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Structural basis of diabetic nephropathy in microalbuminuric NIDDM patients: a light microscopy study

T. Bertani[1, 2], **V. Gambara**[1, 2], **G. Remuzzi**[1, 2]

[1] Mario Negri Institute for Pharmacological Research, Bergamo, Italy
[2] Division of Nephrology and Dialysis, Ospedali Riuniti di Bergamo, Bergamo, Italy

Summary The objective of the study was to evaluate early structural changes occurring in patients with non-insulin-dependent diabetes mellitus (NIDDM) and microalbuminuria by light microscopy. Basal renal biopsy was performed in patients who were subsequently randomized to different antihypertensive treatments. Fourteen NIDDM patients aged 36–65 years (duration of diabetes 9 ± 7 years) with microalbuminuria (mean urinary albumin excretion 66 ± 49 μg/min) underwent percutaneous renal biopsy. Control biopsies were obtained from five patients of similar age undergoing nephrectomy for renal neoplasia with normal renal function and no history of renal disease. Control and diabetic biopsies were processed by light microscopy and stained with haematoxylin and eosin, periodic acid Schiff, Masson's trichrome and silver methenamine. The percentage of globally sclerotic glomeruli was evaluated. Glomerular volume was determined using perimeter analysis. A semiquantitative assessment (range 0 to 3 +) was made of mesangial sclerosis, interstitial fibrosis, tubular atrophy, arteriosclerosis and arteriolar hyalinosis. Glomerular volume was significantly increased in diabetic as compared to control glomeruli (3.2 ± 8 vs 1.8 ± 7, $p < 0.01$). Mesangial sclerosis (0.9 vs 0, $p < 0.0001$) and arteriolar hyalinosis (0.91 vs 0.2, $p < 0.022$) were significantly higher in diabetic compared to control subjects. No significant differences between diabetic and control subjects were found in the percentage of globally sclerotic glomeruli or in the extent of interstitial fibrosis, tubular atrophy and arteriosclerosis. Thus NIDDM patients with microalbuminuria show histological findings consistent with diabetic nephropathy characterized by glomerular hypertrophy, mesangial sclerosis and arteriolar hyalinosis. However, the renal histological changes are mild and appear less marked than in insulin-dependent diabetic patients. [Diabetologia (1996) 39: 1625–1628].

Keywords Glomerulosclerosis, diabetes mellitus, Microalbuminuria, glomerular volume and light microscopy.

Diffuse and nodular glomerulosclerosis are typical lesions of most insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetic patients with clinical proteinuria and renal function impairment [1, 2]. Morphometric studies in the last 20 years have established that the sclerotic process results from an excessive production of mesangial matrix leading to reduction of the glomerular capillary surface and progressive decline of renal function [3–4].

While glomerular lesions in diabetic patients with clinical proteinuria have been well-documented, the nature of renal lesions in those patients who only have microalbuminuria is still elusive.

The few studies available, all in IDDM, found that patients with low levels of microalbuminuria have a heterogeneous pattern of abnormalities ranging from no signs of glomerular damage to glomerulosclerosis [5–7]. Conversely, IDDM patients with higher levels of microalbuminuria most often had diabetic glomerulosclerosis. At present no data are available

Corresponding author: Dr. T. Bertani, Mario Negri Institute for Pharmacological Research, Via Gavazzeni 11, 24125 Bergamo, Italy
Abbreviations: IDDM, Insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus

Table 1. Clinical characteristics of patients at renal biopsy

Age (years)	Sex (female/male)	Diabetes duration (years)	Glucose (mmol/l)	HbA_{1c} (%)	Serum creatinine (μmol/l)	UAE (μg/min)	MAP (mm Hg)	Retinopathy (yes/no)
53 ± 8	6/8	9 ± 7	9.5 ± 2.8	6.9 ± 1.2	0.886 ± 0.088	66 ± 49	116 ± 6	7/7

Data are mean ± SD. UAE; urinary albumin excretion; MAP; mean arterial pressure

by light or electron microscopy of renal lesions in NIDDM patients with microalbuminuria. The aim of the present study is to evaluate the early structural changes occurring in patients with NIDDM and microalbuminuria through the use of light microscopy.

