Stable Renal Function After Islet Transplantation: Importance of Patient Selection and Aggressive Clinical Management

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Background. Proteinuria development and decrease in glomerular filtration rate (GFR) have been observed after successful islet transplantation. The aim of this study was to determine clinical, laboratory, and immunosuppressant-related factors associated with kidney dysfunction in islet transplant recipients.

Methods. A retrospective cohort study was conducted in 35 subjects submitted to pancreatic islet transplantation for treatment of unstable type 1 diabetes mellitus. Demographic, anthropometrical, and laboratory data, as well as immunosuppressive and antihypertensive therapy were recorded. Kidney function was assessed by albuminuria and estimated GFR (eGFR), calculated by modification of diet in renal disease formula.

Results. Age was the only independent risk factor for low eGFR (<60 mL/min/1.73 m²) (odds ratio [OR] = 1.78 [1.22–2.61]). Low-density lipoprotein cholesterol (OR = 2.90 [1.37–6.12]) and previous microalbuminuria (OR = 6.42 [1.42–29.11]) were risk factors for transient macroalbuminuria. Interestingly, tacrolimus was a protective factor for macroalbuminuria (OR = 0.12 [0.06–0.26]). Six of 30 (20%) normoalbuminuric subjects at baseline progressed to microalbuminuria. No subject developed sustained macroalbuminuria. Surprisingly, overall eGFR remained stable during follow-up (before transplant: 74.0 ± 2.0; during immunosuppressive therapy: 75.4 ± 2.8; and after withdrawal: 76.3 ± 5.3 mL/min/1.73 m²; P > 0.05). Even subjects with low eGFR and microalbuminuria at baseline (n = 10) maintained stable values posttransplantation (61.13 ± 3.25 mL/min/1.73 m² vs. 63.32 ± 4.36 mL/min/1.73 m², P = 0.500).

Conclusions. Kidney function remained stable after islet transplantation alone. The unchanged kidney function found in this sample may be attributed to healthier kidney status at baseline and possibly to prompt treatment of modifiable risk factors. Aggressive treatment of risk factors for nephropathy, such as blood pressure, low-density lipoprotein cholesterol, and careful tacrolimus levels monitoring, should be part of islet transplant recipient care.

Keywords: Islet transplantation, Kidney function, Albuminuria, Lipids, Blood pressure.

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Intensive treatment of type 1 diabetes mellitus (T1DM) results in lower hyperglycemia-related micro- and macrovascular chronic complications (1, 2). However, a better metabolic control can only be achieved at the expense of an increase in the number and severity of hypoglycemic episodes (1). There is a subset of subjects with unstable T1DM, who are especially prone to develop severe hypoglycemic episodes because of unawareness of its alert symptoms (3). In these cases, transplantation of allogeneic islets of Langerehans results in stabilization of blood glucose levels with the resolution of severe hypoglycemia and subsequent improvement in their metabolic control and quality of life (4, 5).
Emerging evidence of proteinuria development (6), decrease in glomerular filtration rate (GFR) (7–9), and, in some cases, progression to end-stage renal disease (ESRD) (8) have been observed after successful islet transplantation. Stable kidney function after islet transplantation has also been recently described under a sirolimus-sparing immunosuppressive protocol (10). Diabetic nephropathy (DN) being a major cause of morbidity and mortality in T1DM patients (11), concerns about the patient’s selection based on pretransplant kidney function status (9) and the combinations of immunosuppressive agents (10) have been raised.

The aim of this study was to determine the clinical, laboratory, and immunosuppressant-related factors associated with renal dysfunction in subjects with T1DM after allogeneic islet transplantation alone (ITIA).

**PATIENTS AND METHODS**

**Subjects**

A retrospective cohort study was conducted in 36 subjects with T1DM and hypoglycemia unawareness that have been followed in a single center, before (median of 20 months [minimum–maximum: 0–111]) and after (49 months [7–72]) allogeneic ITA between 2000 and 2007. One subject was excluded because of early withdrawal (102 days after transplant) of immunosuppressive therapy owing to an adverse event (aspergillosis) and subsequent graft failure. Follow-up included 33 subjects at 12 months, 29 at 18 months, 23 at 24 months, 19 at 30 months, 17 at 36 months, 16 at 42 months, and 15 after 48 months. Subjects with baseline renal dysfunction (serum creatinine ≥1.6 mg/dL or albuminuria >300 mg/24 hr) were considered not eligible for islet transplantation.

