Kidney transplantation in Type 1 (insulin-dependent) diabetic patients

Early glomerulopathy

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Summary. The development of diabetic glomerulopathy in kidneys transplanted to diabetic patients was estimated in transplant biopsies and evaluated in relation to suspected clinical risk factors for diabetic nephropathy. Surgical biopsies were taken at baseline and at 24–36 months post-transplantation in 16 Type 1 (insulin-dependent) diabetic patients and 8 non-diabetic control subjects with a glomerular filtration rate more than $30 \text{ ml} \cdot \text{min}^{-1}$ at follow-up. Immunosuppressive therapy included cyclosporine in all but one case. Stereological methods were used to assess basement membrane thickness, volume fraction of mesangium per glomerulus, and volume fraction of matrix per mesangium. The volume fraction of interstitial tissue per cortex was estimated by light microscopy. After 2 years the basement membrane thickness had increased by 55 nm (SD 58 nm) in the diabetic group. This change was significantly different from that of 2 nm (SD 37 nm) in control subjects ($p = 0.02$). Mesangial volume fraction increased significantly by 0.04 (SD 0.03) in diabetic patients, and this change was significantly different from that of -0.01 (SD 0.04) in non-diabetic patients ($p = 0.009$). No change was detectable in the matrix ex-

pressed as fraction of mesangial volume. An increase in interstitial volume fraction from baseline to 2 years was observed, but was significant only in the diabetic group ($p = 0.04$). The changes in structural parameters did not correlate with mean values during follow-up of glycated haemoglobin or estimated protein intake, nor was any pattern discernible in the relationship to graft tissue types. The observed increase in basement membrane thickness corresponds to that observed in native kidneys during the first years of diabetes, whereas an increase in mesangial volume fraction – using a different protocol – was not observed in the early phase of the natural development. Absence of correlation with the various risk factors may reflect an irrelevance of these variables within the current range, or their influence may be offset by stronger mechanisms in the transplant situation, and therefore does not appear in this relatively small series.

Key words: Kidney transplants, diabetic glomerulopathy, basement membrane thickness, mesangial volume fraction, interstitial fibrosis, cyclosporine, stereology.

Transplantation is a mode of renal replacement therapy which has a high success rate in diabetic patients with end-stage renal failure [1–4]. One early concern regarding renal transplantation in diabetic patients was the possibility of a recurrence of diabetic glomerulopathy in the transplanted organs. A specific quality of the transplanted kidney is that it is single, and therefore, according to the hyperfiltration hypothesis [5, 6] might be more prone than native kidneys to developing glomerulopathy.

Previous transplant biopsy studies [7–10] have indicated that within the first 10 years after transplantation the structural changes are modest and unlikely to have any immediate clinical significance. However, histopathological changes develop during the clinically silent phase and analysis of the relationship between structural changes and clinical variables in this early stage

may elucidate factors that significantly influence the process.

The previous studies on development of diabetic glomerulopathy in transplanted kidneys have covered a wide time-range from transplantation to biopsy, and very few biopsies have been performed in the earliest phase, when the first sign of glomerulopathy appears. This stage might be optimal for studies to clarify the pathogenetic mechanisms.

The report from Minneapolis in 1983 [7] of biopsies taken after 2 years was carefully designed and included baseline biopsies taken at transplantation as well as biopsies from non-diabetic control patients who had undergone kidney transplantation. Quantitative electron microscopy was, however, performed in only four diabetic subjects. We have carried out a similar study, comprising

Table 1. Clinical data for kidney transplanted Type 1 (insulin-dependent) diabetic patients