Subjects and methods

We selected 14 patients with mild hypertension (90–104 mmHg diastolic pressure), microalbuminuria (urinary albumin excretion 20–200 μg/min) and serum creatinine under 0.896 ± 0.08 μmol/l for the study. The group consisted of 6 females and 8 males with ages ranging between 36 and 65 years and diabetes duration from 1 to 25 years. Table 1 summarizes clinical and laboratory data. All patients underwent percutaneous renal biopsy using a conventional technique. Five surgical biopsies obtained from patients of similar age (55.03 ± 7.51 years) undergoing unilateral nephrectomy for kidney neoplasia and with no previous history of renal diseases were used for comparison. After the removal of the kidney, small fragments of tissue, which excluded the most superficial glomeruli, were cut and immediately fixed.

All kidney biopsies were fixed in Dubosq-Brazil solution and routinely processed for paraffin embedding. Serial sections of 3 μm thickness were cut and stained with haematoxylin and eosin, Masson's trichome, silver methenamine and periodic acid-Schiff reagent. At least eight glomeruli were examined for each biopsy.

In each patient the number and the percentage of globally sclerotic glomeruli were determined. A semiquantitative assessment of renal damage was performed. The following histological findings were considered: mesangial sclerosis, interstitial fibrosis, tubular atrophy, arteriosclerosis, and arteriolar hyalinosis.

Mesangial sclerosis was graded from 0 to 3 + (0 = no changes, 1 + = mild changes consisting in an expansion of mesangial matrix or cells without reduction of capillary lumina, 2 + = moderate changes consisting in an expansion of mesangial matrix or cells with reduction of capillary lumina, 3 + = severe changes consisting in an expansion of mesangial matrix or cells with marked reduction or occlusion of capillary lumina.

Interstitial fibrosis and tubular atrophy were graded from 0 to 3 + (0 = no changes, 1 + = changes affecting less than 25 % of the biopsy specimen, 2 + = changes affecting 25 to 50 % of the biopsy specimen, 3 + = changes affecting more than 50 % of the biopsy specimen).

Arteriosclerotic changes which consisted of fibrous thickening of the intima often associated with duplication of the lamina elastica, leading to reduction of the vascular lumen, were graded from 0 to 3 + (0 = no changes, 1 + = mild changes, 2 + = moderate changes, 3 + = severe changes).

Although arteriolar hyalinosis can be observed in arteriosclerosis, it is also frequently detected in diabetes and is considered part of diabetic nephropathy. Therefore, arteriolar hyalinosis, consisting of periodic acid Schiff positive material permeating the arteriolar wall and leading to the narrowing or occlusion of vascular lumen, was evaluated with a score ranging from 0 to 3 + (0 = no changes 1 + = mild changes 2 + = moderate changes 3 + = severe changes).

Glomerular volume (V_G) was determined as previously described [8]. Briefly, images of all glomeruli from each biopsy in two sections more than 180 μm apart were digitized from the light microscope using a videocamera (Panasonic, Matsushita Electrical Co., Osaka, Japan) and stored in the computer memory (Macintosh IIfx; Apple Computer Inc., Cupertino, Calif., USA). Glomerular cross sections were displaced on the computer screen at a final enlargement of × 1000 and the area of the capillary tuft outlines was measured using area perimeter analyses (Image v1.55; National Institutes of Health, Bethesda, M. D., USA). The mean number of analysed glomerular sections was 20 (range 8–30). The mean glomerular tuft cross-sectional area (A_m), including area of capillary space, was computed for each biopsy and V_G calculated using the equation:

$$V_G = \frac{\beta}{k} (A_m)^{3/2} \qquad [\mu m^3]$$

where k = 1.1 is a size distribution coefficient, and β = 1.38 is the shape coefficient of glomeruli [9].

