Patterns of renal injury in type 2 diabetic patients with microalbuminuria.

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Patterns of renal injury in NIDDM patients with microalbuminuria

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Summary Microalbuminuria predicts overt nephropathy in non-insulin-dependent diabetic (NIDDM) patients; however, the structural basis for this functional abnormality is unknown. In this study we evaluated renal structure and function in a cohort of 34 unselected microalbuminuric NIDDM patients (26 male/8 female, age: 58 ± 7 years, known diabetes duration: 11 ± 6 years, HbA_{1c}: 8.5 ± 1.6 %). Systemic hypertension was present in all but 3. Glomerular filtration rate (GFR) was 101 ± 27 ml · min^{-1} · 1.73 m^{-2} and albumin excretion rate (AER) 44 (20–199) μg/min. Light microscopic slides were categorized as: C I) normal or near normal renal structure; C II) changes "typical" of diabetic nephropathology in insulin-dependent diabetes (IDDM) (glomerular, tubulo-interstitial and arteriolar changes occurring in parallel); C III) "atypical" patterns of injury, with absent or only mild diabetic glomerular changes associated with disproportionately severe renal structural changes including: important tubulo-interstitial with or without arteriolar hyalinosis with or without global glomerular sclerosis. Ten patients (29.4 %) were classified as C I, 10 as C II (29.4 %) and 14 as C III (41.2 %); none of these patients had any definable non-diabetic renal disease. GFR, AER and blood pressure were similar in the three groups, while HbA_{1c} was higher in C II and C III than in C I patients. Diabetic retinopathy was present in all C II patients (background in 50 % and proliferative in 50 %). None of the patients in C I and C III had proliferative retinopathy, while background retinopathy was observed in 50 % of C I and 57 % of C III patients. In summary, microalbuminuric NIDDM patients are structurally heterogeneous with less than one third having "typical" diabetic nephropathology. The presence of both "typical" and "atypical" patterns of renal pathology was associated with worse metabolic control, suggesting that hyperglycaemia may cause different patterns of renal injury in older NIDDM compared to younger IDDM patients. [Diabetologia (1996) 39: 1569–1576]

Keywords NIDDM, renal structure, microalbuminuria, glomerular filtration rate.

Diabetic nephropathy is the single most frequent cause of end-stage renal disease in western countries, and the proportion of uraemic patients who are diabetic has been increasing in recent years [1, 2]. The United States Renal Data System does not yet separate insulin-dependent (IDDM) from non-insulin-dependent (NIDDM) diabetes mellitus. The European Dialysis and Transplant Association Registry reported that 35 % of diabetic patients requiring renal replacement therapy have NIDDM [2]; however, lack of precision in diabetes classification, originating from insulin-treated (non-insulin-dependent) patients, often classified as having IDDM, may have caused an underestimation of the percent of uraemic diabetic patients affected by NIDDM [3]. Indeed, regional surveys have shown a higher proportion of NIDDM patients on renal replacement therapy, both in the United States [4] and in Europe [3, 5, 6]. In Italy, when diabetes was classified by a specific

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; DN, diabetic nephropathy; GFR, glomerular filtration rate; AER, albumin excretion rate; PAS, periodic acid-Schiff.

