Urinary enzyme measurements as early indicators of renal insult in type 2 diabetes

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ABSTRACT

The association between urine microalbumin, α_1-microglobulin concentration (α1MG) and the urinary enzyme activities of alanine aminopeptidase (AAP), N-acetyl-β-D-glucosaminidase (NAG), α-glutathione-S-transferase (αGST) and π-glutathione-S-transferase (πGST) is investigated in 36 type 2 diabetic and 15 age- and sex-matched non-diabetic subjects. Diabetic subjects were grouped into those with microalbuminuria <3 mg/L (group A: 7M/5F), 3–30 mg/L (group B: 5M/7F) and 30–300 mg/L (group C: 6M/6F). While serum creatinine concentration remained within the laboratory reference range (<115 mmol/L) in all experimental groups, α1MG excretion increased with the severity of microalbuminuria (control group and groups A, B and C mean [SD] values were 1.3 [0.21], 1.6 [0.11], 2.18 [0.42] and 2.8 [0.51] mg/mmol urinary creatinine, respectively). Activities of NAG (U/mmol creatinine) were significantly elevated in groups A, B and C at 98.7 (8.6), 112.8 (12.9) and 147.4(16.2), respectively, compared with the reference range <35 U/mmol creatinine (group C *vs*. groups A and B: $P<0.01$). Activity of AAP (U/mmol creatinine) was significantly elevated in groups B and C at 7.6 (0.5) and 7.9(0.6), respectively (both $P<0.001$), compared to the control and group A values (2.5 [0.2]). Activity of πGST (U/mmol creatinine) was elevated in groups B and C at 2.6 (0.4) and 2.8 (0.5), respectively (both $P<0.001$), compared to the control and group A values (1.1 [0.1]). Similarly, urine πGST activity was also elevated in groups B and C at 2.9 (0.6) and 3.1 (0.5), respectively (both $P<0.001$), compared to control and group A values (1.3 [0.1] and 1.4 [0.2]). These results suggests that site-specific urinary biochemical markers provide valuable information about early renal proximal and distal tubular insult that ultimately may precede enhanced glomerular permeability in subjects with type 2 diabetes.

KEY WORDS: Diabetic nephropathies.
Enzymuria.
Glutathione transferase.

Introduction

Diabetic nephropathy is characterised structurally both by glomerular lesions and changes to the tubulo-interstitial compartment of the kidney, and functionally by increasing severity of microalbuminuria and altered glomerular filtration rate (GFR), the latter usually being assessed in the laboratory by measurements of serum or plasma creatinine concentrations.[1–3] Furthermore, end-stage renal disease (ESRD) in diabetics is increasing and now accounts for approximately 40% of treated ESRD by either transplantation or dialysis.[4] Although incipient diabetic kidney disease is usually characterised functionally by the presence of microalbuminuria, serum creatinine is of limited value in the early detection of renal insult due to its poor sensitivity to early nephron insult and renal dysfunction.[5]

It is now well established that chronic hyperglycaemia, and consequently effective glycaemic control, is the main metabolic determinant associated with irreversible kidney damage in diabetes mellitus.[6] Although microalbuminuria has been used for many years as a predictor of incipient diabetic nephropathy, reflecting the loss of glomerular selectivity,[2] estimation of renal tubular function and integrity may yet provide an early indication of renal insult and thus identify those at risk of developing kidney dysfunction.[7] Consequently, decreased renal tubular reabsorption capacity is characterised by elevated low molecular weight protein (e.g. α_1-microglobulin and β_2-microglobulin) levels in the urine, and early renal tubular insult is characterised by increased proximal tubular enzymuria.[7–9]

In this regard, elevated urine alanine aminopeptidase (AAP) and N-acetyl-β-D-glucosaminidase (NAG) activities have been used to indicate the early onset of proximal renal tubular insult in several clinical states in general (e.g., drug-induced nephrotoxicity),[10] and the diabetic state in particular.[11–18] Other studies indicate that the isoforms of glutathione S-transferase (GST), αGST and πGST, are located in the cells of the proximal and distal renal tubules, respectively, and their urine measurement has allowed the identification of site-specific renal insult following nephrotoxin-induced renal damage.[19–21]

However, renal dysfunction in general and glomerular dysfunction in particular are observed only rarely in early diabetic nephropathy, and the identification of early renal insult and those at risk of developing renal dysfunction is important so that corrective therapies can be applied at an early stage. Consequently, the aim of this study is to investigate the association between the urinary enzyme activities of NAG, AAP, αGST and πGST in subjects with

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type 2 diabetes and the severity of microalbuminuria, the standard laboratory indicator of the onset of nephropathy.