Statistical analysis

All results are expressed as mean ± standard deviation. Student's *t*-test was used to compare glomerular volume and percentage of sclerotic glomeruli.

Chi-square or Fisher's exact tests were used for comparison of scores of histological change.

Results

Mean glomerular volume was significantly increased in diabetic patients as compared to control subjects (3.2 ± 8 vs 1.8 ± 7 μm^3 $p < 0.01$) (Fig. 1). By light microscopy the spectrum of pathological changes was evenly distributed in all patients. Glomeruli were moderately enlarged with a mild diffuse increase of mesangial matrix and few areas of segmental sclerosis (Fig. 2). The increase of mesangial matrix was not associated with mesangial or endocapillary hypercellularity.

The glomerular capillary wall was not markedly thickened. The percentage of globally sclerotic glomeruli in diabetic patients was 6.6 %, not significantly different from that observed in control subjects (2.6 % p = NS). No significant glomerular changes were detected in control biopsies (Fig. 3).

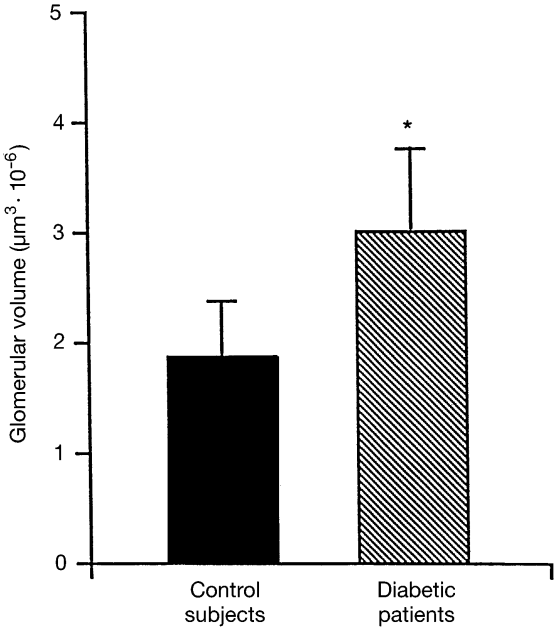

Fig. 1. Distribution of glomerular volume in control subjects and in NIDDM patients * $p > 0.01$

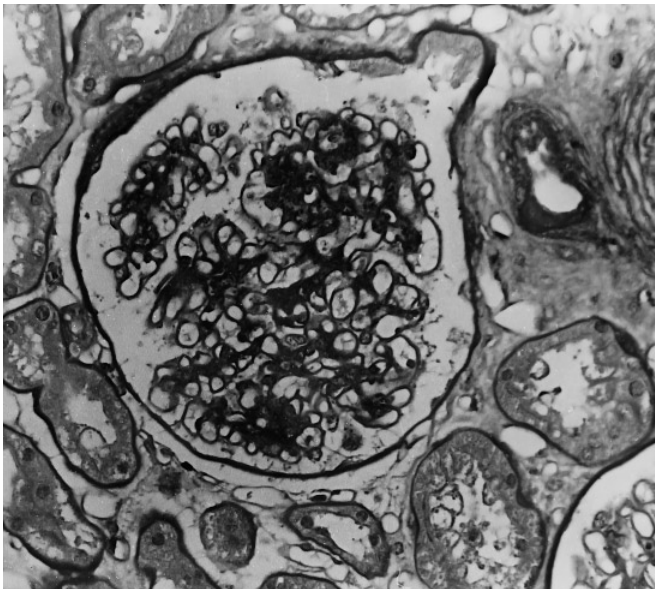

Fig. 2. Glomerulus from a patient with NIDDM and microalbuminuria showing diffuse mesangial sclerosis. Hyalin changes are present in an arteriole (PAS × 250)

In diabetic specimens there was a focal mild interstitial fibrosis and tubular atrophy; however, the score of tubulointerstitial changes was not significantly different from control subjects.

