

# Differentiation between Renal Injury

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## Abstract

Diabetes results in injury to various parts of the renal tubules. By assaying specific biomarkers, injury can be localised to distinct parts of the nephron and the effects of therapy on the kidneys monitored. Based on the early release of the enzyme N-Acetyl- $\beta$ -D-glucosaminidase (NAG) in diabetes, and the presence of tubular proteinuria it was previously believed that proximal tubular injury, was an early event in renal injury. However, NAG can be up-regulated in response to proteinuria and is a marker of increased lysosomal turnover as well as overt injury.

In this study, the release of NAG was compared with that of the cytosolic injury biomarkers alpha glutathione S-transferase ( $\alpha$ GST) and pi glutathione S-transferase ( $\pi$ GST).  $\alpha$ GST is specific for the proximal convoluted tubule and  $\pi$ GST is predominantly found in the distal tubules. Their presence in urine indicates that cytolysis is occurring to cells in distinct parts of the nephron.

In this study, 128 subjects with varying degrees of renal injury were recruited, 57 with no microalbuminuria (group 1), 50 with microalbuminuria (group 2) and 21 with proteinuria (group 3) and 20 age-matched non-diabetic controls. Urinary NAG was measured using a colorimetric method (Roche) and  $\alpha$  and  $\pi$ GST were assayed using the Biotrin Urinary Alpha and Pi GST EIA kits respectively (Biotrin International, Dublin Ireland). Urinary NAG levels expressed as U/mmol urine creatinine and  $\alpha$  and  $\pi$ GST levels, expressed as  $\mu$ g / mmol urine creatinine were (median : range): group 1, NAG 0.54 (0.21-2.96),  $\alpha$ GST 0.21 (0.03 - 4.60),  $\pi$ GST 2.27 (0.13 - 19.2); group 2, NAG 0.69 (0.23 - 2.16),  $\alpha$ GST 0.2 (0.03 - 2.5),  $\pi$ GST 2.55 (0.14 - 33.3) and group 3, NAG 1.12 (0.42-2.60),  $\alpha$ GST 0.18 (0.03 - 1.55),  $\pi$ GST 5.34 (1.37 - 11.98). Urinary NAG,  $\alpha$  and  $\pi$ GST in the control group were 0.33 (0.11-0.53), 0.31 (0.04 - 1.15) and 0.84 (0.18 - 1.32) respectively. NAG and  $\pi$ GST levels in the diabetic subgroups were significantly different to controls ( $p < 0.05$ ), whereas no significant difference between groups was observed for  $\alpha$ GST ( $p > 0.05$ ).

These results suggest that proximal tubular dysfunction or compensation (increased NAG release) rather than damage (no  $\alpha$ GST release) occurs in diabetic renal injury and that distal tubular damage ( $\pi$ GST release) is a very early event in the development of renal injury. By the use of biomarkers released according to different mechanisms and from different sites, new mechanisms of renal injury were shown.

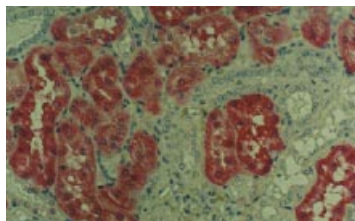
## Introduction

- Diabetic nephropathy is the commonest cause of renal failure. Between 15% - 60% of patients with Type 2 diabetes will develop diabetic nephropathy (DN)<sup>(1)</sup>. However, current tests of renal function and injury are slow to respond to changing renal status in DN and may be inappropriate, for example:
  - Microalbuminuria is an established early marker of DN<sup>(2)</sup>, however it may be more a biomarker of generalised increased vascular permeability rather than renal injury per se.
  - Serum creatinine is an unreliable biomarker of renal injury in early DN as GFR may increase.
  - A large number of new diabetes therapies are being introduced requiring monitoring of potential renal effects and the need to distinguish between adverse drug events and the background diabetic nephropathy.
  - Direct reno-protective therapy, such as ACE inhibitors is becoming important, introducing a need for better renal monitoring.
- Proximal tubular injury is presumed to be an early event in DN, however many current proximal tubule biomarkers may not indicate injury but changed function/compensation:
  - Low molecular weight proteinuria may reflect reduced resorptive function or protein overload, rather than injury
  - Increased urinary N-Acetyl- $\beta$ -D-glucosaminidase (NAG) may indicate increased lysosomal activity due to the increased protein load presented to the renal tubules<sup>(3-4)</sup>.

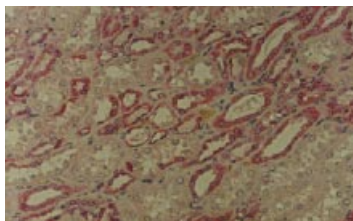
## Glutathione S-transferases

- Glutathione-S-transferases (GSTs) alpha ( $\alpha$ ) and Pi ( $\pi$ ) are located within proximal (PCT) and distal (DCT) convoluted tubules respectively (Figure 1)<sup>(5)</sup>.
- GSTs comprise about 2% of the tubular cytosolic protein and they are rapidly released into the urine in response to renal tubular injury.
- Due to their specific intracellular location, urinary  $\alpha$  and  $\pi$  GST can potentially discriminate between alterations in tubular function and tubular damage and indicate the site of injury<sup>(6)</sup>.
- Urinary GSTs have been shown in many studies to be sensitive and specific biomarkers of renal injury but they have not previously been studied in diabetes.

