Serum cystatin C, enzymuria, tubular proteinuria and early renal insult in type 2 diabetes

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ABSTRACT

This study investigates the association between serum cystatin C, serum creatinine concentrations, N-acetyl-β-D-glucosaminidase (NAG enzymuria), urine α1-microglobulin (α1-MG) and β2-microglobulin (β2-MG) levels in subjects with type 2 diabetes (n=40, 20M/20F, age range 25–65 years; duration of diabetes 8–10 years) and age- and gender-matched healthy controls (n=20). Exclusion criteria were absence of gross proteinuria, hypertension, dyslipidaemia or cardiovascular disease. Fasting blood samples and mid-stream specimen of urine (MSSU) were collected and serum creatinine, cystatin C, urine creatinine, NAG enzymuria, α1-MG and β2-MG were measured. Diabetic subjects were separated into two groups based on albumin:creatinine concentration ratio. Group A: <3.5 (mg/mmol creatinine), group B: 3.5–35 (mg/mmol creatinine). While serum creatinine concentrations remained within the laboratory reference range for all groups, serum cystatin C concentration (mg/L) was significantly increased in group B (1.79 ± 0.42 [mean ± SD] compared to both control [0.81±0.10] and group A values [0.95 ± 0.10]; both P<0.001). NAG enzymuria (units/mmol creatinine) was increased in both diabetic groups compared to control values (group B: 122±7, group A: 70±5, controls 27±2, all P<0.001). α1-microglobulin (μg/mmol creatinine) concentrations, similar in both the control group and group A diabetics at 1.10±0.10 and 1.11±0.21, respectively, were significantly elevated in group B at 2.10±0.41 (both P<0.01). Similarly, elevated β2-MG (μg/mmol creatinine) levels were also observed in group B compared to both group A and control values (3.20±0.21 *vs.* 1.80±0.51 and 0.91±0.11, respectively; both P<0.001). In addition, group B levels were significantly higher than group A (P<0.001). These observations suggest that serum cystatin C is a more appropriate and effective biomarker for the overall estimation of GFR than serum creatinine values. In addition, increased serum cystatin C values were also associated with early renal tubular insult in subjects with type 2 diabetes, as characterised by increased NAG enzymuria, α1- and β2-microglobulin excretion.

KEY WORDS: Cystatin C.
Diabetes, type 2.
Tubular proteinuria.

Introduction

Diabetic nephropathy is characterised structurally 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, the latter usually 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 around 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 dysfunction.[5] The serum concentration of cystatin C, a non-glycosylated protease inhibitor, has been suggested as a more clinically appropriate endogenous marker of renal glomerular dysfunction in general[6–9] and type 2 diabetes in particular.[10]

Deceased renal tubular reabsorption capacity is characterised by elevated low molecular weight protein levels in the urine, such as α1-microglobulin and β2-microglobulin, and early renal tubular insult by increased proximal tubular enzymuria.[11–14] In this regard, elevated NAG enzymuria (the urine excretion of N-acetyl-β-D-glucosaminidase) has been used to indicate the early onset of renal insult in several clinical states in general (e.g., drug-induced nephrotoxicity, and the diabetic state in particular).[12,15] In addition, cystatin C in combination with β2-microglobulin[16] and α1-microglobulin[17] have both been suggested as markers of early diabetic nephropathy.

Consequently, the aim of this study is to investigate the association between serum cystatin C, NAG enzymuria, α1-microglobulin and β2-microglobulin excretion in a cohort of type 2 diabetics.

Materials and methods

Type 2 diabetic subjects (n=40; males [n=20], females [n=20]) were recruited from the diabetic clinic. Exclusion criteria

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included those patients presenting with proteinuria, hypertension, antihypertensive treatments, dyslipidaemia or cardiovascular disease. The age range of diabetics was 25–65 and the duration of diabetes was eight to 10 years. The control group consisted of healthy non-diabetic individuals and was gender- and age-matched to allow appropriate comparisons to be made.

Fasting blood samples and mid-stream specimens of urine (MSSU) were collected and glycated haemoglobin (HbA1c), serum creatinine, cystatin C, urine creatinine and microalbuminuria, urine N-acetyl-β-D-glucosaminidase activity (NAG enzymuria), α1-microglobulin (α1-MG) and β2-microglobulin (β2–MG) were measured. Serum and urine creatinine concentrations were measured using standard laboratory methods, cystatin C was quantitated using a commercial kit (Dako, UK), based on a turbidometric immunoassay,[18] NAG enzymuria was measured by a colorimetric method,[19] and α1-MG and β2-MG were measured using commercial kits (Boehringer Mannheim/Roche Diagnostics, Germany and Roche Diagnostics, Germany, respectively), based on turbidometric assays.[20,21] HbA1c and microalbuminuria were quantitated by turbidometric inhibition immunoassay and immunoturbidometric assays, respectively, using commercial kits provided by Boehringer Mannheim/Roche Diagnostics.

