Glomerular ultrastructure in kidneys transplanted simultaneously with a segmental pancreas to patients with type 1 diabetes

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Abstract

Background. Pancreas transplantation has been reported to prevent development and progression of diabetic glomerulopathy.

Study design. Kidneys transplanted to type 1 diabetic patients were investigated for signs of diabetic glomerulopathy. Biopsies were obtained from 11 patients 2–4 years after simultaneous pancreas and kidney transplantation and from six patients 2–6 years after kidney transplantation alone. During follow-up, glycaemic control was monitored as glycated haemoglobin and, in the pancreas transplant patients, as i.v. glucose tolerance.

Results. Measures of glycaemic control were consistently normal in only two pancreas transplant patients. Four had mean k values < 1.0. In kidney biopsies from the pancreas transplant patients, thickness of the glomerular basement membrane was 395 (0.13) nm (mean, coefficient of variation), which is higher than normal (324 (0.13) nm, P=0.01) and not different from diabetic patients with kidney transplants alone, 418 (0.15) nm. The mean calculated annual increase in thickness did not differ between patients with and without a pancreas transplant, 26 (0.77) versus 29 (0.54) nm/year. Estimates of the mesangium and mesangial matrix were in the normal range in both groups while the interstitial volume fraction was increased and to a similar extent.

Conclusion. The increase in thickness of the glomerular basement membrane in kidneys transplanted simultaneously with a segmental pancreas is probably an expression of diabetic glomerulopathy caused by the modest impairment in glucose metabolism present in most patients.

Key words: Key words: basement membrane thickness; diabetic glomerulopathy; glycaemic control; pancreas transplantation

Introduction

Indications for pancreas transplantation to subjects with type 1 diabetes have been much debated [1–3]. Reported beneficial effects include improved quality of life [4], improved survival of patients [5], and reduced neuropathy [6]. Some of these effects are limited and others have been disputed as possibly being the effect of selection of patients. Prevention of early glomerulopathy in the transplanted kidney would therefore be an important contribution. Early reports by Bohman et al. with such message were based on a small biopsy series [7,8]. Recently an extended study has been published by the group, modifying their conclusion to say that a functioning pancreas transplant can prevent or reduce the various signs of diabetic nephropathy that eventually develop in diabetic patients with a kidney graft only [9]. The present investigation is another study of this subject.

Subjects and methods

Patients

Eleven Type 1 diabetic patients with end-stage renal failure who had received simultaneous pancreas and kidney transplants at Rikshospitalet, Oslo (n=7) or Sahlgrenska University Hospital, Göteborg (n=4) underwent follow-up biopsies of the kidney transplant 2–4 years later (PK group). All patients had received segmental pancreas grafts with Anastomoses to the iliac vessels. Demographic data on patients and details on kidney and endocrine pancreas function are presented in Table 1. The time from transplantation to biopsy was 30±9 months (mean±SD). Biopsies from six kidneys transplanted to type 1 diabetic patients in Oslo (K group) served as control material. Clinical data are presented in Table 2. In this group, time since transplantation was 39±19 months. All patients received cyclosporin A (CsA) as part of their immunosuppressive therapy.

Transplant function

Glomerular filtration rate (GFR) was measured as the plasma clearance of $^{51}$Cr EDTA following single bolus injection.
The biopsies were fixed in 2% glutaraldehyde in buffer and mailed in the fixative to the laboratory for embedding into Vestopal.

Electron microscopy. The thickness of the peripheral glomerular basement membrane (BMT) and volume fractions (Vv) of mesangium per glomerulus and matrix per mesangium were determined as expressions of diabetic glomerulopathy. Sampling of tissue for study was done according to a protocol that ensured unbiased independent sampling. From each biopsy in the present series 3 glomeruli were sampled and thin sections for electron-microscopy were prepared at two levels, 60 μm apart, with a random position within glomeruli [12].

The entire profiles were photographed at a low magnification to produce photomontages at 2360 × magnification. Volume fractions of mesangial regions and of the tuft, i.e. the capillaries plus mesangial area, were estimated using the circumscribed, minimal, convex polygon as reference space [13]. Fractions were estimated by point counting, using an 8:1 grid, the denser points being used for mesangial areas. The estimate of volume fraction of mesangium per glomerulus, Vv (mes/glom), was based on sum of points from all three levels in each biopsy. No biopsies were taken on clinical indication. Informed consent was obtained from the patients and the procedure was approved by the Regional Committees of Ethics. Biopsies from Oslo were core needle biopsies while those from Göteborg were surgical wedge biopsies handled as previously described [10,11]. The biopsies were fixed in 2% glutaraldehyde in buffer and mailed in the fixative to the laboratory for embedding into Vestopal.

