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Urinary Excretion of Transferrin by Non-Insulin-Dependent Diabetics: a Marker for Early Complications?

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We measured concentrations of transferrin (TRF, in micrograms), and creatinine (Cr, in millimoles) in samples of untimed urine from 53 healthy subjects and 157 non-insulin-dependent diabetic (NIDD) subjects. The urinary TRF/Cr ratio was significantly higher in the NIDD group ($P < 0.001$). If NIDD subjects are grouped according to their Alb/Cr ratio into normal albuminuria (Group A, Alb/Cr <2.5 mg/mmol), microalbuminuria (Group B, Alb/Cr 2.5–26.8 mg/mmol), and macroalbuminuria (Group C, Alb/Cr >26.8 mg/mmol), the TRF/Cr ratios in all three groups exceeded those for healthy controls. Moreover, this ratio was higher in Group B than in Group A and higher in Group C than in Group B. The value for TRF/Cr was clearly abnormal (i.e., exceeded the 95th percentile value found in healthy subjects) in 61%, 95%, and 100% of Group A, B, and C subjects, respectively. The TRF/Cr ratio was significantly higher in those NIDD subjects with clinical retinopathy, and it correlated with arterial pressure. Evidently, TRF/Cr may be increased early in NIDD subjects, and it may be a sensitive marker for detecting development of complications of diabetes.

An early manifestation of diabetic nephropathy, increased excretion of albumin (*1*), is now generally believed to be sufficiently specific, particularly in subjects with insulin-dependent diabetes mellitus, to predict the subsequent development of clinically overt diabetic nephropathy (*1–3*). However, certain other proteins besides albumin may also be excreted in abnormal amounts during this early phase of diabetic nephropathy. In some cases these may precede abnormal excretion of albumin (*4*). For example, excessive transferrinuria was recently reported (*5, 6*) in insulin-dependent diabetes; thus transferrinuria may be an additional marker of early nephropathy.

We measured the excretion of transferrin (TRF) in a group of non-insulin-dependent diabetic (NIDD) subjects, all of them Chinese, in whom NIDD is common but insulin-dependent diabetes is relatively uncommon.

Materials and Methods

Subjects

During a three-week period, we studied 157 NIDD subjects (112 men and 45 women, age range 21–88 years) who were attending the diabetic clinic at the Prince of Wales Hospital. During their visit to the clinic, their height and weight were recorded, and blood pressure was measured while the subject was in the sitting position. Any retinopathy was noted during clinical examination by fundoscopy. Blood was sampled at the clinic by venipuncture for determination of plasma glucose and serum fructosamine and in some patients for estimation of glycated hemoglobin ($Hb A_1$) as required for clinical management.

The healthy nondiabetic subjects were 17 men and 36 women from laboratory and clinical staff (ages 21–41 years). All subjects (patients and controls) collected an untimed urine specimen, which was stored at −20 °C until analysis.

Methods

Concentrations of TRF and albumin (Alb) in urine were measured by automated immunoturbidimetry in a Cobas Bio centrifugal analyzer (Roche Diagnostics, Basle, Switzerland) with use of antitransferrin and antialbumin antibodies from Dako, Glostrup, Denmark (*7*). For measurement of TRF, antibody to TRF was diluted 21-fold with phosphate buffer (pH 7.4, 0.1 mol/L) containing 70 g of polyethylene glycol 6000 (Sigma, St. Louis, MO) per liter. We mixed 150 μL of the diluted antibody with 40 μL of standards or samples and measured the rate of change of absorbance at 340 nm at 37 °C. The between-batch coefficients of variation (CV) for the method were 8.7% and 4.2% for concentrations of 460 and 2676 μg/L, respectively. The detection limit for the assay was 130 μg/L. Between-batch CVs for the albumin method were 3.3% and 6.7% for concentrations of 2.5 and 80 μg/L, respectively. Urinary creatinine (Cr) concentration was measured by use of the Jaffé reaction in an Astra 8 analyzer (Beckman Instruments, Brea, CA) according to the manufacturer's protocol.

We measured $Hb A_1$ by agar gel electrophoresis (Corning, Palo Alto, CA) and fructosamine and glucose by previously described methods (*8*).