Diabetics were separated into two groups depending on their albumin:creatinine concentration ratio: group A <3.5 (mg/mmol creatinine), group B 3.5–35 (mg/mmol creatinine). Results, expressed as mean (SD), were analysed using one-way analysis of variance (one-way ANOVA), followed by the Bonferroni's multiple comparison test. $P<0.05$ was considered significant.

Results

Mean HbA1c values were significantly increased in the diabetic groups compared to control group values (both $P<0.001$) and also varied as the magnitude of microalbuminuria increased. Group B (3.5–35 mg albumin/mmol creatinine) HbA1c values were significantly greater than those in group A ($P<0.001$), which in turn were significantly different from the control group values ($P<0.001$) (Table 1). Elevated serum cystatin C concentrations were observed in group B compared to values from the control group and group A (both $P<0.001$), and group B values were higher than group A values ($P<0.01$). However, serum creatinine concentration remained within the laboratory reference range for all groups (<110 μmol/L).

NAG enzymuria was elevated two-fold in group A and four-fold in group B diabetic groups compared to the non-diabetic control group values (both $P<0.001$ compared to control group values and $P<0.001$ group A *vs.* group B) (Table 1). Interestingly, NAG enzymuria was elevated in group A subjects in the absence of any increase in serum cystatin C concentrations. Urinary concentrations of α1-MG were also significantly elevated in group B compared to group A and control group values (both $P<0.001$), whereas β2-MG concentrations in both diabetic groups were significantly elevated compared to control values (both $P<0.01$). In addition, urine β2-MG concentrations were significantly higher in group B when compared to group A values ($P<0.01$).

Discussion

The pathophysiological processes underlying the increased microalbuminuria in diabetes mellitus are related to haemodynamic alterations, structural, functional and biochemical changes observed in the glomerular basement membrane and mesangium of the glomerulus.[1–3] The results of the present study demonstrate that elevated serum cystatin C and albumin:creatinine ratio (microalbuminuria) were present in the absence of any changes in serum creatinine concentration, an insensitive measure of renal glomerular function, in a cohort of type 2 diabetics. As it has been proposed that ≥50% of nephrons must cease to function before alterations in serum creatinine concentrations and GFR occur,[5] the observation of reference range serum creatinine concentrations in the presence of elevated microalbuminuria and cystatin C suggests that serum creatinine is of limited value in the early detection of renal dysfunction in diabetes mellitus, similar to results of previous studies.[10,22] Consequently, the results of the present study are consistent with other studies which suggest that serum cystatin C is a more appropriate and sensitive marker of glomerular function in diabetes.[5, 13, 22, 23]

Table 1. Glomerular and tubular function in type 2 diabetic subjects with and without significant microalbuminuria.

	Control group (CG, $n=20$)	Group A (GA, $n=20$)	Group B (GB, $n=20$)
Microalbuminuria (mg/mmol urinary creatinine)	<3.5	3.5–35	
Disease duration (years)	–	8.5±0.8	9.2±0.7
HbA1c (%)	5.2±0.4	6.9±0.8†,‡	8.7±0.5†
Serum cystatin C (mg/L)	0.81±0.10	0.95±0.10§	1.79±0.42†
NAG enzymuria (U/mmol creatinine)	27±2	70±5†,‡	122±7†
α1-microglobulinuria (g/mmol creatinine)	1.10±0.10	1.11±0.21‡	2.10±0.41†
β2-microglobulinuria (μg/mmol creatinine)	0.91±0.11	1.80±0.51*,‡	3.2±0.21†

Results, expressed as mean (SD), were analysed using one-way analysis of variance (one-way ANOVA), followed by the Bonferroni's multiple comparison test.

CG *vs.* GA or GC *vs.* GB: $^{*}P<0.01$, $^{\dagger}P<0.001$.

GA *vs.* GB: $^{\ddagger}P<0.01$, $^{\S}P<0.001$.

Although it has been suggested that proteinuria itself might cause renal tubular cell injury, the site of the injury is unknown. The specific low molecular weight proteins α1- and β2-MG have been used widely in renal toxicology and nephrotoxicity studies as an estimate of proximal renal tubular protein absorption, and increased values have been shown to reflect both early renal damage and site-specific insult in the kidney.[11,12,21] The present study demonstrates elevated excretion of both these low molecular proteins associated with microalbuminuria, which is consistent with increased glomerular permeability and renal tubular dysfunction.

The elevated NAG enzymuria observed in the present study is also consistent with renal proximal tubular insult and with the results of previous studies.[11,12,13,19] It has been proposed that increased NAG enzymuria reflects lysosomal proliferation associated with an autophagic response to cellular insult in the renal proximal tubules, in this case in type 2 diabetics. Furthermore, the patterns of excretion of NAG and β2-MG were similar and both were elevated above control values before those of microalbumin, α1-MG or cystatin C, suggesting their use as sensitive markers of renal tubular insult predating glomerular dysfunction in type 2 diabetes.