Glycated haemoglobin was tested as HbA1c in Oslo, (Bio-Rad, Munich, normal value <8.0%), and as Hba1c in Göteborg, normal value <5.5%. Intravenous glucose tolerance tests were performed at least yearly. The disappearance rate of blood glucose was expressed as the k value, normal value >1.0.

Biopsy procedure

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Glomerulopathy in kidneys transplanted simultaneously with a pancreas cell membrane. On average 350 intercepts were classified per biopsy. Further, the volume fraction of matrix in mesangial regions was estimated by point counting, using a 2:1 grid.

Biopsies taken at the time of transplantation were available from three patients. In the remaining 14 cases the BMT in biopsies from 16 kidneys at the time of living donor transplantation, 324 nm, was used for estimation of change over time.

Light microscopy. From each biopsy semithin plastic sections from 3–5 different blocks of tissue were used for estimation of interstitial volume fraction. The sections at the level 20 μm from the baseline sections were studied in a light-microscope with a drawing tube, projecting a point grid onto the visual fields. The sections were measured, counting points hitting cortex, interstitium, glomeruli including Bowman’s capsule, and large vessels. The definition of interstitium was the entire space between tubular epithelium, glomeruli and larger vessels, i.e. including interstitial cells and capillaries. The interstitial space was expressed as fraction of cortex minus glomeruli and large vessels, i.e. the ‘tubular cortex’. The magnification was 292 × and the distance between points corresponded to 51 μm. The average total area measured per biopsy was 1.8 mm².

Statistics

If not otherwise stated values are mean ± SD or mean with coefficient of variation (CV) = SD/mean. The Mann-Whitney U non-parametric test was used for comparisons between groups. Linear correlations were studied by least-square regression.

Results

Results of the quantitative morphological study are presented in Table 3. BMT in biopsies from the PK patients was not different from values in the K patients, 418 (0.15) nm and higher than in baseline biopsies from living donor kidneys at the time of transplantation [10], 395 (0.13) nm versus 324 (0.13) (P = 0.01). With compensation for the small difference in follow-up time, the mean calculated annual increase in BMT did not differ, 26 (0.77) nm/year in PK patients versus 29 (0.54) in K patients. Individual BMT values are plotted in relation to follow-up time in Figure 1, which also includes data from our previous investigations of transplanted kidneys [10,11].

No correlation was seen between mean k values in i.v. glucose tolerance tests in the PK group and the rate of increase in BMT (r = 0.31, P = 0.31). Due to different methods for measurement of glycated haemoglobin in the two centres the correlation coefficient

Table 3. Structural findings in follow-up biopsies from kidneys transplanted to type 1 diabetic patients simultaneously with a pancreas, and from kidneys transplanted to type 1 diabetic patients without a pancreas. For comparison, findings in biopsies obtained from 16 kidney donors aged 38, 21–65 years (median, range) at the time of transplantation are shown

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Unless otherwise indicated values are mean (coefficient of variation, CV). There are no significant differences between the groups.
could not be calculated. However, the thickest BM was observed in the patient with clearly pathological mean HbA₁ as well as mean k value (Table 1 patient no. 6). Conversely, the only two patients with all individual k values and glycated haemoglobin normal (Table 1, patients nos. 8 and 10) had BMT definitely within the normal range.

Estimates of the mesangium and mesangial matrix were in the normal range and did not differ between the two groups. The interstitial volume fraction was increased in both groups and to a similar extent.

Discussion

Biopsies from renal allografts show a variety of histopathological changes, some existing already in the donor [15], others developing as a result of ischaemia, hypertension, rejection, and toxic effects of drugs. The expansion of the interstitium observed in kidneys transplanted alone or with a pancreas is certainly an expression of such mixed damage. A nephropathy score combining several structural variables, as used by the Stockholm group [9], will to some extent be unspecific. We will only consider the more specific measures, and separately.

Thickening of the glomerular basement membrane is the earliest sign of diabetic glomerulopathy [16] and has previously been reported to occur in kidneys transplanted to patients with diabetes [8,9,11,17,18]. Other factors that might affect BMT are rejection in the form of transplant glomerulopathy and membranous glomerulonephritis, which may appear de novo in kidney transplants [19]. Both types of changes will cause gross proteinuria, which was not found in our patients, and will be conspicuous on histopathological examination. Minor early forms might exist but should not be related to diabetes. No increase in BMT was seen in kidneys transplanted to non-diabetic patients [8,11]. The immunosuppressive therapy, notably CsA, does not affect BMT [20]. Therefore the most likely interpretation of the demonstrated increase in BMT is that it is a manifestation of early diabetic glomerulopathy.

