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Heterogeneous nature of microalbuminuria in NIDDM: studies of endothelial function and renal structure

P. Fioretto[1], C. D. A. Stehouwer[2], M. Mauer[3], M. Chiesura-Corona[4], E. Brocco[1], A. Carraro[1], E. Bortoloso[1], V. W. M. van Hinsbergh[5], G. Crepaldi[1], R. Nosadini[1]

[1] Department of Internal Medicine and Center for the Study of Aging, National Research Council, University of Padova, Italy
[2] Department of Internal Medicine and Institute for Cardiovascular Research, Vrije Universiteit, Amsterdam, The Netherlands
[3] Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota, USA
[4] Institute of Radiology, University of Padova, Italy
[5] Gaubius Laboratory, TNO Prevention and Health, Leiden, The Netherlands

Summary Microalbuminuria (MA) is associated with microangiopathy (renal and retinal lesions) in insulin-dependent diabetic (IDDM) patients. In contrast MA does not reflect microvascular damage in a substantial number of non-insulin-dependent diabetic (NIDDM) patients. MA predicts cardiovascular disease in NIDDM patients with increased von Willebrand factor (vWF) plasma levels which are hypothesized to reflect endothelial dysfunction. However, it is not known whether MA is consequent to generalised endothelial dysfunction or to renal injury. Thus, this study evaluated vWF plasma levels in relation to renal and retinal structural abnormalities in NIDDM patients with MA. Kidney biopsies, fundoscopy and measures of vWF plasma levels were performed in 32 NIDDM patients with MA. These patients were allocated to two renal structural categories: A) Without renal structural abnormalities (C I, $n = 10$): normal or near-normal renal structure, and B) With renal structural abnormalities ($n = 22$), further divided into: C II ($n = 12$) with typical diabetic nephropathology, predominantly glomerulopathy, and C III ($n = 10$) with atypical patterns of renal injury (more advanced tubulo-interstitial and arteriolar than glomerular changes). vWF plasma levels were significantly higher in category B (C II: 195 ± 49 % and C III: 161 ± 46 %) than in category A (C I: 119 ± 42 %), (chi-square, $p < 0.05$). Diabetic retinopathy was also related to vWF plasma levels (ANOVA, $p < 0.05$). These data suggest that there are two types of MA in NIDDM: one associated with increased vWF levels, established renal injury and frequently retinopathy, and the other characterized by normal vWF levels, normal renal structure and absent or mild diabetic retinopathy. We propose that vWF plasma levels in NIDDM patients with MA may help to identify patients with important renal structural changes, increased retinopathy risk and, perhaps, generalised endothelial dysfunction. Whether vWF plasma levels predict end-stage renal disease and cardiovascular events deserves longitudinal studies. [Diabetologia (1998) 41: 233–236]

Keywords Non-insulin-dependent diabetes mellitus, microalbuminuria, von Willebrand factor, endothelial function, renal structure.

Microalbuminuria (MA) antedates overt nephropathy in both insulin-dependent (IDDM) and non-insulin-dependent diabetes (NIDDM) [1]; MA predicts clinical proteinuria in up to 80 % of IDDM patients [1] and MA levels exceeding 30 μg/min are associated with established diabetic glomerulopathy [2]. MA in IDDM is also associated with increased von Willebrand Factor (vWF) plasma levels [3]. In IDDM MA is a weaker predictor of renal disease. In fact only 20 % of patients with MA progress to overt nephropathy, in part because of premature death from cardiovascular disease [1]. The renal structural abnormalities underlying MA in NIDDM were essentially unknown until we recently described heterogeneity in

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Corresponding author: Dr. P. Fioretto, Department of Internal Medicine, University of Padova, via Giustiniani 2, 1-35128 Padova, Italy

Abbreviations: MA, Microalbuminuria; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; vWF, von Willebrand factor; AER, albumin excretion rate.