In conclusion, these observation suggest that serum cystatin C is a more appropriate and effective biomarker than serum creatinine for overall estimation of GFR. However, early renal tubular insult characterised by increased small molecular weight proteinuria and NAG enzymuria are early manifestations of renal insult in type 2 diabetics, and in the present study these predated alterations in cystatin C and serum creatinine. □

References

1 Panchapakesan U, Xin-Ming C, Pollock CA. Drug insight: thiazolidinediones and diabetic nephropathy – relevance to renoprotection. *Nat Clin Pract Nephrol* 2005; **1**: 33–43.

2 Mogenson CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease. *Diabetes* 1993; **32**: 64S–78S.

3 Mogensen CE, Christensen CK. Predicting diabetic nephropathy in insulin-dependent diabetic patients. *N Engl J Med* 1984; **311**: 89–93.

4 Friedman EA, Friedman AL. Is there really good news about pandemic diabetic nephropathy? *Nephrol Dial Transplant* 2007; **22**: 681–3.

5 Perrone RD, Madias NE, Levy AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem* 1992; **38**: 1933–53.

6 Finney H, Newman DJ, Gruber W, Merle P, Price CP. Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the Behring nephelometer systems (BNA, BN II). *Clin Chem* 1997; **43**: 1016–22.

7 Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function – a review. *Clin Chem Lab Med* 1999; **37**: 389–95.

8 Price CP, Finney H. Developments in the assessment of glomerular filtration rate. *Clin Chim Acta* 2000; **297**: 55–66.

9 Chantrel F, Agin A, Ofner M, Koehl C, Moulin B, Hannedouche T. Comparison of cystatin C versus creatinine for the detection of mild renal failure. *Clin Nephrol* 2000; **54**: 374–81.

10 Harmoinen A, Kouri T, Wirta O *et al*. Evaluation of plasma cystatin C as a marker of glomerular filtration rate in patients with type 2 diabetes. *Clin Nephrol* 1999: **52**: 363–70.

11 Whiting PH, Price RG. Importance of early detection of renal dysfunction: value of markers of early renal disease and damage. *Clin Biochem* 2001; **3**: 3–8.

12 Jung K, Pergande M, Schimke E, Ratzman KP, Illus A. Urinary enzymes and low molecular mass protein as indicators of diabetic nephropathy. *Clin Chem* 1988; **34**: 544–77.

13 Uslu S, Efe B, Alatas O *et al*. Serum cystatin C and urinary enzymes as screening markers of renal dysfunction in diabetic patients. *J Nephrol* 2005; **18**: 559–67.

14 Price RG, Whiting PH. Urinary enzymes and nephrotoxicity in humans. In: Jung K, Matternheimer H, Burchards U eds. *Urinary enzymes*. Heidelberg: Springer-Verlag, 1992: 203–21.

15 Whiting PH, Ross IS, Borthwick L. Serum and urine N-acetyl-β-D-glucosaminidase in diabetics on diagnosis and subsequent treatment, and stable insulin-dependent diabetics. *Clin Chim Acta* 1979; **92**: 459–63.

16 Hong C-Y, Hughes K, Chia K-S, Ng V, Ling S-L. Urinary α1-microglobulin as a marker of nephropathy in type 2 diabetic Asian subjects in Singapore. *Diabetes Care* 2003; **26**: 338–42.

17 Aksun SA, Özmen, Özmen B *et al*. β2-microglobulin and cystatin C in type 2 diabetes: assessment of diabetic nephropathy. *Exp Clin Endocrinol Diabetes* 2004; **112**: 195–200.

18 Kyhse-Anderson J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindstrom V. Serum cystatin C determined by a rapid automated particle-enhanced turbidometric method is a better marker than serum creatinine for glomerular filtration rate. *Clin Chem* 1994; **40**: 1921–6.

19 Whiting PH, Ross IS, Borthwick L. N-acetyl-β-D-glucosaminidase levels and the onset of diabetic microangiopathy. *Ann Clin Biochem* 1983; **20**: 15–9.

20 Jung M, Jung K. Low molecular weight proteins in serum as markers of glomerular filtration: cystatin C, α1-microglobulin and β2-microglobulin. *Lab Med* 1994; **18**: 463–70.

21 Kurrle-Weittenhiller A, Engel W. Immunoturbidometric determination of urinary α1-microglobulin on Hitachi analysers. *Clin Chem* 1992; **38**: 1090–1.

22 Christenssen AG, Grubb AG, Nilsson, J-Å, Norrgren K, Sterner G. Serum cystatin C is advantageous compared with serum creatinine in the detection of mild but not severe diabetic nephropathy. *J Intern Med* 2004; **256**: 510–8.

23 Page MK, Bukki J, Luppa P, Neumeier D. Clinical value of cystatin C determination. *Clin Chim Acta* 2000; **297**: 67–72.