**Transplant Related Procedures**

The pancreatic islet isolation, infusion, and immediate posttransplant management were performed as described previously (4). The mean number of infusions per patient was 2.0±0.9 (single n=12; two n=13; three n=8; and four infusions n=2, respectively). The maintenance immunosuppressive regimen was based on tacrolimus (Prograf, Astellas-Pharma US, Inc., Deerfield, IL; target trough level 4–6 ng/mL) and sirolimus (Ra-pamune, Wyeth Pharmaceuticals, Inc., Madison, NJ; target trough level 10–15 ng/mL for 3 months, 8–12 ng/mL thereafter).

Twelve subjects were converted from tacrolimus or sirolimus to mycophenolate mofetil (MMF; CellCept, Roche, Nutley, NJ) or mycophenolate sodium (Myfortic, Novartis, East Hanover, NJ), targeting maximum tolerable dosage (maximum of 2000 mg and 1440 mg, respectively). The reason for conversion in five subjects was tacrolimus-related side effects (nephrotoxicity [n=2], eczema [n=1], depression [n=1], and neurotoxicity [n=1]), and in two was due to sirolimus side effects (mouth ulcer [n=1] and migraine [n=1]). The other five subjects received alemtuzumab (Campath-1H, Genzyme, Cambridge, MA) induction and were converted 3 months after islet infusion from tacrolimus to MMF or mycophenolate sodium as per protocol, except for one subject in whom sirolimus was substituted for tacrolimus because of gastrointestinal intolerance.

Six of eight subjects who received comanitator bone marrow cell (CD34+ enriched) infusion from the same islet donor discontinued immunosuppressive drugs per protocol at 1 year after the transplant (12). Other variations within protocols were related to the induction agents: five-dose course of daclizumab (1 mg/kg biweekly; Zenapax, Roche, Nutley, NJ; n=30); or alemtuzumab (20 mg intravenously, two doses before the transplant, n=5); and infliximab (5–10 mg/kg 2 hr before islet infusion, single dose; Remi-cade, Centocor, Malvera, PA; n=11) or etanercept (50 mg intravenously 1 hr before islet infusion and 25 mg twice a week for 2 weeks; Enbrel, Amgen, Thousand Oaks, CA; n=11). All subjects received prophylaxis for cytomegalovirus (13) and *Pneumocystis carinii pneumonia*.

In addition to the interventions directly related to the transplant, all recipients received aggressive management of risk factors for nephropathy, which included treatment of hypertension and dyslipidemia, and tight control of tacrolimus trough levels, because this immunosuppressive agent is known to cause nephrotoxicity. The aggressive management was defined according to the American Diabetes Association guidelines for preventing/treating DM chronic complications (14) and included treating hypertension with a target blood pressure (BP) less than 130/80 mm Hg, using preferentially angiotensin-converting enzyme inhibitors (ACEi) and angio-tensin receptor blockers (ARB) and treating dyslipidemia with a target low-density lipoprotein (LDL) cholesterol level less than 100 mg/dL, using statins. Monitoring of tacrolimus levels was also performed with an intensive approach (two to three times a week in the first month, once a week in the second month, twice a month in the third month, and every 3 month thereafter), guaranteeing that the subjects really receive a low-dose tacrolimus protocol (target trough level 4–6 ng/mL).

All protocol procedures were approved by the University of Miami health research ethics board and appropriate informed consent was obtained from each subject.

**Clinical Data Assessment**

Clinical variables (age, gender, ethnicity, diabetes duration, body mass index [BMI], and systolic and diastolic BP), antihypertensive therapy (ACEi and ARB), and immunosuppressive medication data were recorded. Diabetic retinopathy (DR) was classified as absent, nonproliferative, or proliferative based on ophthalmologic report. Peripheral neuropathy was diagnosed by clinical symptoms and compatible physical examination and cardiovascular disease by history and stress test.

