoing inclination, both in research centers and clinical

laboratories, to stress on the value of determining the urinary content of low molecular weight (LMW) proteins and high molecular weight (HMW), non-filterable enzymes in detecting early tubular damage and assessing minimal renal dysfunction (*Morgan, 1982*).

Finally, it would be helpful to record the assumption stated by many researchers in the field of diagnostic enzymology, that the determination of renal enzymatic activity in blood plasma seems to offer little, or practically nothing, in the recognition and follow up of the different renal pathologies (*Mattenheimer, 1977; Dubach and Lehir, 1984*).

*** Aim of the work**

This study is carried out with the aim of determining the significance of certain urinary enzymes, namely: -N-acetyl-B.D. glucosaminidase (NAG), B-glucuronidase (B-gl) and alkaline phosphatase (A.P.), as well as the L.M.W. protein, B₂-microglobulin (B₂-m) in the diagnosis of some renal diseases and in the assessment of the eventual persistence of damage after treatment. Furthermore, we hope to be able to elucidate or nullify any significant difference between our research parameters and the conventional parameters of renal investigations, such as (blood urea, serum creatinine, serum cholesterol and daily amount of albumin in urine) in the accuracy of diagnosing

the studied renal pathologies and in estimating their prognosis.

In addition, we wish to determine whether the diagnostic potential of these enzymes is enhanced by the simultaneous assay of more than one renal enzyme with different localizations in the subcellular compartments and different activities along the nephron, i.e. assaying lysosomal and brush-border enzymes.

Another aim of this study is to compare the urinary excretion of B_2 -m and tubular enzymes as parameters for tubular damage and alterations in tubular function with different renal pathologies. Theoretically, any tubular lesion would result in abnormal B_2 -microglobulinuria as well as enzymuria, particularly enzymes located on the surface of the brush-border of proximal tubular cells, i.e. brush-border enzymes.

Hopefully, this work could meaningfully share in the ongoing enthusiasm of clarifying the imigma that is increasingly put forward regarding the expected applicability of recommending urinary enzyme assay as a routine and reliable investigatory tool in clinical practice.

Review of Literature

CHAPTER 1

INTRODUCTION TO URINARY ENZYMOLOGY

In human urine, a great number of enzymatic activities have been demonstrated; In many instances, isoenzymes could be separated. Because of the ongoing research attempts in urinary enzymology, at least 40 enzymes have been assessed so far, and nearly 10 of these showed promising diagnostic sensitivity in urorenal diseases (*Dubach and Lehir., 1984*).

In 1968, *Raab* proposed a classification for urinary enzymes according to their biochemical nature into: oxidoreductases, transferases, lyases and hydrolases; this biochemical classification is still commonly accepted and applied (*Raab, 1968*).

The group of hydrolases have received special interest. Its subgroup, the glycosidases and the phosphatases, were particularly emphasized for showing considerable value in detecting early pathological processes of the renal parenchyma. Specific enzymes belonging to this group are now identified and their diagnostic potentials are being evaluated (*Mattenheimer, 1977*).

Once more, in 1972, *Raab* reviewed urinary enzymatic activities and their changes in disease states; His publication concerning the origin of enzymes in urine

proposed several contributing sources both under normal and pathological conditions.

Under normal conditions, enzymatic activities of urine may originate from 4 sources:

1. Kidneys:

Most urinary enzymes derive from the kidneys. Renal tubular cells contain high activities of many enzymes in order to fulfill their numerous biochemical functions. According to *Mattenheimer (1977)*, 3 factors account for the renal excretion of enzymes in urine:

- * The rate of enzyme synthesis in the tubular cells.
- * Turnover rate of the tubular cells.
- * Physiological alterations in the permeability of tubular cell membrane.

2. Epithelial cells of the urogenital tract

Cellular turnover in various epithelia (renal pelvis, ureter and urinary bladder) leads to desquamation of epithelial cells; Decomposition of these cells allows their enzymatic content to escape in urine, however, the enzymatic activities in these cells are relatively low and their contribution to urinary enzyme activity is insignificant.

3. Glandular secretions of the urogenital tract

With the exception of the prostatic gland whose fluid is rich in acid phosphatase activity, the sexual secretory

glands contribute little to the enzymatic composition of urine.

4. Serum

Similar to the known limitations for serum protein escape via the glomeruli, only serum enzymes with molecular weights smaller than 70,000 are filtered in the glomeruli. However, only small amounts of these enzymes escape reabsorption in the nephron and appear in the final urine. Renal excretion of serum enzymes occurs in very few instances and does not affect total enzymatic activity in urine.

Under pathological conditions, marked changes may occur in the enzymatic pattern of urine. These changes may reflect alterations (increase or decrease) in activities of normally detected urinary enzymes, or the appearance of new enzymes:

In disease states, 5 sources for urinary enzymes are recognized:

1. Kidneys

In illness, as in health, the kidney is the main source of urinary enzymes, 4 pathobiochemical principles are probably responsible for the changes in urinary enzyme activities occurring with different renal pathologies (*Mattenheimer, 1977*).

- The changes in rate of enzyme synthesis (stimulation / inhibition)