Patient	Age (years)	Sex	Diabetes duration (years)	Kidney source ^a	Age of donor (years)	At 24–36 months		Antihypertensive therapy at biopsy ^b
						GFR (ml · min ⁻¹)	Urinary protein (g · 24 h ⁻¹)	
1	30	M	29	C	36	52	0.7	L + β + Ca
2	34	M	22	L	42	40	0	L
3	24	M	16	L	58	43	1.2	L + β + hydralazine
4	42	M	35	C	62	34	0	L + β + Ca
5	45	M	40	C	42	39	0	L + β
6	50	M	33	C	48	38	1.4	L + β
7	40	M	19	C	28	51	0.8	L
8	34	M	17	C	58	60	trace	L + β + Ca + ACE ^c
9	46	M	15	C	39	53	0.3	L + β + Ca
10	46	M	35	C	12	48	0	L + β
11	27	F	17	L	51	32	0	L + β + ACE ^d
12	47	M	27	L	38	45	0	L
13	49	M	15	C	69	36	trace	L
14	38	F	30	L	45	41	0	L
15	41	F	27	L	54	73	0	L + β
16	32	F	21	C	47	62	0	L
median	41		25		46	44		
range	24–50		15–40		12–69	32–73		

^a L = Living donor, C = Cadaveric donor; ^b L = Loop diureticum, β = β_1 blocking agent, Ca = calcium blocker, ACE = angiotensin converting enzyme inhibitor; ^c duration of therapy 31 months; ^d duration of therapy 7 months.
GFR = glomerular filtration rate

Table 2. Clinical data for kidney transplanted control patients

Patient	Age (years)	Sex	Diagnosis ^a	Kidney source ^b	Age of donor (years)	At 24–36 months biopsies		Antihypertensive therapy ^c
						GFR (ml · min ⁻¹)	Urinary protein (g · 24 h ⁻¹)	
1	36	M	PCK	C	11	60	0	L, β , Ca
2	33	M	CPN	L	61	40	0	L, β
3	37	M	CGN	C	48	45	1.2	0
4	41	F	CPN	C	14	77	0	0
5	30	M	NUD	C	40	45	0	β
6	38	F	CGN	L	55	72	0	β
7	41	M	CGN	C	24	60	— ^d	β
8	51	F	PCK	C	47	47	0	L
median	38				44	54		
range	30–51				11–61	40–77		

^a PCK = Polycystic kidney disease, CGN = Chronic glomerulonephritis, CPN = Chronic pyelonephritis, NUD = Unknown; ^b L = Living donor, C = cadaveric donor; ^c L = Loop diuretic, β = β_1 blocking agent, Ca = calcium blocker; ^d Low grade, not quantified

larger groups of patients, in whom a close clinical follow-up was also performed.

Subjects and methods

Subjects

According to the protocol established for this study in 1983, baseline biopsies should be taken from all kidneys transplanted to Type 1 diabetic patients, and to selected control patients. Repeat biopsies should be performed after 6 and 24 months. The present series is a group of 16 consecutive diabetic patients in whom biopsies were taken at transplantation and at 24–36 months follow-up. Clinical data are given in Table 1. Patients were accepted for this series only if their glomerular filtration rate (GFR) exceeded 30 ml · min⁻¹ at the time of the last biopsy. Control subjects were selected preferentially as recipients of one kidney from a cadaveric donor, when the other kidney was transplanted to a diabetic patient. Repeat biopsies were obtained from eight control subjects (Table 2). Only two of the

baseline pairs underwent follow-up biopsies (diabetic patient 6 – control subject 3, and diabetic patient 16 – control subject 8). Reasons for drop-out were graft function loss or – insufficiency, intercurrent disease or the patient's decision not to participate. The study was approved by the local Ethics Committee.

Clinical programme and laboratory methods

The immunosuppressive protocol included cyclosporine A in all patients except diabetic patient 15 who received an HLA-identical graft and was treated with only azathioprine and prednisolone. Blood concentration of cyclosporine was monitored using a radioimmunoassay method with a polyclonal antibody. Trough levels were similar in diabetic patients and control subjects, 331 ± 188 vs 336 ± 106 (mean ± SD) µg/l at the time of the 2–3 year biopsy.