**Laboratorial Analysis**

Kidney function was evaluated by serum creatinine (Jaffé method, Roche Diagnostics, Roche Cobas-Mira, inter- and intra-assay variations: 1.4% and 2.1%; four [1–15] and 17 [4–39] measurements/patient available pre- and posttransplant, respectively) and urinary albumin excretion rate (UAER; immunoturbidimetry, Beckman-Synchron/CX9, Ramsey, MN; three [1–10] and 15 [3–31] measurements/patient available pre- and posttransplant, respectively) was measured in 24-hr urine collections. The subjects were classified as normoalbuminurics (UAER <30 mg/24 hr), microalbuminurics (30–299 mg/24 hr), or macroalbuminurics (≥300 mg/24 hr) based on two of three pretransplant measurements, and the same criterion was used for kidney status definition during follow-up. Albuminuria progression was considered when the elevation, based on this criterion, has persisted until the end of the
study. If the increment in albuminuria was followed by a regression to the previous stage, the elevation was considered transient. The estimated GFR (eGFR) was calculated by the modification of diet in renal disease formula (15): 186 × (serum creatinine) × 0.139 × age −0.203 × 0.742 if female × 1.210 if Afro-American. Subjects were classified following the National Kidney Foundation guidelines for chronic kidney disease (CKD) (stage 1: >90, stage 2: 60–89, stage 3: 30–59, stage 4: 15–29, and stage 5: <15 mL/min/1.73 m²) (16). Glycemic profiles were evaluated by fasting plasma glucose (hexokinase method) and A1c (high performance liquid chromatography, Variant II Hemoglobin Testing System, BioRad, Richmond, CA, inter- and intra-assay variation: 1.7% and <2.0%, normal values 4.2%–6.1%). Fasting lipids (total cholesterol, high-density lipoprotein cholesterol, and triglycerides) were measured by the enzymatic method, and LDL-cholesterol was determined by the Friedewald equation (17).

### Statistical Analysis

Statistical analysis and graphics were performed using Excel for Windows, SAS 9.1 (SAS Institute Inc., Cary, NC) and SSPS 15.0 software. Results of continuous variables were expressed as means ± SEM (SD in Table 1) except for albuminuria and triglycerides (median [minimum–maximum]) and categorical as number of cases (%). To assess changes in binary outcomes (abnormal eGFR [CKD stage 3] or albuminuria [UAER ≥300 mg/24 hr] values anytime posttransplant), multiple logistic regressions using generalized estimating equations were used, in a total of 734 observations from the 35 subjects. Stepwise model building techniques were used initially considering as covariates the factors found to be associated with each variable in bivariate analysis. Covariates considered in these models as potential explanatory or confounding factors include the following: time interval (pre- and posttransplant time-points), age, diabetes duration, gender, BMI, systolic and diastolic BP, A1c, lipids (LDL, high-density lipoprotein and triglycerides levels), use of ACEi, ARB, sirolimus, and tacrolimus at each time point, and pretransplant kidney function (CKD stage 1 or 2 and microalbuminuria). To verify if kidney function parameters (eGFR and albuminuria) have varied posttransplantation, we used the pretransplant values as controls. The comparison between postversus pretransplant time points was performed by repeated measures analysis. For continuous outcomes where normality assumptions were appropriate, linear mixed models regression were used. Appropriate interaction terms were included in repeated measures regression models and were assessed for statistical significance to compare the slopes between regression lines. P values of less than 0.05 (two-tailed) were considered to be statistically significant.

### RESULTS

Main clinical and laboratory characteristics of the 35 subjects enrolled are described in Table 1. Patients’ age at transplant was 42.5 ± 8.6 years and the duration of diabetes was 26.5 ± 13.4 years. All subjects were white and 13 (37%) were males. BP means were 119.5 ± 12.5 mm Hg (n = 9; stage 2: n = 9; stage 3: n = 9). A1c was normal in all patients after transplant (pre: 8.6 ± 0.9%, P < 0.01). A mild but statistically significant increment in LDL-cholesterol levels after transplantation was found (pre: 35.4 ± 6.6 mg/dL, P = 0.008), despite higher statins use (20% vs. 83%, P < 0.001). The BP remained stable during the follow-up (pre: 113.9 ± 16.6 mm Hg vs. post: 119.3 ± 119.5 ± 12.5 mm Hg, nine subjects (26%) had a history of hypertension, and 13 (37%) were on ACEi or ARB at baseline. Chronic diabetes complications at baseline included DR in 60% of the subjects (n = 21; 11 proliferative). Nine subjects (26%) had peripheral neuropathy and none had cardiovascular disease. Thirty subjects (86%) were normoalbuminuric (UAER: 5.6 [0–26.3] mg/24 hr) and five (14%) microalbuminuric (UAER: 50.6 [31.7–104.0] mg/24 hr). Baseline eGFR was 76.2 ± 22.5 mL/min/1.73 m², six subjects (17%) had a moderate decrease in eGFR (CKD stage 3) because baseline and the other 29 had preserved kidney function (CKD stage 1: n = 9; stage 2: n = 20).