**Figure 1. Immunohistochemical Localisation of GSTs in the Kidney**



$\alpha$ GST in Proximal Tubules



$\pi$ GST in Distal Tubules

## Aim

- To determine urinary levels of NAG,  $\alpha$  and  $\pi$ GST in patients with Type 2 diabetes.
- To investigate the localisation of renal tubular injury in diabetic nephropathy.

## Materials and Methods

- SUBJECTS
- CONTROLS: n = 20 (10 male, 10 female) age male (63:5) female (60:6) (mean : SD).
- DIABETIC SUBJECTS: n = 128 (83 male, 45 female) age male (62:11) and female (65:11) (mean : SD).
- Patient grouping:
  - group 1. n = 57, microalbumin < 20 mg/L
  - group 2. n = 50, microalbumin 20 - 200 mg/L
  - group 3. n = 21, urinary protein > 0.2g/L.
- All subjects had normal serum creatinine.

## Results

- Urinary NAG (U/mmol creatinine),  $\alpha$ GST and  $\pi$ GST results ( $\mu$ g/mmol creatinine) are shown in table 1 and figure 2.

**Table 1.**

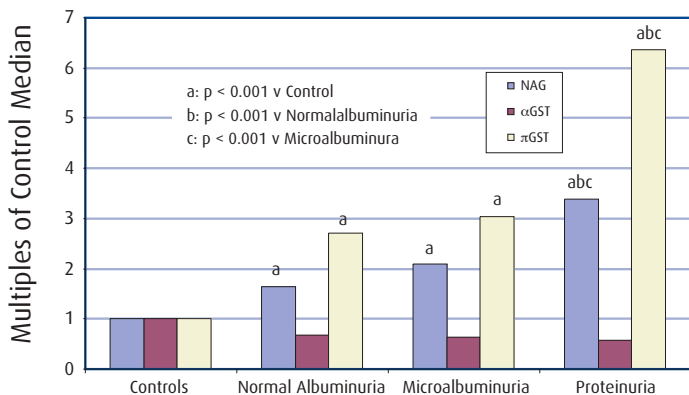
|   | Control group         | Group 1                            | Group 2                            | Group 3                                 |
|---|-----------------------|------------------------------------|------------------------------------|---|
| $\alpha$ GST<br>$\mu$ g/mmol creatinine<br>Median (range) | 0.31<br>(0.04 - 1.15) | 0.21<br>(0.03 - 4.60)              | 0.2<br>(0.03 - 2.5)                | 0.18<br>(0.03 - 1.55)                   |
| $\pi$ GST<br>$\mu$ g/mmol creatinine<br>Median (range)    | 0.84<br>(0.18 - 1.32) | 2.27<br>(0.13 - 19.2) <sup>a</sup> | 2.55<br>(0.14 - 33.3) <sup>a</sup> | 5.34<br>(1.37 - 11.98) <sup>a,b,c</sup> |
| NAG<br>U/mmol creatinine<br>Median (range)                | 0.33<br>(0.11 - 0.53) | 0.54<br>(0.21 - 2.96) <sup>a</sup> | 0.69<br>(0.23 - 2.16) <sup>a</sup> | 1.12<br>(0.42 - 2.6) <sup>a,b,c</sup>   |

a p < 0.001 v Control,

b p < 0.001 v Group 1,

c p < 0.01 v Group 2.

Figure 2. Tubular Biomarkers in Diabetic Nephropathy



## Summary

- Urinary NAG concentrations were found to be elevated in all patient groups indicating increased lysosomal activity.
- The absence of increased urinary αGST indicates minimal proximal tubular cytolysis in early diabetic nephropathy.
- Increased πGST seems in all diabetic groups seems to indicate that distal tubular injury is an early event in diabetic nephropathy, regardless of proteinuria.
- The early elevation of πGST in diabetic nephropathy makes it a potentially useful biomarker for studying it and its therapy.
- Since urinary αGST release seems to be normal in diabetic nephropathy, it could be a more sensitive biomarker of nephrotoxicity and other renal effects in diabetic subjects.

## Discussion and Conclusions

- The combined use of biomarkers of different types shows great promise for distinguishing between compensation and injury.
- The use of biomarkers with different origins can enable injury to be localised to specific tissues.
- This system shows great promise for studying DN and other chronic renal injuries including chronic nephrotoxicity.
- The early elevation of πGST in diabetic nephropathy makes it a potentially useful biomarker for studying it and its therapy.
- Since urinary αGST release seems to be normal in diabetic nephropathy, it could be a more sensitive and discriminating biomarker of nephrotoxicity and other renal effects in diabetic subjects.

## References

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