The excretion of TRF was expressed in terms of micrograms per millimole of creatinine. The distributions of the data were non-gaussian and the data were log transformed before analysis. Differences between groups were compared by Student's *t*-test and the Mann–Whitney U test, as appropriate.

Results

The TRF/Cr ratio in NIDD subjects significantly ($P < 0.001$) exceeded that in healthy subjects (Table 1). If the 95th percentile value seen in healthy subjects was taken as the upper limit of reference, then 85.4% of the NIDD subjects had an abnormally high TRF/Cr ratio and 69.4% a high Alb/Cr ratio.

The diabetic subjects were divided into three subgroups according to Alb/Cr ratio: Group A <2.5 mg/mmol, Group B between 2.5 and 26.8 mg/mmol, Group C >26.8 mg/mmol—values chosen to correspond to albumin excretion rates of 20 and 250 mg/24 h (*9*). The value for TRF/Cr in all three groups significantly exceeded those for the healthy subjects (Table 1, Figure 1). In Group A (normal albumin excretion), 61% of subjects had high TRF/Cr ratio (higher than the 95th percentile value seen in healthy subjects). Values for TRF/Cr in Groups B and C significantly exceeded those in

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[3] Nonstandard abbreviations: NIDD, non-insulin-dependent diabetes; TRF, transferrin; Cr, creatinine; $Hb A_1$, glycated hemoglobin; and Alb, albumin.
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Table 1. Median (and Range) of Urinary Alb/Cr and TRF/Cr in Healthy Subjects and NIDD Subjects

	n	Alb/Cr, mg/mmol	TRF/Cr, μg/mmol
Healthy subjects	53	1.05	33.9
		(0.53–2.80)	(6.0–114.3)
NIDD subjects	157	4.13	233[a]
		(0.12–463)	(6.7–166 900)
Group A	49	1.82[a]	78.8[a]
Alb/Cr <2.5 mg/mmol		(1.06–2.5)	(6.7–41 700)
Group B	85	5.09[a]	285[a,b]
Alb/Cr 2.5–26.8 mg/mmol		(2.55–26.6)	(7.6–33 830)
Group C	23	54.3[a]	5368[a,b]
Alb/Cr >26.8 mg/mmol		(27.6–462.6)	(1303–166 910)

[a] Significantly (P <0.001) different from healthy subjects by Mann–Whitney U test.

[b] Significantly (P <0.001) different from Group A by Mann–Whitney U test.

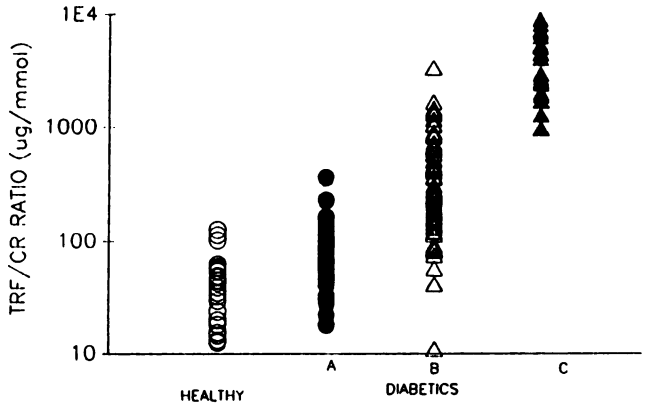

Fig. 1. TRF/Cr ratio in healthy subjects and in three groups of non-insulin-dependent diabetics, divided according to their Alb/Cr ratio

Group A, Alb/Cr <2.5 mg/mmol; Group B, Alb/Cr 2.5–26.8 mg/mmol; Group C, Alb/Cr ratio >26.8 mg/mmol

Group A (Table 1). Of the subjects in Group B (microalbuminuria group), 95% had a high TRF/Cr ratio, as did *all* the subjects in Group C (macroalbuminuria). The increase in TRF/Cr was greater than the increase in the Alb/Cr ratio. For example, the median TRF/Cr ratio in Group B was 8.4 times that for the healthy subjects. In comparison, Alb/Cr was 4.8 times higher. In NIDD subjects, the TRF/Cr ratio and the Alb/Cr ratio were correlated (r = 0.798).