A1c was normal in all patients after transplant (pre: 7.45 ± 0.11 vs. post: 6.09 ± 0.9%, P < 0.001). A mild but statistically significant increment in LDL-cholesterol levels after transplantation was found (pre: 101.6 ± 2.3 mg/dL, P = 0.008), despite higher statins use (20% vs. 83%, P < 0.001). The BP remained stable during the follow-up (pre: 121.7 ± 2.3 vs. post: 119.3 ± 16.6 mm Hg, P = 0.005), mainly at the expense of increasing the number of antihypertensive medications per patient (0.34 ± 0.48 vs. 0.71 ± 0.86, P = 0.002) and ACEi/ARBs use (37% vs. 66%, P = 0.029).

### Variables Associated With Kidney Function After Islet Transplantation

Variables associated with decreased eGFR or microalbuminuria at any time point during posttransplant follow-up are summarized in Table 2 (bivariate analysis). Age and pretransplant values expressed as the mean ± SD, median (minimum–maximum) or number of subjects (%).

#### TABLE 1 - Clinical and laboratory characteristics of the subjects (n = 35) at baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>42.5 ± 8.6</td>
</tr>
<tr>
<td>Diabetes duration (yr)</td>
<td>26.5 ± 13.4</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>13 (37)</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>35 (100)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.0 ± 2.3</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>9 (26)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>119.5 ± 12.5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>69.3 ± 6.8</td>
</tr>
<tr>
<td>ACEi/ARB, n (%)</td>
<td>13 (37)</td>
</tr>
<tr>
<td>A1c (%)</td>
<td>7.4 ± 1.1</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>181.9 ± 43.5</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>102.6 ± 35.4</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>66.6 ± 16.8</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>55.58 ± 116.0</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>76.2 ± 22.5</td>
</tr>
<tr>
<td>Albuminuria (mg/24 hr)</td>
<td>6.9 (0–104.0)</td>
</tr>
<tr>
<td>Nephropathy (normo-/ microalbuminuria), n (%)</td>
<td>30 (86)/5 (14)</td>
</tr>
<tr>
<td>Retinopathy (normal/ nonproliferative/proliferative), n (%)</td>
<td>14 (40)/10 (29)/11 (31)</td>
</tr>
<tr>
<td>Peripheral neuropathy, n (%)</td>
<td>9 (26)</td>
</tr>
<tr>
<td>Cardiovascular disease, n (%)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

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plant microalbuminuria were associated with lower eGFR. In contrast, better pretransplant kidney function was found to be a protective factor (odds ratio [OR] = 0.22, 95% CI = 0.05–0.97, *P* = 0.050). An association trend between low eGFR and use of ARBs (OR = 2.80, 95% CI = 0.99–7.94, *P* = 0.052) or ACEi (OR = 2.45, 95% CI = 0.98–6.14, *P* = 0.056) was observed, as expected because of their mechanism of action. No other clinical or laboratory variables, neither immunosuppressive agent in use (Table 2) nor combination (tacrolimus/sirolimus: 74.4±2.7 mL/min/m² vs. MMF/tacrolimus or sirolimus: 69.0±3.3 mL/min/m², *P* = 0.070), showed statistically significant association with low eGFR. Multiple regression analysis indicated that only age remained significantly associated with low eGFR (OR = 1.78, 95% CI = 1.22–2.61, *P* = 0.002) and all other variables, including pretransplant kidney function markers, were not statistically significant.

In the case of albuminuria, a higher BMI, LDL-cholesterol, and pretransplant microalbuminuria resulted as risk factors for macroalbuminuria. Interestingly, the current use of tacrolimus was associated with an 80% reduction in macroalbuminuria occurrence risk (OR = 0.20, 95% CI = 0.09–0.41, *P* = 0.001). A borderline association between microalbuminuria and higher number of islet transplants (three or four infusions) was found (OR = 3.87, 95% CI = 0.99–15.12, *P* = 0.051). However, after adjustments in multiple regression, only LDL-cholesterol (OR = 2.90, 95% CI = 1.37–6.12, *P* = 0.005) and previous microalbuminuria (OR = 6.42, 95% CI = 1.42–29.11, *P* = 0.001) remained as risk factors for macroalbuminuria. The protective effect of tacrolimus maintained statistical significance in multiple regression analysis (OR = 0.12, 95% CI = 0.06–0.26, *P* = 0.001). A deleterious effect of sirolimus on kidney function was demonstrated in the multiple regression analysis for microalbuminuria (OR = 3.68, 95% CI = 1.17–10.70, *P* = 0.020) but not for macroalbuminuria.