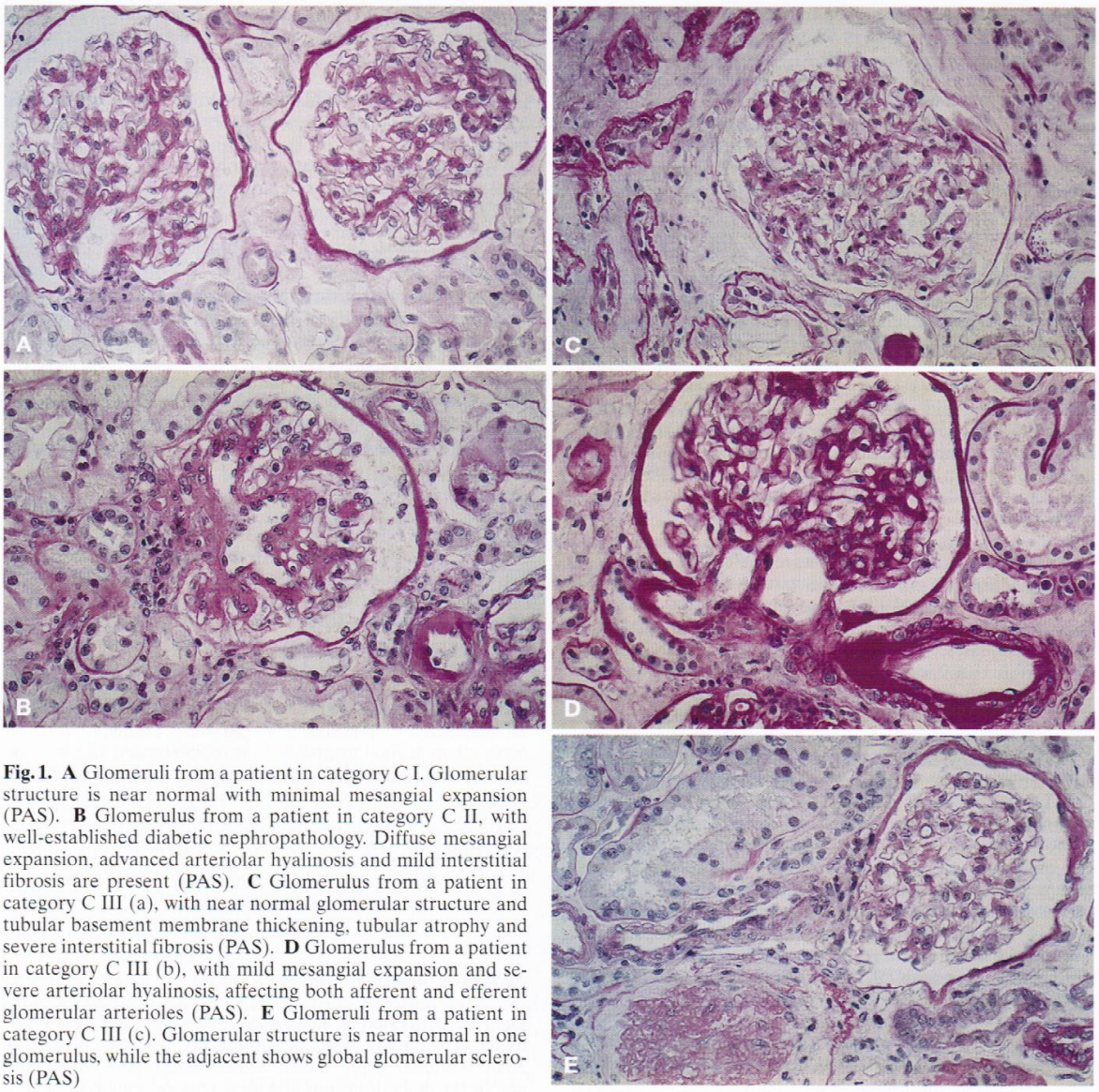

Fig. 1. **A** Glomeruli from a patient in category C I. Glomerular structure is near normal with minimal mesangial expansion (PAS). **B** Glomerulus from a patient in category C II, with well-established diabetic nephropathology. Diffuse mesangial expansion, advanced arteriolar hyalinosis and mild interstitial fibrosis are present (PAS). **C** Glomerulus from a patient in category C III (a), with near normal glomerular structure and tubular basement membrane thickening, tubular atrophy and severe interstitial fibrosis (PAS). **D** Glomerulus from a patient in category C III (b), with mild mesangial expansion and severe arteriolar hyalinosis, affecting both afferent and efferent glomerular arterioles (PAS). **E** Glomeruli from a patient in category C III (c). Glomerular structure is near normal in one glomerulus, while the adjacent shows global glomerular sclerosis (PAS)

group of 19 age and sex-matched normal control subjects: 85–135) and AER was 44 (20–199) μg/min (median, range) (normal values: 5 [0–14]).

All but 6 patients were receiving antihypertensive therapy, and the majority of them were on ACE inhibition. Overall, only 3 patients were normotensive, according to the criteria described above.