The fact that baseline biopsies were not available in the majority of cases studied here is a definite drawback. However, the BMT values used for calculation of change over time were from kidneys handled and investigated by the same method as used for the follow-up biopsies. Similar baseline values were found in our investigation of cadaveric kidneys [10] and have been reported by others [17].

The first study of BMT in kidneys transplanted concomitantly with a pancreas is that by Bohman et al. in Stockholm [7,8]. BMT was found to be normal, in contrast to biopsies from diabetic patients without a pancreas transplant, and the pancreas was considered to have had a protective effect by improving glycaemic control. However, only six biopsies from three patients were obtained 2 years or more after transplantation, when a BMT increase could be expected. All these showed BMT within the normal range. Recently a larger series has been published by the same group [9]. Biopsies in the kidney transplant patients had significantly increased mean BMT. In contrast, of 12 biopsies obtained in the pancreas transplant group later than 2.5 years after transplantation, 11 had BMT values below normal the mean ± 2SD. However, the mean value was 383 ± 47 nm, close to our patients' 395 ± 53 nm, which is significantly increased compared with our normal controls although within 2 SD. Furthermore, when repeat biopsies had been obtained in the Stockholm series, a significant increase in BMT was recorded in pancreas transplant patients too. The annual increase was 19 nm versus 42 nm in patients with kidney transplants only, i.e. a clearly significant difference. The outcome in the pancreas transplant group was thus similar to our result, whereas kidney transplant patients developed increased BMT more rapidly than in our series. This might be related to less efficient insulin treatment in the Stockholm series, which was partly historical. We find it reasonable that the effects of improving glycaemic control by pancreas transplantation should be evaluated in comparison with modern diabetes treatment, i.e. the patients should take multiple insulin injections, use self-monitoring of blood glucose, and be aware of their HbA₁c values.

Another study in this field is that by Fioretto et al. reporting no further increase in GBM width in native kidneys within 5 years following pancreas transplantation. Their baseline values were high, however, and with high variability among patients [21].

In a previous series we not only found an increase in BMT compared with baseline biopsies; the mesangial volume fraction was also found to have increased [11]. This increase depended entirely on the paired comparison because the follow-up values were still within the normal range. The more elaborate method used in the present study similarly showed normal volume fractions in kidneys transplanted to diabetic patients, whether simultaneously with a pancreas or solitary.

Bilous et al. [18] investigated kidneys transplanted to diabetic patients before and after the addition of a pancreas allograft. During a mean of 4.4 years with the pancreas graft no significant further increase occurred in any of the structural glomerular variables measured. However, there was a trend for a further increase in BMT (P = 0.07). The mesangial volume fraction values reported for pancreas transplant patients were similar to those obtained in our study, but whereas we found no difference between patients with or without a pancreas transplant, the previously investigated biopsies from diabetic kidney transplant patients, which served as controls in the study by Bilous et al., showed significantly more mesangial volume as fraction of glomerular volume, interpreted as most probably an expression of diabetic glomerulopathy. However, these results were based on measurements with a low sampling fraction at high magnification, making the estimate rather uncertain, since the sampling error may be considerable. The same methodological considerations may apply to the
recent Stockholm study [9] which also reported more increased volume fraction of mesangium in recipients of kidney grafts only.

With prolonged follow-up in our patients, glomerulopathy is likely to progress, and probably more so in patients with more disturbed metabolism, i.e. with kidney transplants only. Barbosa et al. in their randomized prospective 5-year post-transplant renal allograft biopsy study noted a significant difference in the accumulation of mesangial matrix between patients with multiple or continuous insulin administration and those with only one or two daily injections [17]. Differences in structural measures between patients with and without pancreas transplants, which were not seen in our study, can be expected to develop. The point we want to make is that recipients of pancreas grafts are not exempt, unless metabolic control is truly normalized. BMT remained normal in the two patients with perfect metabolic control, one of which had the longest observation time following transplantation in this series.

It is a fact that normoglycaemia is often not attained by pancreas transplantation. Insulin independence is the rule, but glucose metabolism is not completely normalized in the majority of patients, neither in this series nor in the general experience [18, 22–24]. The problem may be more pronounced when a segmental rather than a whole pancreas is transplanted, but exists with both techniques. In the Stockholm series, mean fasting blood glucose, mean HbA1c and mean IVGTk value were all within, but close to, the upper normal limit, which indicates that individual observations were sometimes elevated. This modest impairment of glucose tolerance seems to be harmful to the transplanted kidney — and may be so to other organs. In fact the findings in the pancreas-transplant patients may be analogous to the experience in patients with undiagnosed type 2 diabetes who may covertly develop diabetic glomerulopathy [25, 26].

Our study indicates that there may be untoward consequences of deficient glucose control following pancreas transplantation, and argues for attention and correction.

References