### Kidney Function After Islet Transplant

Unexpectedly, the overall eGFR remained stable during all pre- and posttransplant follow-up (line equations pre-: y = 76.512 ± 0.034x vs. posttransplant: y = 76.109 ± 0.015x, *P* = 0.400 for slopes comparisons) (Fig. 1A). To analyze the eGFR in detail and evaluate possible acute nephrotoxic effects of immunosuppressive drugs in the early posttransplant period (when sirolimus target levels are higher), pretransplant eGFR means were compared with those of posttransplant time points (3-month intervals) and no significant differences were found (pre: 70.5±6.3; 0–3: 72.0±3.3; 3–6: 78.0±3.3; 6–12: 72.7±3.1; 12–18: 74.2±3.3; 18–24: 72.9±3.5; 24–30: 76.5±3.8; 30–36: 82.1±3.8; 36–42: 80.3±4.1; 42–48: 77.1±3.9 and >48 months: 76.4±3.5 mL/min/1.73 m²; *P* > 0.05 for all comparisons).

To verify the specific effect of immunosuppressive therapy on eGFR, we performed a separate analyzes only with subjects off immunosuppressive drugs at the most recent follow-up (due to graft failure, n = 3; adverse events, n = 1; per protocol, n = 5 or patient option, n = 1; total n = 10, 25 ± 17 months after first transplant) and compared the eGFR means before immunosuppressive treatment initiation (pretransplant values), during and after its withdrawal. There were no statistically significant differences among the three periods (before: 74.0±2.0 mL/min/1.73 m², during: 75.4±2.8 mL/min/1.73 m², and after withdrawal: 76.3±5.3 mL/min/1.73 m², *P* > 0.05).

### Table 2. Variables associated with CKD stage 3 and/or macroalbuminuria in any time point after islet transplant

<table>
<thead>
<tr>
<th>Variable</th>
<th>CKD stage 3, OR (95% CI)</th>
<th><em>P</em></th>
<th>Macroalbuminuria, OR (95% CI)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (each 10 yr)</td>
<td>2.18 (1.19–3.98)</td>
<td>0.011</td>
<td>1.20 (0.59–2.45)</td>
<td>0.617</td>
</tr>
<tr>
<td>Diabetes duration (yr)</td>
<td>1.04 (0.99–1.09)</td>
<td>0.088</td>
<td>1.03 (0.98–1.08)</td>
<td>0.222</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.44 (0.09–2.24)</td>
<td>0.324</td>
<td>0.30 (0.04–2.10)</td>
<td>0.225</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1.00 (0.93–1.08)</td>
<td>0.982</td>
<td>1.28 (1.00–1.64)</td>
<td>0.046</td>
</tr>
<tr>
<td>Systolic BP ≥130 mm Hg</td>
<td>1.74 (0.83–3.65)</td>
<td>0.144</td>
<td>1.37 (0.31–6.08)</td>
<td>0.679</td>
</tr>
<tr>
<td>Diastolic BP ≥80 mm Hg</td>
<td>0.82 (0.50–1.34)</td>
<td>0.437</td>
<td>0.63 (0.17–2.28)</td>
<td>0.477</td>
</tr>
<tr>
<td>A1c ≥6%</td>
<td>0.76 (0.46–1.26)</td>
<td>0.294</td>
<td>0.51 (0.14–1.86)</td>
<td>0.304</td>
</tr>
<tr>
<td>LDL-cholesterol ≥100 mg/dL</td>
<td>0.91 (0.66–1.24)</td>
<td>0.546</td>
<td>2.63 (1.11–6.25)</td>
<td>0.028</td>
</tr>
<tr>
<td>Low HDL cholesterol</td>
<td>0.96 (0.40–2.32)</td>
<td>0.936</td>
<td>0.70 (0.22–2.17)</td>
<td>0.535</td>
</tr>
<tr>
<td>Triglycerides &gt;150 mg/dL</td>
<td>1.52 (0.96–2.45)</td>
<td>0.073</td>
<td>0.24 (0.04–1.69)</td>
<td>0.153</td>
</tr>
<tr>
<td>CKD stage 1 or 2³</td>
<td>0.22 (0.05–0.97)</td>
<td>0.050</td>
<td>0.70 (0.12–3.94)</td>
<td>0.681</td>
</tr>
<tr>
<td>Microalbuminuria³</td>
<td>13.12 (3.53–48.80)</td>
<td>&lt;0.001</td>
<td>5.44 (1.58–18.70)</td>
<td>0.007</td>
</tr>
<tr>
<td>ACEi</td>
<td>2.45 (0.98–6.14)</td>
<td>0.056</td>
<td>2.38 (0.89–7.82)</td>
<td>0.231</td>
</tr>
<tr>
<td>ARB</td>
<td>2.80 (0.99–7.94)</td>
<td>0.052</td>
<td>3.00 (0.79–11.40)</td>
<td>0.107</td>
</tr>
<tr>
<td>Statins</td>
<td>1.76 (0.96–3.21)</td>
<td>0.067</td>
<td>3.25 (0.90–11.75)</td>
<td>0.071</td>
</tr>
<tr>
<td>3 or 4 islets transplants</td>
<td>2.2 (0.83–6.24)</td>
<td>0.110</td>
<td>3.87 (0.99–15.12)</td>
<td>0.051</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.69 (0.43–1.09)</td>
<td>0.111</td>
<td>0.20 (0.09–0.41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>1.13 (0.61–2.11)</td>
<td>0.704</td>
<td>3.09 (0.37–24.35)</td>
<td>0.289</td>
</tr>
</tbody>
</table>