renal structure in these patients [4]. We observed that MA is associated with significant glomerular, arteriolar and tubulo-interstitial lesions in approximately 70 % of NIDDM patients, while the remaining 30 % have normal or near-normal renal structure [4]. Although quantitative measurements of renal structures by morphometric analysis will be performed in future studies, the classification by light microscopy is likely to be reliable, given the strict correlations between quantitative and semi-quantitative estimates of renal structures described in IDDM. Increased levels of urinary albumin excretion are also markers of an increased risk for cardiovascular disease in both types of diabetes [5, 6]. Indeed MA in NIDDM patients is a more precise predictor of cardiovascular events than of overt nephropathy [5, 6]. It has been suggested that MA NIDDM patients with increased vWF levels are at especially high risk for cardiovascular disease and those with normal vWF levels are not [7]. Increased vWF concentrations have been hypothesized to reflect generalised endothelial dysfunction, perhaps thereby being related to both MA [7, 8] and cardiovascular disease in NIDDM patients. Microalbuminuria in NIDDM is thus heterogeneous: it can occur with and without increased vWF levels and with and without renal structural abnormalities [4, 7]. It is not known, however, whether MA NIDDM patients with renal structural changes are those with abnormal vWF levels or, conversely, whether those without renal injury are those with normal vWF levels and, perhaps, normal endothelial function. This distinction indicating two types of MA in NIDDM with very different renal and cardiovascular prognosis is important. We therefore compared vWF plasma levels, as an indirect estimate of endothelial function, in MA NIDDM patients with and without renal structural abnormalities.

Subjects and methods

Patients and procedures. Thirty-two Caucasian NIDDM patients were studied. Patients were diagnosed as having NIDDM when onset was after age 40 years, when they were not ketosis prone and when they were not receiving insulin in the first two years after diagnosis. Inclusion criteria were NIDDM for at least 2 years, age 70 years or less, serum creatinine lower than 2 mg/dl, persistent microalbuminuria [albumin excretion rate (AER) 20–200 μg/min in at least two of three consecutive 24 h urine collections] and absence of liver disease. Patients with any contraindication for performing kidney biopsy were excluded.

These studies were approved by the ethical committee of the University of Padova and all patients were asked to give written informed consent. The patients were admitted to the Department of Internal Medicine at the University of Padova Hospital where percutaneous renal biopsy and renal functional studies were performed. Antihypertensive treatment was withdrawn in those patients receiving therapy 3–5 days before admission to the hospital; after the studies were completed, this therapy was resumed. During admission patients underwent at least three 24 h urine collections for measurement of AER (by radioimmunoassay) after urine culture was determined to be sterile. vWF plasma samples were blinded and measured by ELISA. They were obtained after an overnight fast. Glomerular filtration rate was determined by the plasma clearance of [51] Cr-EDTA, as described in detail elsewhere. HbA_{1c} was measured by high-performance liquid chromatography (DIAMAT Analyzer; Bio-Rad, Hercules, Calif. USA) to assess metabolic control. Blood pressure was measured at least 10 times in the supine position during admission, and the values provided represent the mean of these repeated measurements. Ischaemic heart disease was diagnosed in presence of clinical symptoms plus new Q waves on ECG and/or diagnostic enzyme changes (miocardial infarction) or of clinical symptoms plus ECG changes during angina and/or a positive exercise test (angina pectoris). All patients had funduscopy performed through dilated pupils at the Department of Ophthalmology of the University of Padova and, when required, fluoroangiographic studies were carried out. Patients were classified as follows: 1) absence of diabetic retinopathy; 2) background diabetic retinopathy; 3) proliferative diabetic retinopathy.

Renal structure

Percutaneous kidney biopsies were performed under ultrasound guidance by experienced investigators (P. F. and M. C.).

Light microscopy. Renal tissue was processed as previously described, evaluated by two observers (P. F. and M. M.) without knowledge of the patient's identity, and classified [4] as follows:

A. *Without renal structural abnormalities*
Category I (C I). Normal or near-normal renal structure. These biopsies were normal or showed very mild mesangial expansion, tubulo-interstitial changes or arteriolar hyalinosis in any combination.

B. *With renal structural abnormalities*
Category II (C II). Typical diabetic nephropathology. These biopsies showed established diabetic glomerulopathy with proportionally severe tubulo-interstitial and arteriolar changes. This picture is typical of that seen in IDDM patients.

Category III (C III) Atypical patterns of renal injury. These biopsies revealed mild diabetic glomerular changes with disproportionately severe tubular and vascular involvement including tubular atrophy, tubular basement membrane thickening and reduplication and interstitial fibrosis; advanced glomerular arteriolar hyalinosis commonly associated with atherosclerosis of larger vessels; global glomerular sclerosis. These changes were present in any possible combination.