Following transplantation, 17 diabetic patients injected insulin twice per day, most often as a combination of rapid and medium long-acting insulin, six patients injected three or four times a day. During follow-up one patient changed from two injections to the

Table 3. Suspected risk factors in Type 1 (insulin-dependent) diabetic patients who underwent kidney transplant biopsies at 2–3 years post-transplant

Patient	Mean HbA _{1c}		Protein intake (g · kg body weight ⁻¹ · 24 h ⁻¹)	HLA DR type recipient/ donor	Δ BMT ^a (nm)	Δ V _v (mes/glom) ^a
	%	no of observations				
1	9.4	9	1.1	4/4,7	210	0.01
2	9.8	15	1.8	3/3	116	0.02
3	10.9	14	2.0	3,4/4,7	44	0.04
4	8.8	16	1.0	3,7/1,7	26	0
5	8.5	6	2.1	3,8/1,3	-11	0.03
6	10.7	21	2.4	3,4/5,8	46	0.07
7	9.4	14	1.9	3,4/3,4	133	0.04
8	10.0	5	1.1	1,4/2,4	13	0.07
9	9.0	5	1.9	4,6/2,6	56	0.10
10	8.5	5		3,8/5,6	53	0.01
11	10.5	14	0.9	3,4/4,6	54	0.06
12	9.1	4	1.8	4/4	81	0.08
13	11.2	7	1.2	3,4/7	13	-0.01
14	9.6	16	0.7	3/3,7	41	0.05
15	9.4	8		3/3	13	0
16	9.5	16	1.2	4/2,4	-15	0.01
median	9.5	12			45	0.035
range	8.5–11.2	4–21			-15–210	-0.01–0.10

^a corrected to 24 monthsBMT = basement membrane thickness; V_v (mes/glom) = volume fraction of mesangium in the glomerulus

subcutaneous insulin pump, and six patients from two or three to four daily injections. Metabolic control was evaluated by multiple HbA_{1c} analysis, 4–21 times per patient (median 12 times), excluding the first 3 months, when erythropoiesis is very active. All determinations were made at the Department of Clinical Chemistry, Sahlgrenska Hospital, using ion exchange chromatography on Mono S (Pharmacia Fine Chemicals AB, Uppsala, Sweden). The reference interval in young adults being 3.7–5.1%. The frequency of determinations depended on whether the patients attended our out-patient clinic or were seen here only for scheduled follow-up at 6, 12, 18 and 24 months post-transplant (see Table 3).

Blood pressure was closely monitored in all the diabetic patients and control subjects. Hypertension was treated aggressively and diastolic values above 90 mm Hg in the supine position were not accepted unless orthostatic blood pressure fall (in some diabetic patients) prevented treatment. Antihypertensive drugs were used at the time of the last biopsy in all diabetic patients and all but two control subjects (Tables 1 and 2). Any angiotensin converting enzyme (ACE) inhibitor medication during follow-up is listed separately. None of the control subjects received such treatment.

Fig. 1. Semithin plastic section as used for the estimate of interstitial volume fraction. Three such sections were used per biopsy. The section shown is from the baseline biopsy from control subject 6, illustrating fibrosis in a biopsy from a living donor, with normal renal function tests. The magnification is 75 ×

Protein intake was estimated at 12 or 24 months in most diabetic patients by dietary history interview, covering habits over the past year and evaluated as previously described [11] (see Table 3).

Glomerular filtration rate (GFR) was determined at regular intervals, including all biopsy occasions, as the plasma concentration slope following a single bolus injection of ⁵¹CrEDTA [12]. Tables 1 and 2 show the individual values.

Urinary protein excretion was determined by the Tsuchiya method [13] on 24 h samples (Tables 1 and 2).

Biopsies

All biopsies were cortical wedge specimens, obtained in the operating room, the kidney surface being exposed for inspection. The baseline biopsies were taken about 30 min after revascularization. The tissue was mailed in 2.5% glutaraldehyde fixative in 0.1 mol/l cacodylate buffer, pH 7.4, to the laboratory in Århus, Denmark where preparation for light- and electron microscopy was completed, using paraffin and Vestopal as embedding media. Paraffin sections were used for estimation of glomerular volume and frequency of glomerular occlusion (results to be published).