Based on the light microscopic findings 10 patients (5 male/5 female) were allocated to category I (29.4 %), 10 patients (9 male/1 female) to category II (29.4 %) and 14 (10 male/2 female) to category III (41.2 %). Important tubulo-interstitial changes were observed in all but 1 patient, who had very severe arteriolar hyalinosis lesions, in category III; these tubulo-interstitial lesions were often associated with arteriolar changes and in some ($n = 3$) patients to severe (> 25 %) global glomerular sclerosis.

The frequency of global glomerular sclerosis was 0 (0–20) (median, range) in CI, 0 (0–36) in CII and 4 (0–46) in CIII.

The clinical features of the patients as divided into the three structural groups are summarized in Tables 1 and 2.

CI patients tended to be younger than patients in groups CII and CIII, although the difference was not significant by ANOVA ($p = 0.13$); 50 % of patients in

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

Category	male/female	%	Age (years)	Known NIDDM duration (years)	BMI (kg/m^2)	HbA_{1c} (%)
C I	5/5	23.4	54 ± 9	8 ± 3	31 ± 4[b]	7.5 ± 0.8
C II	9/1	29.4	60 ± 6	14 ± 6[a]	26 ± 4	9.6 ± 1.8[d]
C III	12/2	41.2	61 ± 6	10 ± 8	30 ± 3[b]	8.5 ± 1.3[c]
p values						

Data are mean ± SD
[a] $p < 0.05$ vs C I and C III; [b] $p < 0.03$ vs C II; [c] $p < 0.05$ vs C I; [d] $p < 0.005$ vs C I

Table 2. Renal function and blood pressure of patients divided into three renal structural categories

Category	AER (μg/min)	GFR ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	Blood pressure	
			Systolic (mmHg)	Diastolic (mmHg)
C I	45 (20–198)	111 ± 20	142 ± 19	91 ± 10
C II	50 (22–190)	91 ± 36	154 ± 13	92 ± 7
C III	39 (20–198)	101 ± 21	155 ± 16	90 ± 10

Data are mean ± SD (AER is median and range) NS for all comparisons

Table 3. Diabetic retinopathy in relation to patterns of renal injury

Category	Diabetic retinopathy		
	Absent	Background	Proliferative
C I	5	5	0
C II	0	5	5
C III	6	8	0

CI were females, while in groups CII and CIII there was a clear preponderance of males (Table 1). Known duration of NIDDM tended to be different among groups (ANOVA, $p = 0.08$), with group CII patients having the longest duration (*t*-tests, $p < 0.05$ vs CI and CIII). HbA_{1c} levels were significantly different among groups (ANOVA, $p < 0.01$); subsequent analysis showed that CII and CIII patients had higher HbA_{1c} values than CI patients (*t*-tests, $p < 0.005$ and $p < 0.05$, respectively). BMI was also different among groups (ANOVA, $p < 0.02$); BMI was only mildly increased in CII patients (range: 18.1–31.5) and was significantly higher in CI (range: 27.7–40.1) and CIII patients (24.5–35.1) compared to CII (*t*-tests, $p < 0.05$ for both). AER levels were superimposable in the three groups (Table 2). GFR was not different in the three groups of patients; however, 5 of the 10 patients in CII had GFR values under 85 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$ compared to 1 of 10 in CI and 2 of 14 in CIII. Systolic and diastolic blood pressure values were similar in the three groups; two of the 3 normotensive patients were in group CI and one in group CIII. Seven of 10 patients in group CI, 10 of 10 in group CII and 11 of 14 in group CIII were receiving antihypertensive therapy.

Results of the funduscopic or fluoroangiographic evaluations are given in Table 3. In several patients varying degrees of hypertensive retinopathy were present with or without diabetic retinopathy.

Normal control subjects. Based on the scores for interstitial fibrosis, 3 of 36 (8 %) subjects had important tubulo-interstitial changes. Several normal control subjects had mild arteriolar hyalinosis lesions; 6 (16 %) had more advanced arteriolar lesion scores, comparable to those observed in patients in categories II and III (scores ≥ 1.0 according to our previously described scoring system [13]). However, none of the control subjects had arteriolar hyalinosis lesions of comparable severity to those observed in the patients categorized in group III because of vascular changes.