No patient in this study had immunofluorescent or electron microscopy findings of any definable renal disease other than typical diabetic nephropathy or the patterns described above.

Statistical analysis. Data are expressed as mean ± 1SD. Values for AER, not normally distributed, were logarithmically transformed before analysis and are expressed as median and range. Data were analysed using the statistical package SPSS for Macintosh. Comparisons among the diabetic groups first used a one-way analysis of variance (ANOVA) and then unpaired Student's *t*-test for parameters shown to be different by ANOVA. Chi-square analyses were performed to test differences in

Table 1. Clinical characteristics of NIDDM patients divided into three renal structural categories

Category Number	Age (years)	Known duration (years)	HbA_{1c} (%)	AER (μg/min)	GFR ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	SBP (mm Hg)	DBP (mm Hg)
C I (10)	51 ± 8	9.7 ± 3.8	8.0 ± 1.2	42 (21–196)	125 ± 24	146 ± 18	89 ± 10
C II (12)	57 ± 5	15.3 ± 4.6	9.8 ± 2	51 (23–190)	97 ± 35	151 ± 19	91 ± 9
C III (10)	55 ± 5	10.6 ± 6.1	8.9 ± 1.4	40 (20–198)	102 ± 23	151 ± 9	91 ± 9
ANOVA *p* values	NS	< 0.03	< 0.05	NS	0.085	NS	NS

Data are expressed as mean ± 1SD, except for AER presented as median (range). HbA_{1c}, Glycated hemoglobin; AER, albumin excretion rate; GFR, glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure

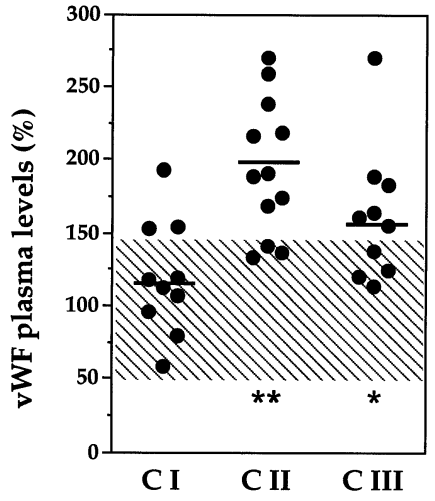

Fig. 1. von Willebrand Factor (vWF) plasma levels in NIDDM patients divided into three renal structural categories. ** = CI vs CII, $p < 0.005$; * = CI vs CIII, $p < 0.05$, CII vs CIII, NS. The shaded area represents the normal range in our laboratory

the frequency of the presence or absence of renal injury (using two simplified categories A and B) and diabetic retinopathy (present or absent) in relation to vWF levels (< vs ≥ 150 %, i.e. the upper limit of normal). Values for $p < 0.05$ were considered significant.

Results

Clinical features. On the basis of light microscopic findings, 10 patients (6 male/4 female) had no renal structural changes and were allocated to C I (31 %); the remaining 22 (69 %) had significant renal structural abnormalities: 12 of these (11 male/1 female) were allocated to C II (38 %) and 10 (7 male/3 female) to C III (31 %) (Table 1).

Age was similar in the three groups (ANOVA, $p = 0.48$). Known duration of NIDDM differed among groups (ANOVA, $p < 0.03$), with C II patients having the longest duration ($p < 0.01$ vs CI and $p = 0.06$ vs C III) (Table 1). HbA_{1c} levels differed among groups (ANOVA, $p < 0.05$); C II patients had higher HbA_{1c} values than did C I patients ($p < 0.03$). AER levels were similar in the three groups (Table 1). GFR was not different among the three groups (ANOVA, $p = 0.085$); however, C II and C III patients tended to have lower GFR values than did C I patients ($p = 0.054$ and $p = 0.05$, respectively). Systolic and diastolic blood pressure values were not significantly different in the three groups (Table 1). All but five patients (2/10 in C I, 1/12 in C II and 2/10 in C III) had been receiving antihypertensive therapy, the majority angiotensin converting enzyme inhibitors (6 in C I, 9 in C II and 7 in C III), before admission. Duration of antihypertensive treatment was similar in the three groups (4.6 ± 3.2 years in C I, 5 ± 3.5 in C II and 4.9 ± 2.7 in C III). There were three smokers in C I, 3 in C II and 2 in C III. There was no correlation between HbA_{1c} and vWF plasma levels ($r = 0.14$, NS). vWF plasma levels differed among groups (ANOVA, $p < 0.005$); C I patients had values similar to normal control subjects, while values for patients in C II and C III were abnormal and significantly higher than those in CI ($p < 0.005$ and 0.05, respectively) and in normal control subjects (Fig. 1). Chi-square analysis confirmed that patients with increased vWF plasma levels more frequently than patients with normal vWF levels had renal structural abnormalities (15 vs 7, $p < 0.03$): among patients with increased vWF levels 15 had and 3 did not have renal structural changes, while among patients with normal vWF levels 7 had and 7 did not have renal structural abnormalities.