When we divided NIDD subjects according to presence or absence of retinopathy, TRF/Cr ratio was significantly higher in those with clinical retinopathy (179 μg/mmol vs 368 μg/mmol, P <0.05). The TRF/Cr ratio was not correlated with duration of diabetes (r = 0.074), randomly measured plasma glucose (r = 0.024), serum fructosamine (r = 0.054), or Hb A_1 (r = 0.059), but it correlated significantly with systolic (r = 0.253, P <0.01) and diastolic blood pressure (r = 0.307, P <0.01).

Discussion

NIDD accounts for at least 95% of the diabetes seen among the Hong Kong Chinese population. It frequently occurs in persons younger than 30 years, and indeed is commoner than classical IDD in this age group. However, complications such as retinopathy and nephropathy are similar in their frequency to Caucasian populations.

To assess the prevalence of transferrinuria in this population, we measured the TRF/Cr ratio in an untimed urine specimen and also compared this ratio with the Alb/Cr ratio. There have been several studies on the suitability of untimed urine samples and first morning urine for the detection of microalbuminuria (perhaps better termed "paucialbuminuria"). The Alb/Cr ratio in early morning urine has been shown to give a reliable estimate of 24-h albumin excretion rate (*10, 11*). The use of untimed urine specimens is not accepted by all investigators, some of whom suggest that they are unreliable as a measure of albumin excretion rate (*12, 13*). However, other studies have shown that protein/Cr and Alb/Cr ratios for untimed urine samples correlate well with those for 24-h urine (*9, 14–16*). On the basis of these latter studies we used such samples in this study.

Our results show the TRF/Cr ratio to be abnormal in the great majority of patients: 95% of patients with Alb/Cr ratio between 2.5 and 26.8 (equivalent to albumin excretion of 25–250 mg/24 h) and all patients with Alb/Cr >26.8 (equivalent to albumin excretion >250 mg/24 h) had an abnormally high TRF/Cr ratio, and in addition 61% of NIDD subjects with normal Alb/Cr ratios had high TRF/Cr ratios as compared with healthy controls. Walton et al. (*17*) also reported TRF excretion to be increased in insulin-dependent diabetic children without microalbuminuria. Bernard et al. (*6*) observed a greater prevalence of increased values for TRF than for albumin. The increased excretion of TRF, the relative molecular mass of which is similar to that of Alb, 77 000, but is less anionic, suggests that there may be alteration in the charge of the glomerular membrane in NIDD.

An important unanswered question is the degree of albuminuria or other protein excretion that indicates the development of early nephropathy. In the case of *N*-acetyl-β-glucosaminidase (EC 3.2.1.30), for example, increased urinary excretion may reflect poor glycemic control rather than incipient nephropathy (*18*). In our population, the values for TRF did not correlate significantly with any of three variables of recent metabolic control—glucose, fructosamine, and Hb A_1—suggesting that excess transferrinuria is not explained by short- or medium-term fluctuations in glycemic control but more probably by an intrinsic abnormality of glomerular function. The lack of correlation with duration of diabetes may be explained by the nature of our population. In many of our patients diabetes was diagnosed incidentally or diagnosed only after many years of symptoms, and a substantial proportion have evidence of complications at presentation. Thus, assessment of duration of diabetes is very difficult and is likely to be underestimated in most patients.

The Alb/Cr ratio was found to correlate with arterial blood pressure in our population (r = 0.365, Cheung et al., unpublished observations), and the TRF/Cr ratio also correlated significantly with arterial blood pressure. In NIDD subjects with clinical retinopathy the Alb/Cr ratio (3.64 vs 5.19 mg/mmol, P <0.05) and TRF/Cr ratio were higher. Increased excretion of albumin in diabetic retinopathy has been reported (*19, 20*), and in one study increased TRF excretion was also found in insulin-dependent diabetes mellitus (*20*).

We conclude that excessive transferrinuria is very com-

mon in NIDD subjects and frequently occurs before microalbuminuria. Our results suggest that this may be a sensitive marker of incipient nephropathy. A longitudinal study is now in progress to further address this point.

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