Materials and methods

Thirty-six type 2 diabetic subjects and 15 age- and sex-matched non-diabetic control subjects were recruited. Mean age (standard deviation [SD]) was 48 (10) years and duration of diabetes was between eight and 10 years. Diabetic subjects with renal disease characterised by proteinuria, hypertension (with or without antihypertensive treatments), dyslipidaemia or cardiovascular disease, evidence of renal insufficiency, chronic urinary tract infection, renal stones and renal tumours were excluded from the study. Diabetic subjects were grouped according to urine protein concentration into those with microalbuminuria <3 mg/L (group A: seven males, five females), 3–30 mg/L (group B: five males, seven females) and 30–300 mg/L (group C: six males, six females). These microalbuminuria ranges were chosen, after consultation with a consultant diabetologist, to reflect minimal, moderate and severe loss of glomerular integrity in diabetic subjects.

Fasting blood and urine samples (mid-stream) were taken and plasma creatinine and microalbuminuria concentrations were quantitated by standard laboratory methods, while urine activities of AAP and NAG and urine α_1-microglobulin (α1MG) levels were measured, as previously described,[11,22,23] and αGST and πGST urine activities were measured using a commercial enzyme immunoassay (EIA) kit (Biotrim, Dublin, Ireland). Enzyme activities were expressed on the basis of urinary creatinine to allow for inter-individual variations in urine flow rate. Results, expressed as mean (SD), were analysed using two-way analysis of variance (ANOVA) with post hoc analysis performed using Dunnett's test. P values <0.05 were considered significant.

Results

All the diabetic subjects had serum creatinine concentrations within the laboratory reference range (<115 μmol/L for men and <75 μmol/L for women), regardless of the severity of microalbuminuria observed. Urine excretion of α1MG was significantly increased in all diabetic groups, and it increased with the magnitude of microalbuminuria (control group and groups A, B and C values were 1.3 [0.21], 1.6 [0.11], 2.18 [0.42] and 2.8 [0.51] mg/mmol urinary creatinine, respectively). Compared to the control values, a small but significant increase in α1MG excretion was noted in group A ($P<0.05$), with further increases noted in groups B and C (both $P<0.001$). Although mean α1MG values were highest in group C, they were not significantly different from those in group B.

Urine activities of NAG, a lysosomal hydrolase found predominantly in the renal proximal tubule, were significantly increased (three-fold) in diabetic subjects with microalbuminuria <3 mg/L. A four- and five-fold increase in NAG enzymuria was also observed in groups B and C, respectively, compared to control values (all $P<0.01$) (Table 1). Overall, the magnitude of NAG enzymuria increased with the severity of microalbuminuria. However, urine activity of AAP, a brush border mitochondrial enzyme, was only increased in patients with a microalbuminuria value >3mg/L. Similar and significant four-fold elevations in AAP activity were noted in groups B and C, compared to the control values and those obtained from diabetic subjects with microalbuminuria <3 mg/L (all $P<0.01$).

A 1.3-, two- and three-fold increase in urinary αGST activity was observed in groups A, B and C, respectively ($P<0.05$, $P<0.001$ and $P<0.001$, respectively) compared to control values. Again, a small but significant increase in urinary αGST activity was noted in diabetic subjects with microalbuminuria <3 mg/L. However, a different pattern of enzyme activity was noted with πGST. While πGST activities were similar to control values in group A, a similar three- to four-fold elevation was noted in groups B and C (both $P<0.001$).

The proximal tubular enzyme NAG and the small molecular weight protein α1MG demonstrated similar patterns of urinary excretion with increasing severity of microalbuminuria, albeit with serum creatinine concentrations within the reference range, but showed elevated values in the absence of significant microalbuminuria. αGST and the distal tubular marker πGST were elevated only when significant proteinuria was present in a manner similar to that seen with AAP excretion.

Discussion

Incipient diabetic nephropathy is characterised by structural, functional and biochemical changes associated with glomerular and tubular elements of the kidney.[3] The results of the present study demonstrated clearly that increased severity of microalbuminuria, the standard clinical predictor of incipient diabetic nephropathy,[3] and increased glomerular permeability, are associated with early changes in urinary parameters (i.e. α1MG proteinuria, NAG, AAP, αGST and πGST enzymuria), indicating renal tubular dysfunction in a cohort of type 2 diabetic subjects. Furthermore, urine NAG activity and α1MG concentration were elevated in those diabetic subjects with microalbumuria <3mg/L, suggesting that renal tubular insult could predate overt glomerular changes, indicating the potential use of these parameters in the recognition of early renal insult associated with diabetes mellitus.