Five patients in C I had background diabetic retinopathy and 5 had no retinopathy; 7 patients in C II had proliferative and 5 had background diabetic retinopathy; 6 patients in C III had background and 4 did not have diabetic retinopathy. ANOVA revealed differences in vWF plasma levels among patients without retinopathy (132 ± 41 %), with background (159 ± 59 %) and proliferative (199 ± 37 %) retinopathy ($p < 0.05$), with values in patients with proliferative retinopathy being significantly higher than in those without retinopathy ($p < 0.005$). Chi-square analysis confirmed that patients with increased vWF plasma levels more frequently had diabetic retinopathy (background and proliferative, $p < 0.05$). Ischaemic heart disease was diagnosed in 1 of 10 patients in C I, in 4 of 12 patients in C II and in no one in C III.

Discussion

This study demonstrates that MA is of a heterogeneous nature in NIDDM. Increased vWF plasma levels in MA patients are associated with important renal structural abnormalities and, frequently, with diabetic retinopathy. On the contrary, MA alone does not predict the underlying renal pathology. Indeed, despite the presence of persistent MA, a substantial proportion of NIDDM patients have normal renal structure and normal vWF levels. Similarly MA does not predict the grade of retinopathy.

These findings are consistent with two clinical observations. The low predictive value of MA for overt nephropathy in NIDDM [1, 5] is in keeping with the existence of a subset of patients without renal injury and without endothelial dysfunction. Also, MA is less frequently associated with diabetic retinopathy in NIDDM than in IDDM [9]. The typical changes of diabetic glomerulopathy (microangiopathy) were found, in the present study, in less than half of the MA patients. These patients had the highest vWF levels and all also had evidence of microangiopathy at the retinal level (background retinopathy in 42 % and proliferative in 58 %). Similarly in IDDM patients MA was associated with increased vWF levels only in presence of retinopathy [10].

Increased vWF plasma levels have previously been observed in MA and in proteinuric NIDDM patients by Chen et al. [8]; however, kidney biopsies were not performed in the MA patients in that study.

The exact nature of the relationships between endothelial dysfunction, vWF levels and renal structural changes in NIDDM remains to be established. It can be hypothesized that renal structural abnormalities are consequent to endothelial dysfunction in NIDDM; however, the contrary may also be possible. Each may be related to another causative factor(s) and not causally related to each other. Longitudinal studies with repeated measurements of renal structure and factors reflecting endothelial function will help to clarify this issue. Nevertheless, it seems reasonable to postulate that the development of renal injury and endothelial dysfunction in NIDDM may be related to common pathogenetic mechanisms, such as metabolic control, leading to widespread and generalized microangiopathy. Indeed MA patients with normal renal structure and, on average, normal vWF levels had the lowest HbA_{1c} levels, even though their AERs were similar to those of patients in the other categories.

In conclusion, the results of this study suggest the existence of two types of MA in NIDDM: the first is associated with serious renal structural abnormalities, including both typical diabetic glomerulopathy and tubulo-interstitial and arteriolar changes, and increased vWF plasma levels, suggestive of generalised endothelial dysfunction; the second is associated with normal renal structure and normal vWF plasma levels, consistent with normal endothelial function. Thus, in NIDDM MA 'per se' is a poor indicator of endothelial dysfunction and end-organ damage.

Although long-term longitudinal studies are required to clarify whether these two types of MA have different prognostic values for end-stage renal disease and cardiovascular disease, we propose that measuring vWF plasma levels in NIDDM patients with MA may be important in clinical practice to identify patients with important renal structural abnormalities, frequently diabetic retinopathy, and, perhaps, generalised endothelial dysfunction.

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