Discussion

In IDDM patients with long disease duration (≥ 10 years) and overt nephropathy, non-diabetic renal disease is rare (Mauer M, unpublished data). Thus, in the vast majority of long-term IDDM patients, the loss of kidney function is related to a well-defined pattern of diabetic nephropathology, including glomerular basement membrane and tubular basement membrane thickening and mesangial expansion, especially matrix accumulation, but also arteriolar hyalinosis affecting both afferent and efferent glomerular arterioles [9–15]. Interstitial fibrosis is frequently present, especially in patients with advanced glomerulopathy and typically in areas with global glomerular sclerosis and tubular atrophy [11, 13]. This pattern of renal lesions is quite monotonous and predictable in IDDM patients with clinical nephropathy.

Three studies have reported that non-diabetic renal disease is quite frequent in NIDDM patients with overt nephropathy. Parving et al. [16] found that 1 of 4 of such patients had renal diseases other than diabetes; Gambara et al. [17] confirmed these

data reporting that only 1 of 3 of NIDDM patients with overt nephropathy had typical patterns of DN. Khan et al. [33] recently observed the presence of non-diabetic renal disease in 42 % of 153 NIDDM patients with overt nephropathy; the occurrence of non-diabetic renal disease was much lower (12 %) in the series of 33 proteinuric patients studied by Olsen and Mogensen [34]. In all these studies, however, patients were referred to the nephrologist and kidney biopsies were not performed on the basis of research protocols, but for clinical indications. Thus, these studies do not describe the usual NIDDM patients with nephropathy, but those with an unusual clinical course; also the different results may reflect differences in the criteria for kidney biopsy. A large autopsy study on NIDDM patients did not confirm a high incidence of non-diabetic renal diseases [18]. Thus, the available data on renal structure in NIDDM patients with proteinuria are still inconclusive.

Microalbuminuria in NIDDM has been shown to predict mortality, mainly cardiovascular [22, 24, 25] and, in approximately 20 % of patients, the development of overt proteinuria [23]. However, whether the raised urinary albumin excretion is an expression of underlying diabetic renal lesions is still unknown. Only one study to date evaluated renal structure in microalbuminuric NIDDM patients [35]; surprisingly these authors, who reported diagnostic heterogeneity in proteinuric NIDDM patients [17], found that all 16 microalbuminuric NIDDM patients had classic lesions of diabetic glomerulopathy.

Since 1992 we have been performing kidney biopsies on the basis of a research protocol rather than clinical indications. The present paper summarizes the preliminary analysis in the microalbuminuric patients studied to date.

The initial reading of the light microscopy tissue of these NIDDM patients made apparent the inadequacy of current descriptive formulations, largely based on observations of research biopsies in IDDM (of which the authors have reviewed several hundreds). Virtually all IDDM patients with at least 10 years of diabetes and overt nephropathy have obvious diabetic glomerulopathy. Diabetic glomerulopathy, although less severe, is usually quite advanced in microalbuminuric IDDM patients. We recently reported that all IDDM patients with AER over 30 μg/min had electron microscopic morphometric measures of diabetic glomerulopathy above the normal range [32]. Since electron microscopic morphometric analysis has not been completed in these patients, it is currently impossible to make any precise comparison between diabetic glomerulopathy in IDDM and NIDDM microalbuminuric patients. Nevertheless, many NIDDM patients with microalbuminuria did not have glomerulopathy, or they had very mild mesangial expansion by light microscopy. Thus, 70 % of the microalbuminuric NIDDM patients had normal or near normal glomerular structure by light microscopy, with or without tubulo-interstitial and arteriolar changes. The remaining 30 % had renal changes typical of DN in IDDM, with glomerular, tubulo-interstitial and vascular lesions occurring in parallel.

