LABORATORY MONITORING OF GENTAMYCIN AND ITS NEPHROTOXICITY

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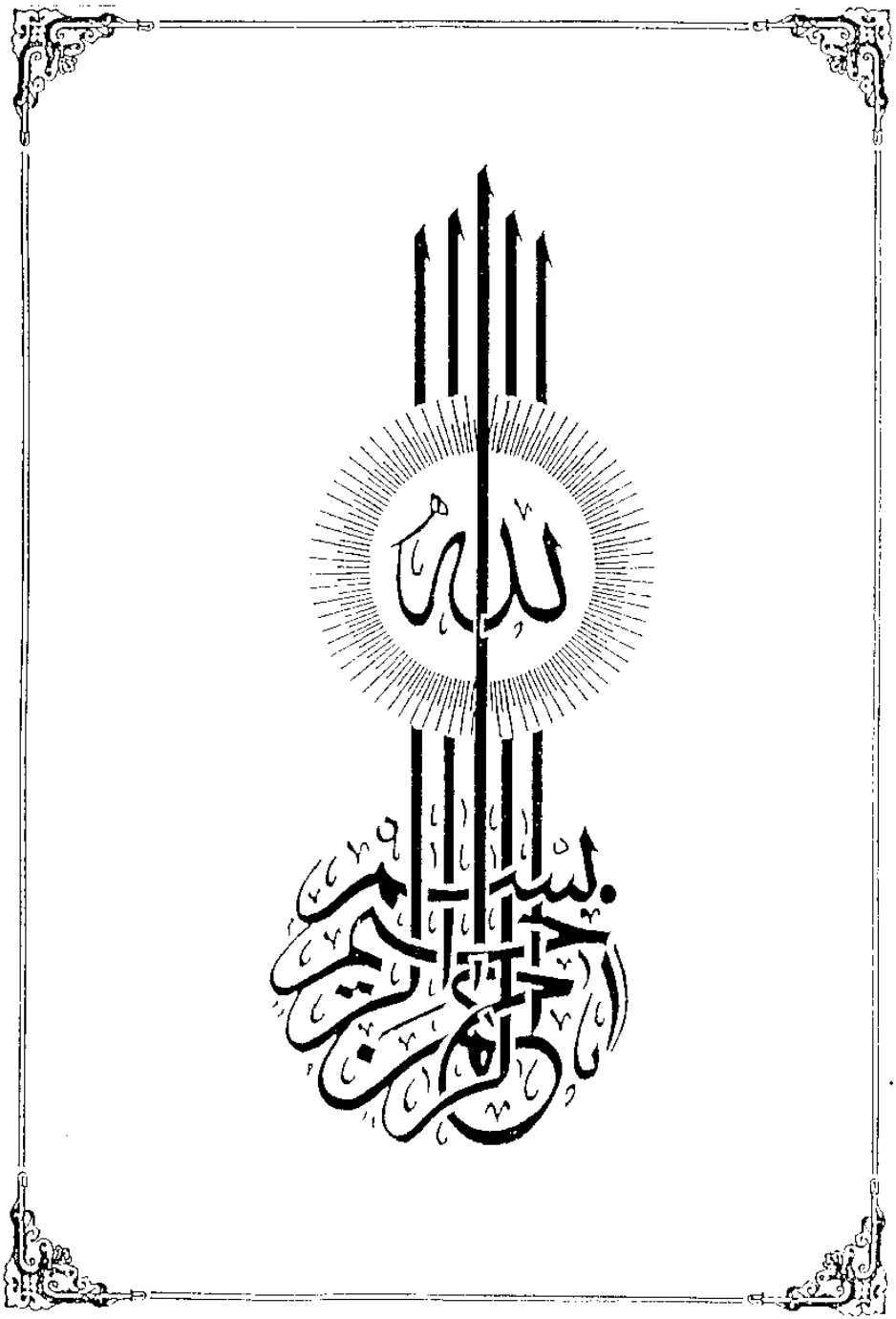

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Abbreviations

AAP:	**Alanine aminopeptidase**
ALT:	**Alanine aminotransferase**
ALP:	**Alkaline phosphatase**
AST:	**Aspartate aminotransferase**
β-glu:	**β-glucuronidase**
Cr. Cl.:	**Creatinine clearance**
GGT:	**Gamma-glutamyl transferase**
LAP:	**Leucine aminopeptidase**
LDH:	**Lactate dehydrogenase**
NAG:	**N-acetyl β-D-glucosaminidase**
rpm:	**Rotation per minute**

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INTRODUCTION:

Gentamycin is an aminoglycoside antibiotic frequently used for control of severe bacterial infections. However, aminoglycosides have a low toxic to therapeutic ratio; thus serum levels for producing clinical efficacy are often near the levels at which toxicity is encountered. Administration of aminoglycosides is a frequent cause of iatrogenic renal failure and may be a leading reason for drug-related hemodialysis ***(Wilson, 1990)***.

Urinary enzymes excretion is proved to correlate to tubular structural integrity and constitutes a reliable parameter linking biochemical, anatomical and functional events in tubular damage, thus enzymuria could be the first sign of aminoglycoside-induced nephrotoxicity while the usual routine renal function tests are still unaffected ***(Emanuelli et al., 1988)***.

AIM OF WORK:

In this study we aim at monitoring of serum gentamycin levels by an enzyme immune assay technique.