Data expressed as OR and 95% CI.

³ HDL cholesterol <40 mg/dL for males or <50 mg/dL for females.

² Based on pretransplant classification.

CKD, chronic kidney disease; OR, odds ratio; CI, confidence interval; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BP, blood pressure; ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers.
Because pretransplant kidney function could influence the outcomes posttransplantation, subjects were stratified based on pretransplant kidney status. Those with low eGFR (<60 mL/min/1.73 m²) or microalbuminuria (n=10) showed lower but stable eGFR, when compared with patients with normal kidney function pretransplant (pre: 61.13±3.25 vs. 78.81±2.46; and post: 63.32±4.36 vs. 81.34±2.73 mL/min/1.73 m², respectively, P<0.05 for comparisons between normal and abnormal baseline kidney function). Both groups maintained stable eGFR after transplantation (P>0.05) (Fig. 1C). The frequency of CKD stage 3 in the final evaluation was not statically higher than at baseline (9 [26%] vs. 6 [17%], P=0.532). None of the patients progressed to CKD stage 4 or 5 in this sample.

Urinary albumin excretion values are depicted in Figure 1(B). A transitory increment in albuminuria seemed to occur in the period between 24 and 48 months after first transplant (0–24 months: 11.7 [0–632.5]; 24–48: 25.6 [0–1112.3]; and >48 months: 13.4 [0–387.4] mg/24 hr, P=0.065). When the subjects were analyzed based on the baseline DN classification, from the 30 normoalbuminuric subjects, 24 (80%) remained in normal range at the most recent evaluation, 21 (70%) within normoalbuminuric range during all period and three (10%) with a transient increase in albuminuria, reaching the microalbuminuric range and then regressing to normal levels. Six subjects (20%) experienced sustained increase in albuminuria (two subjects reaching transient macroalbuminuria) and have progressed to microalbuminuria. One of five patients with microalbuminuria at baseline showed transient macroalbuminuria, although all of them were in the microalbuminuric range at final evaluation and sustained macroalbuminuria was not detected. Higher posttransplant albuminuria levels, although not statistically significant, were observed in subjects with poorer baseline kidney function (eGFR <60 mL/min/1.73 m²) or microalbuminuria (31.1 [0–121.0] vs. 74.4 [0–1112.3] mg/24 hr, P=0.080), whereas it remained stable in the others (0 [0–996] vs. 7.9 [0–632.5] mg/24 hr, P=0.510) (Fig. 1D).