Thus, we developed a new classification system which included three major groups. Category I was defined as normal or near-normal renal structure, category II as "typical" patterns of renal injury (similar to the changes in IDDM). Category III was defined as "atypical" patterns of renal injury, including severe tubulo-interstitial and/or arteriolar hyalinosis and/or global glomerular sclerosis lesions in the presence of absent or mild glomerular changes.

Thus, despite comparable renal function, NIDDM patients with microalbuminuria are structurally heterogeneous: only 29 % had "typical" DN, 29 % had near normal renal structure and 42 % severe tubulo-interstitial and/or vascular lesions disproportionate to the mild glomerular involvement. In this series of patients we did not find cases of any definable non-diabetic renal disease. The difference between our findings and those in previous reports in proteinuric NIDDM patients (see above) may be explained by the study design, in that patients in the present study had kidney biopsies performed on the basis of a research protocol as opposed to clinical indication for atypical course.

The "atypical" patterns of renal injury observed in many of our patients are probably related to hyperglycaemia, since HbA_{1c} levels were higher both in patients with "typical" and with "atypical" patterns of lesions compared to patients without lesions. Thus, hyperglycaemia may cause different patterns of renal injury in older NIDDM compared to younger IDDM patients. The tubulo-interstitial and vascular changes could also be related to aging and systemic hypertension. However, hypertension was present in almost all patients (except for 3) in all 3 structural categories, and "per se" cannot account for the different lesions observed in category III. Further, mean age was similar in category II and III patients, despite the different patterns of renal injury in the two groups. Also, we examined a large number of age-matched normal control subjects and found that severe lesions, as observed in NIDDM patients, were uncommon in the non-diabetic subjects. Nevertheless, aging and blood pressure may have varying impact in different patients, and therefore may contribute, in presence of other factors, to renal injury in this subset of patients.

The reasons why the kidney may react differently to hyperglycaemia in different patients with NIDDM are not clear. It can be hypothesized that the heterogeneity in renal structure might reflect the heterogeneous nature of NIDDM "per se". Patients with "typical" DN lesions had the longest known diabetes duration, worse metabolic control and they all had

diabetic retinopathy; interestingly their BMI only slightly exceeded normal values, as opposed to clearly increased BMI values in categories I and III.

The nature of this interrelationship of diabetic milieu and renal structural changes remains enigmatic; however, these relationships also extend to diabetic retinopathy. Diabetic retinopathy was present in all C II patients, background in 50 % and proliferative in 50 %. None of the patients in C I and C III had proliferative retinopathy, while background diabetic retinopathy was observed in 50 % of C I and 57 % of C III patients. Thus, all C II patients had diabetic retinopathy and all patients with proliferative retinopathy had "typical" DN.

A high proportion of microalbuminuric NIDDM patients (29 %) had normal or near-normal renal structure. They tended to be younger and had shorter diabetes duration and better metabolic control than patients with renal lesions (categories II and III). Although we do not have an explanation for the abnormal AER in these patients, it is possible that microalbuminuria in this subset is a clinical manifestation of generalized endothelial dysfunction rather than of renal damage "per se". Parving et al. [16] observed what they termed "minimal lesion" nephropathy in 4 of 36 NIDDM patients with clinical proteinuria. It is possible that the patients diagnosed as having "minimal lesion" nephropathy in this latter study are similar to our category I patients, however this will require longitudinal studies to be addressed. Suffice it to say here that a substantial proportion of NIDDM patients with microalbuminuria or proteinuria may have increased glomerular capillary wall permeability to protein for reasons not currently understood.

In summary, in this unselected series of 34 cases biopsied for research reasons, NIDDM patients with microalbuminuria do not have non-diabetic renal diseases; however they may have different patterns of renal injury compared to IDDM patients. More "typical" diabetic nephropathy patterns are seen among microalbuminuric NIDDM patients with proliferative retinopathy and normal BMI, while "atypical" patterns of renal injury are more common among those with increased BMI and background or no retinopathy. Finally, a subset of microalbuminuric NIDDM patients have near-normal renal structure by light microscopy; in these patients the increased renal permeability to protein might be expression of generalized endothelial dysfunction.

Long-term longitudinal studies are needed to determine the course of renal function in these patients with different patterns of renal injury.

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