Nephrotoxicity of the drug will be assessed by measuring urinary enzymes as an indicator of tubular damage.

Finally, correlation of serum gentamycin level with the level of released urinary enzymes will be carried out.

REVIEW OF LITERATURE

ENZYMURIA

The aminoglycoside-induced renal damage occurs at the level of the proximal tubular cells ***(Palla et al., 1985)***. Few anatomical and physiological facts about the proximal tubules may help us to understand how urinary enzymes can be considered as markers of renal cell damage or death.

I- ANATOMY OF THE RENAL PROXIMAL TUBULES:

The proximal tubule begins abruptly at the urinary pole of the glomerulus. It consists of a convoluted portion (pars convoluta), which is a direct continuation of the parietal epithelium of Bowman's capsule, and a straight portion (pars recta), which is located in the medullary ray. The length of the proximal tubule is approximately fourteen mm in humans ***(Rouillier, 1969)***.

The proximal tubule includes three morphologically distinct segments, namely, S1, S2 and S3. The S1 segment is the initial portion of the proximal tubule. It begins at the glomerulus and constitutes two thirds of the pars convoluta. The S2 segment consists of the remainder of the

pars convoluta and the initial portion of the pars recta. The S3 segment consists of the remainder of the proximal tubule ***(Tisher and Madsen, 1991)***.

A- Pars Convoluta:

The individual cells of the pars convoluta are extremely complex in shape. From the main cell body, large primary ridges extend laterally from the apical to the basal surface of the cells and they interdigitate with those of adjacent cells ***(Bulger, 1965 ; and Welling and Welling, 1976)***.

The mitochondria are located in the lateral wall processes close to the basolateral plasma membrane. The high voltage electron microscope shows the mitochondria to be branched and anastomose with one another ***(Bergeron et al., 1980)***. Paramembranous cisternal system is often observed between the plasma membrane and the mitochondria suggesting the continuity of the mitochondria with the smooth endoplasmic reticulum ***(Bergeron and Thiery, 1981)***.

Smooth and rough endoplasmic reticulum together with the free ribosomes are abundant in the cytoplasm. A well developed Golgi apparatus is located as well in the cytoplasm ***(Tisher and Madsen, 1991)***.

A well developed brush border forms the apical or luminal surface of the proximal convoluted tubule. It is formed by numerous finger-like projections of the apical plasma membrane and are called microvilli ***(Welling and Welling, 1975)***. Biochemical studies have demonstrated 5'-nucleotidase, alkaline phosphatase, aminopeptidase, magnesium adenine triphosphatase activity within the brush border membranes from the kidney

cortex ***(Tisher and Madsen, 1991)***, as well as gamma glutamyl transferase and muramidase ***(Palla et al., 1985)***.

The pars convoluta of the proximal tubule contains a well developed endocytic-lysosomal apparatus that is involved in the reabsorption and degradation of macromolecules from the ultrafiltrate that are absorbed by endocytosis. The lysosomes contain a variety of acid hydrolases including acid phosphatase, various proteases, lipases, and glycosidases ***(Maunsbach, 1973)***. Studies have demonstrated that the lysosomes have an acidic interior ***(Mellman et al., 1986)***. This may explain the mechanism behind the accumulation of amphiphilic cationic drugs such as aminoglycoside antibiotics, chloroquine and tricyclic antidepressants in the proximal convoluted tubule ***(Kaloyanides and Munoz, 1980)***.

The proximal convoluted tubule plays a major physiologic role in the reabsorption of the ions sodium, bicarbonate, chloride , potassium, calcium, phosphate, and organic solutes as glucose and amino acids. Approximately half of the ultrafiltrate is reabsorbed in the proximal convoluted tubule ***(Burg, 1986)***.

B- Pars Recta:

In this part of the proximal tubule, the basolateral invaginations of the plasma membrane are virtually absent, mitochondria are small and randomly scattered throughout the cytoplasm ***(Tisher and Madsen, 1991)***. The pars recta has peroxisomes which differ from lysosomes. They are irregular and surrounded by thick membrane. They do not contain acid hydrolases and their function is not known with certainty. However, they are believed to be involved in lipid metabolism. They also have a high

content of catalase which is involved in the degradation of in addition to various oxidative enzymes such as L-α-hydroxy acid oxidase and D-amino acid oxidase ***(Ohmo, 1985)***. It is worth saying that the pars recta is involved in the secretion of organic acids and bases. ***Woodhall et al. (1978)*** reported that the pars recta is also liable to damage by nephrotoxic compounds and drugs.

II- URINARY ENZYMES:

Enzymes are normally excreted into the urine and this enzymuria is increased in various disease states. The amount of enzymuria is expressed as the ratio of enzyme activity to milligrams of creatinine per ml of urine. Significant enzymuria is confirmed when this ratio exceeds $\overline{X}\pm 2SD$ ***(Kunin et al., 1978)***.

A- Sources of Urinary Enzymes:

1- In Health:

Under normal conditions, enzymatic activity in urine may originate from four sources namely the kidneys, the epithelial cells of the urogenital tract, the glandular secretions of the urogenital tract, and the serum.

a- The kidneys:

Most of the urinary enzymes are derived from the kidneys, where the tubular cells are rich in many enzymes essential for the fulfillment of their biochemical functions. ***Mattenheimer (1977)*** suggested that the rate of enzyme synthesis in the tubular cells together with the physiological alterations in the permeability of the tubular cell membrane and the