**FIGURE 1.** (A) Pre- and posttransplant eGFR (line equations pretransplant: y=76.512±0.034x vs. posttransplant: y=76.109±0.015x, P=0.400 for slopes comparisons), (B) pre- and posttransplant albuminuria (0–24 months: 11.7 [0–632.5], 24 to 48 months: 25.6 [0–1112.3], >48 months: 13.4 [0–387.4] mg/24 hr, P=0.065), (C) posttransplant eGFR, and (D) albuminuria in patients with baseline normoalbuminuria and CKD stage 1 or 2 (white circles) versus baseline microalbuminuria and/or CKD stage 3 (black circles). P less than 0.05 for differences between groups (white circles vs. black circles) and P more than 0.05 for differences between pre- and posttransplant values for both groups. eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease.
As discussed earlier, clinically important deterioration of kidney function, requiring immunosuppressive regimen modification was seen in two subjects (5.7%). In both cases, a history of microalbuminuria and CKD stage 2 was present at baseline. In these cases, intermittent increases in creatinine, albuminuria, and proteinuria, starting approximately 1 year after transplant, were observed. The immunosuppressive therapy was modified when a clinical significant increase in creatinine was observed. These subjects were maintained on sirolimus, whereas tacrolimus was replaced with MMF. Both subjects returned to microalbuminuria and showed a mild decrease in eGFR (pretransplant: 64 and 66; most recent evaluation: 51 and 59 mL/min/1.73 m², with 54 and 51 months of follow-up, respectively).

To better understand if BP and lipid levels, as well as their respective treatments, could play a role in transient increments in albuminuria, we compared means before, during, and after the higher albuminuria period (24–48 months). A progressive decrease in LDL-cholesterol levels (105.38±2.84, 94.03±4.10 and 87.72±4.32 mg/dl; P=0.004 for comparison between 0–24 and 24–48 months) along with higher frequency in statins use per patient (0.45±0.07, 0.75±0.08, 0.68±0.11; P=0.003) was observed. Additionally, BP levels remained stable, whereas an increment in the number of antihypertensive drugs/patient was found in the late period (0.57±0.12, 0.65±0.13, 1.07±0.21; P=0.007 for comparison between 24–48 months and >48 months).

**DISCUSSION**

In this sample of ITA recipients, abnormal values of eGFR and albuminuria at baseline were predictors of less favorable kidney outcomes during the follow-up. Moreover, LDL-cholesterol was found to be an important modifiable risk factor and immunosuppressive maintenance therapy with tacrolimus protected from transient macroalbuminuria. Notably, there was no significant deterioration in overall kidney function in the posttransplant period and none of the subjects progressed to sustained macroalbuminuria or ESRD.

Intensive insulin therapy and whole-organ pancreatic transplantation can prevent chronic complications of T1DM (1, 18). Because clinical islet transplantation restores blood glucose homeostasis (4, 7, 19, 20), it would be expected to reduce microvascular diabetes chronic complications, at least in the same way as seen in intensive insulin therapy trials. In earlier reports, progression of both DR and DN after islet transplantation was attributed to natural history of the disease (20). Later on, proteinuria was described in three patients from Edmonton cohort (6) and its resolution after sirolimus discontinuation and increase in tacrolimus dosage called attention for immunosuppressive-related nephrotoxicity, already known in the kidney transplantation setting (21). These concerns were amplified because of the description of decrease in eGFR (9) and the unexpected evolution to ESRD in two patients (10% of the sample) observed in another cohort (8). Recently, the Vancouver group reported no GFR decline in islet transplant recipients (10), under a sirolimus-sparing immunosuppressive protocol. Notably, the lack of worsening in GFR in their study was based on a borderline statistical difference (P=0.07) between baseline and posttransplant GFR levels instead of comparison between groups (10).

It is conceivable that the impairment in renal function observed in recent islet transplantation trials be related to the combination of sirolimus and tacrolimus. Tacrolimus is a calcineurin-inhibitor with well-established propensity to cause CKD of both native and transplanted kidneys (22, 23). Sirolimus was thought to be a renal-safe drug, but lately it has been associated with kidney damage through glomerular (24) and tubular (25) mechanisms (26). The combination of both drugs might synergize enhancing renal damage (27).

A systematic evaluation of risk factors for kidney dysfunction after ITA had not been conducted so far. An association between baseline GFR and the eGFR delta in the first year posttransplant has been suggested, but clear correlations with immunosuppressive drugs levels could not be established (9). In our sample, classical risk factors for DN (namely, age, previous microalbuminuria, and higher LDL cholesterol) (28) were associated with a decrease in eGFR or macroalbuminuria posttransplantation. Additionally, another variable of interest, even though with weak association not sustained after adjustments in multiple regression, was the recipient’s BMI, formerly associated with islet graft failure (29) and potentially with DN, through its relation with metabolic syndrome (30).