Light microscopy. From the plastic embedded blocks semithin sections (1 μm) were cut and stained with toluidine blue for the identification and unbiased sampling of glomerular profiles for electron microscopy [14]. The sections cut immediately before the trimming around the sampled glomerular profile (Fig. 1) were used for estimation of interstitium as proportion of cortical tissue. This was done with a drawing tube attached to the microscope, enabling the projection of a point-grid to the visual fields. Points hitting the interstitium, defined as the space outside tubular epithelium, Bowman's capsule and large vessels (i.e., including capillaries and cells), and total number of points hitting the cortex (excluding renal corpuscles) were counted to provide the volume fraction of interstitium per cortex (minus renal corpuscles): V_v (interst/cortex). The counting was done by one person, unaware of the source of the biopsy, at 142 × magnification with a point distance corresponding to 105 μm.

Electron microscopy. The three glomerular profiles were photographed at magnification of 2400 × to produce photomontages of

Fig. 2. Glomerular basement membrane thickness in sequential biopsies from eight non-diabetic (left) and 16 diabetic patients (right). The group of diabetic patients shows an increase over the 2-year period ($p = 0.0011$), significantly different from the change in control subjects ($p = 0.023$)

Fig. 3. Estimates of mesangial volume fraction, i.e. the fraction of the glomerulus occupied by the solid mesangial space, made from biopsies from eight non-diabetic (left) and 16 diabetic (right) patients. The increase in the diabetic patients is significant ($p = 0.0006$), and is different from the change in the group of control subjects ($p = 0.009$)

the entire profiles. The montages were used for the estimation of mesangial regions as fraction of the total glomerulus, defined as the circumscribed, minimal convex polygon [15]. For this purpose a 1:8 point-grid was used with a distance between coarse points corresponding to 22 μm . The fine points were used for hits on mesangium. Another set of micrographs, with an independent position on the in-

dividual profiles, was photographed at 10000 $\times$ magnification. The set covered approximately 45% of the total profile and was used for the estimation of basement membrane thickness by the orthogonal intercept method [16], and volume fraction of matrix in the mesangium, $V_v(\text{matrix/mes})$, using an integral test system with different sets of points: 1:1:2 for glomerular tuft, mesangium and matrix, respectively. The distance between coarse points corresponded to 5 μm .

Statistical analysis

The change over time in follow-up biopsies was tested by paired t -tests. Differences between groups were tested by unpaired t on delta-values and correlations expressed by Spearman's correlation coefficient, r . Group values are presented in the tables with mean, or median, and SD or coefficient of variation (CV) = SD/mean.

Results

Although age of donor and the proportion of living to cadaveric donors differed between diabetic patients and control subjects the baseline biopsies showed no significant differences in the three parameters expressing diabetic glomerulopathy: basement membrane (BM) thickness was 328 nm (CV 0.12) for control subjects and 312 nm (CV 0.14) for diabetic patients. Mesangial volume fraction, $V_v(\text{mes/glom})$, was 0.20 (CV 0.22) vs 0.18 (0.15), and $V_v(\text{matrix/mes})$ 0.54 (CV 0.16) vs 0.49 (CV 0.18). Interstitial volume fraction, $V_v(\text{interst/cortex})$, in the areas of the kidney used for electron microscopy was of the same order in the two groups, and showed considerable variation between biopsies: 0.27 (CV 0.34) and 0.29 (CV 0.32), respectively.

Biopsies obtained after 6 months in a subset of patients showed no significant changes in the glomerulopathy parameters.

The duration from transplantation to the last biopsy spanned a period from 23 to 36 months, on average 25.8 months in diabetic patients (SD 3.8) and 28.6 months (SD 6.1) in control subjects. The structural parameters estimated in the last biopsies were 'normalized' to a 24-month standard, assuming a linear rate of development from baseline to end-point.