In diabetic kidney disease, increased NAG and AAP enzymuria has been observed.[7,8,13] Both enzymes are localised predominantly to the proximal renal tubule, with NAG being of lysosomal origin and AAP being of mitochondrial origin in the renal brush border. Both have been shown to be sensitive indicators of renal proximal tubular insult in several disease processes,[7] and in early diabetic nephropathy in particular.[7–15] In the present study, while increased AAP activity was only observed in individuals with significantly elevated microalbuminuria, elevated NAG enzymuria was seen in the absence of significant microalbuminuria, but with serum creatinine concentrations still within the laboratory reference range. These findings are consistent with altered lysosomal function due to an autophagic response to early epithelial cell injury initiated by chronic hyperglycaemia.

Table 1. Results of parameters tested in the control and experimental groups.

Parameter	Experimental groups			
	Control Group	A	B	C
Microalbuminuria	<3	<3	3–30	30–300
α1MG	1.30 (0.21)	1.60 (0.11)[a]	2.18 (0.42)[b]	2.80 (0.51)[b]
Urine activities of NAG	21.1 (7.2)	98.7 (8.6)[b,d]	112.8 (12.9)[b]	147.4 (16.2)[b,c]
AAP	2.5 (0.2)	2.6 (0.2)[b,d]	7.6 (0.5)[b,*]	7.9 (0.6)[b]
αGST	1.1 (0.1)	1.1 (0.1)[b,d]	2.6 (0.4)[b,*]	2.8 (0.5)[b]
πGST	1.3 (0.1)	1.4 (0.2)[a,d]	2.9 (0.6)[b,*]	3.1 (0.5)[b]

NAG (N-acetyl-β-D-glucosaminidase), AAP (alanine aminopeptidase), αGST (α-glutathione S-transferase), πGST (π-glutathione S-transferase) activities and α1MG (α_1-microglobulin) are expressed as units of activity/mmol urinary creatinine and mg/mmol urinary creatinine, respectively. Microalbuminuria is expressed as mg/L.

Results are expressed as the mean (SD) and were compared using ANOVA.

Results from diabetic groups were compared to control values: [a]$P<0.05$, [b]$P<0.001$.

Group A versus group B: [*]$P<0.001$, group B versus group C: [c]$P<0.01$.

Indeed, while previous studies have suggested that increased NAG enzymuria is a consequence of chronic or fluctuating hyperglycaemia, and not necessarily a reflection of diabetic renal insult,[24,25] it is now accepted that hyperglycaemia resulting from poor long-term glycaemic control is a major underlying cause of diabetic complications, fuelling interrelated pathophysiological processes such as increased polyol pathway activity, protein glycation, advanced glycation end-products and free radical effects.[26–28]

However, while elevated GST activities have been noted in the diabetic state,[19,29,30] the present study also demonstrated elevated activity of the cytosolic isoenzymes of GST. These different patterns of GST isoenzyme excretion are especially interesting because αGST is only found in the proximal renal tubule, whereas the intrarenal localisation of πGST is to the distal tubule.[19–21] Consequently, the presence of elevated πGST suggests the presence of distal tubule insult in diabetics with proteinuria, and suggests that while proximal tubular and glomerular insult may be initial events in the natural history of diabetic nephropathy, as demonstrated by increased α1MG excretion, elevated NAG, AAP and αGST urine activities and microalbuminuria, this is followed by distal tubular insult indicated by increased πGST activity.

In conclusion, the present study demonstrates clearly that proximal tubular insult, characterised by increased NAG, AAP and αGST enzymuria, is present in type 2 diabetics and that generally it is associated with increasing proteinuria, as indicated in other studies.[19] However, NAG activity was elevated in patients without significant proteinuria or increased serum creatinine concentration, which is consistent with its role as an early indicator of diabetic renal insult. Furthermore, the results suggest that renal tubular insult may precede increased glomerular permeability in diabetic renal disease. Consequently, as these urinary biomarkers assess different areas of the renal architecture, estimation of renal tubular function and integrity may provide a site-specific and early indication of renal insult in subjects with diabetes. □

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