The protective role of tacrolimus observed in our series was an unexpected finding. An improvement in albuminuria and proteinuria after sirolimus withdrawal and increase in tacrolimus dose has been described previously, even though a cause-effect relationship could not be established because both medications were modified simultaneously (6). Interestingly, tacrolimus has recently been reported to be effective as a steroid-sparing agent drug for minimal changes in nephrotic syndrome (31). Furthermore, the known hemodynamic effects of tacrolimus (32) might have contributed, at least in part, to the observed positive effects on albuminuria in our series.

Progression to microalbuminuria was higher (20%) in our sample than in the Diabetes Control and Complications Trial (<10% in a similar period of observation) (1). However, patients from the Diabetes Control and Complications Trial cannot be directly compared with those included in islet transplantation trials, because unstable T1DM control was an exclusion criterion and also duration of the disease was shorter than in islet transplant recipients (1). Our results are in fact between classical DN progression studies (17% in 5–10 years) (33) and those from the International Multicenter Trial (36%) (7) and the Edmonton cohorts (26%) (9).

Unexpectedly, we did not observe a decrease in kidney function after islet transplantation in our cohort of recipients. The unchanged kidney function found in our series when compared with previous reports could be attributed to healthier kidney status at baseline. In our series, most patients were normoalbuminuric and no patient had macroalbuminuria, contrasting with pretransplant values in macroalbuminuric range found on 5%, 7%, and 29% of the Milan (8), Edmonton (9), and Vancouver (10) cohorts, respectively. Another aspect that may have contributed to the better results observed in our study was the prompt treatment of renal side effects, by switching the immunosuppressive regimen (two cases) or by adding nephroprotective medications. Furthermore, in our experience, aggressive management of conventional risk factors for kid-
ney dysfunction, aiming a BP less than 130/80 mm Hg, LDL-cholesterol less than 100 mg/dL, and tacrolimus target levels less than 6 ng/mL, resulted in stability of BP and progressive decrease in LDL-cholesterol levels. This approach could have possibly contributed to the observed improvement in albuminuria levels at the late follow-up (>48 months).

Based on the results of the present study, a practical recommendation regarding patient selection and posttransplant clinical care can be given. Indication of islet transplant for a patient with previous kidney abnormalities should take into account fully risks and benefits and a posttransplant close surveillance with proactive management of possible complications should be planned, including kidney biopsies when clinically indicated (28). If a modification in the immunosuppressive therapy is required, maintenance of tacrolimus over sirolimus should be considered. Intensive treatment of conventional risk factors for nephropathy, such as tight BP and LDL-cholesterol control and careful tacrolimus target levels monitoring, might be part of clinical islet transplantation care to minimize other aggressors in addition to immunosuppressive therapy.

Limitations of our study included the relatively small sample size, the retrospective analyses, and the lack of a control group. The sample size restrictions were overcome by using a large number of assessments per patient and using statistics models specifically created for repeated measurements. The retrospective design prevented us from having a more comprehensive and prospective kidney function evaluation, through direct function measurements (i.e., EDTA Chromium, ioheoxol, and iodothalamate) or kidney biopsies. Although the use of indirect methods to estimate GFR (namely, the modification of diet in renal disease formula) may diminish the accuracy of the results by underestimating values when GFR is higher than 60 mL/min/m², it is largely accepted as a kidney function estimator and is currently the formula of choice in the diabetic population (34). Furthermore, our aim was to analyze changes in kidney function during the follow-up and not to describe the frequency of low GFR in this population. Thus, a measurement bias, if present, would be applied to all values, and it would not affect the final observation. Finally, as the subjects were followed up pretransplant for a median of 20 months, we used pretransplant eGFR values as recipient's own controls.

In conclusion, kidney function remained stable after ITA, even with the combination of two nephrotoxic drugs. A mild increase in microalbuminuria incidence was seen, but no decline in eGFR could be detected. Possibly, treatment of modifiable risk factors, as pretransplant microalbuminuria and LDL-cholesterol, and tight BP control, may minimize the deleterious effects of immunosuppressive drugs. These results could probably only be accomplished because of aggressive treatment of conventional risk factors for nephropathy.

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REFERENCES

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