In the two year biopsies basement membrane thickness had not increased in the control group (2 ± 37 nm, mean $\pm$ SD), whereas the increase of 55 ± 58 nm in the diabetic group was statistically significant whether the entire group was considered ($p = 0.0019$), or patient 1 was omitted from the calculation ($p = 0.0011$). Individual data are shown in Table 3, and illustrated in Figure 2. Comparison of delta-values in diabetic patients vs control subjects was also statistically significant ($p = 0.023$).

The diabetic group also showed a significant increase in mesangial volume fraction by 0.04 ± 0.03 (mean $\pm$ SD), $p = 0.0006$, to a mean value for the group of 0.21 (CV 0.15) (Table 3 and Fig. 3). No significant change was observed in the control subjects (-0.01 ± 0.04). The difference between control subjects and diabetic patients was statistically significant ($p = 0.009$). The change in $V_v(\text{mes/glom})$ observed in individual diabetic patients showed no signifi-

Fig. 4. Volume fraction of interstitial tissue in cortex, excluding renal corpuscles, estimated in the areas used for quantitative electron microscopy. The eight non-diabetic patients are shown (left) and the diabetic patients (right). The increase over time was statistically significant only in the diabetic group ($p = 0.04$)

cant correlation to the change in basement membrane thickness (Table 3).

The volume fraction of matrix in the mesangium did not change significantly in either group, -0.03 ± 0.12 in control subjects and $+0.06 \pm 0.14$ in diabetic patients, and the difference in delta-values between groups was not statistically significant.

The interstitial volume fraction increased on average by 0.10 (SD 0.19) in the control group, and by 0.13 (SD 0.22) in the diabetic group (Fig. 4). The change reached statistical significance only in diabetic patients ($p = 0.04$). Current $V_V(\text{interst/cortex})$ at 2 years did not correlate with basement membrane thickness or $V_V(\text{mes/glom})$ in the diabetic group, whereas variation in $V_V(\text{interst/cortex})$ could account for 32% of the variation in $V_V(\text{mes/glom})$ in control subjects, ($r = 0.57$).

There was no correlation between structural findings and any of the clinical presumed risk factors recorded, HbA_{1c} , dietary protein intake or tissue type.

Discussion

The aim of this study was to follow the development of diabetic glomerulopathy in the initial phase after a renal transplantation to diabetic patients. Diabetic glomerulopathy is characterized by three structural parameters, basement membrane thickness, volume fraction of mesangium per glomerulus and volume fraction of matrix per mesangium. Each of these parameters is a continuous variable, showing a certain range in control subjects [17,

18], due to the combined effect of a true biological variation, and the errors in the estimate consequent to sampling and measurements. Therefore, to detect subtle changes in the early phase consideration of the precision of the parameters in question, as well as determination of the baseline values are important [19]. The inclusion of cadaveric donors in whom clinical information is sometimes sparse, broadens the range of baseline values [18].

Previous studies of glomerular structure in the native kidney from diabetic patients have shown an early increase in basement membrane thickness [19]. The expansion of mesangial regions is a very important aspect of diabetic glomerulopathy with respect to the late demise in glomerular function [20, 21]. This change however, is demonstrable only in fairly advanced stages in the native kidney [20, 22], whereas the present series of transplanted kidneys showed an increase in both parameters after only 2 years. The basement membrane thickening corresponded well with findings in native kidneys [19], albeit with a much larger variation within the group, as well as with those of the Minneapolis series of transplanted diabetic patients [7]. The renal transplant series published from Minneapolis did not show an increase in mesangial volume fraction in groups of diabetic patients with 1–7 years' duration [7, 10]. This conforms, however, with the fact that the estimates in diabetic patients after 2 years are still completely within the normal range, so that the positive outcome depended on the paired comparison. An earlier detection in the present series compared with the native kidneys could relate to the larger series and the use of three rather than two random profiles. Yet, a large variation remained between the three individual profiles, and the question remains, if the observed increase in mesangial volume fraction is in fact an expression of incipient diabetic glomerulopathy, or alternatively, the finding is a random outcome – irrespective of statistical significance – in a parameter with a low precision. Changes of similar magnitude were observed in the control group, where they seemed to represent a “regression towards the mean”. The lack of correlation between the increase in $V_V(\text{mes/glom})$ and basement membrane thickening in this series is noteworthy.