Arteriolar hyalinosis was observed in almost all patients and was significantly higher than in control subjects ($p < 0.022$).

Arteriosclerotic changes were similarly represented in diabetic and control patients. The spectrum and the extent of renal damage are summarized in Table 2.

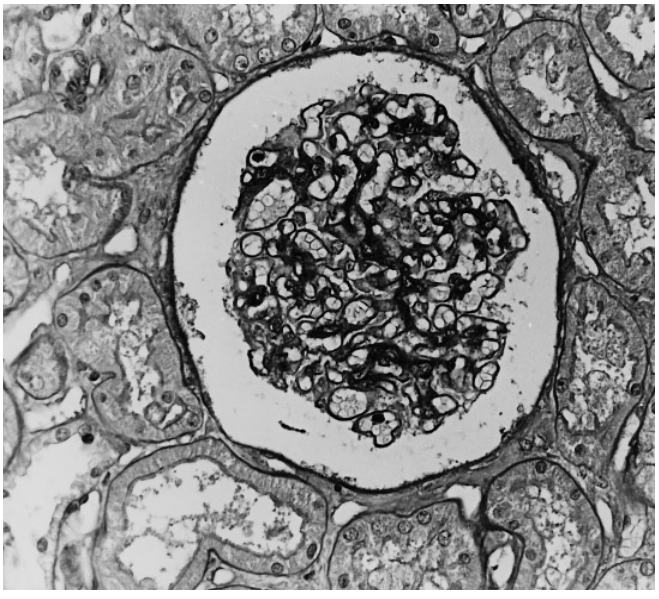

Fig. 3. Glomerulus from a control patient showing no significant changes (PAS × 400)

Discussion

Our study through the use of light microscopy, demonstrates results for patients with NIDDM, microalbuminuria and mild hypertension consistent with a diagnosis of diabetic nephropathy with glomerular hypertrophy, mesangial sclerosis and arteriolar hyalinosis.

While all patients had a remarkable increase in glomerular volume, glomerulosclerosis and arteriolar hyalinosis were mild. Renal lesions also included some chronic tubulointerstitial lesions and arteriosclerosis which, however, were also seen in control subjects and could be regarded as non-specific, age-related changes. The few available data on light microscopy findings in NIDDM microalbuminuric diabetic patients have mostly been published in abstract form [10, 11]. Some showed lesions of diffuse or nodular glomerulosclerosis that we have been unable to confirm in the present study [10, 11].

Our light microscopy findings of mild glomerular lesions are at variance with those observed in IDDM patients with similar levels of microalbuminuria, in whom more marked glomerular structural changes have been detected [5–7].

However, pathological findings here are in agreement with a preliminary study performed in 69 NIDDM patients with microalbuminuria by Fioretto and co-workers [12] who demonstrated that in these patients glomerular changes were less pronounced than in IDDM.

The reasons why glomerular structure might be more protected in NIDDM require further studies.

Table 2. Semiquantitative assessment of pathological changes

	n	Glom. volume	Globally sclerotic glom	Mesangial sclerosis	Interstit. Fibr.	Tub. atrophy	Arteriosc.	Art. hyal.
Control subjects	5	1.8 ± 7	2.6	0	1	1	0.8	0.2
Diabetic patients	14	3.2 ± 8[b]	6.6	0.9[c]	1.1	1.06	0.4	0.91[a]

[a] $p < 0.022$; [b] $p < 0.01$; [c] $p < 0.0001$ vs control subjects
Data are expressed as mean score (range 0 to 3 +)
Glom., Glomerular; Fibr., fibrosis; Tub., tubular; Arteriosc., arteriosclerosis; Art. hyal., arteriolar hyalinosis

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