Another important point to take into account in studying changes in these parameters is that none of them is ‘specific’ for diabetic glomerulopathy, and the transplanted kidney is exposed to many factors that may influence glomerular structures, independent of the diabetic state. Changes in the large vessels may present a main problem, whether due to rejection phenomena, cyclosporine toxicity and effects of other drugs, or pre-existing nephrosclerosis in the transplanted kidney. A relative vascular insufficiency could influence glomerular structural integrity directly or by an interaction via an increase in the interstitial space. The more dramatic graft reactions were excluded in the present series, since only patients with GFR in excess of $30 \text{ ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$ body surface were accepted in the study. Even this limit, however, allows the inclusion of biopsies which were damaged at the outset or became so during follow-up.

The cyclosporine nephrotoxicity has rightly been a matter of great concern. Myers et al. [23] have described

hyalinization of arterioles and mesangial expansion as an effect of cyclosporine treatment in cardiac transplant recipients. This shows the necessity of having a control group of non-diabetic renal transplant recipients. The control group in our series, although treated with cyclosporine, did not show increase in mesangial volume fraction over the 2-year period. The dosage of cyclosporine taken by our patients, reflected as blood cyclosporine concentration was, however, much lower than in the cardiac transplanted patients. This is probably of great importance for the development of cyclosporine nephrotoxicity [24].

An increase in interstitial volume fraction has been described as one of the frequently encountered consequences of cyclosporine treatment [23, 25]. Our estimate concentrated upon the areas within the biopsies immediately adjacent to the glomeruli studied by electron microscopy in order to test a possible correlation with the structural quantities in those particular glomeruli. The estimates do not necessarily represent the overall fibrosis in the individual kidneys, since one characteristic of the interstitial changes is the patchy localization. In our control group a positive correlation was obtained between the $V_v(\text{interst/cortex})$ and the $V_v(\text{mes/glom})$ at 2 years. In our study we were careful to sample any glomerular profile, irrespective of its structural appearance, to get unbiased estimates valid for the population of open glomeruli. Only totally occluded glomeruli had to be excluded from the analysis, since the structural definitions are lost in such corpuscles, and quantitations are therefore not possible.

There is now accumulating evidence that the development of late diabetic complications is related to the degree of diabetic control [26, 27]. It may then seem surprising that HbA_{1c} values recorded during follow-up were not related to the basement membrane thickening. One possible explanation is that the range of HbA_{1c} values was high and rather narrow. An alternative explanation would be that the transplanted kidneys differ in their sensibility to hyperglycaemia. Although we could not identify such sensibility on the basis of HLA-tissue type, this remains a possibility. Mauer et al. [9] have suggested that intrinsic factors in the transplanted kidney may play a major role.

As for protein intake, the suggested harmful effect has not been clearly proven, although there is experimental evidence from diabetic rats [31] and clinical data suggesting a beneficial effect of protein restriction on the expression of the marker of early nephropathy, elevated urinary albumin excretion. In the transplanted kidney model no correlation was seen between protein intake and the development of diabetic glomerulopathy, in spite of the fact that protein intake ranged from very low to very high values. For reasons mentioned above, this does not, however, exclude the possibility of high protein intake as a risk factor in the long run.

The finding of a significant increase in basement membrane thickness also in the transplant model demonstrated that the pathogenic mechanisms of diabetic glomerulopathy are active also in this setting.

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