New-Onset Diabetes Mellitus after Kidney Transplantation in Denmark

Mads Hornum,* Kaj Anker Jørgensen,† Jesper Melchior Hansen,‡ Finn Thomsen Nielsen,§
Karl Bang Christensen,‖ Elisabeth R. Mathiesen,‖ and Bo Feldt-Rasmussen*‡

Departments of *Nephrology and †Endocrinology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; ‡Department of Renal Medicine C, Århus University Hospital, Skejby, Århus, Denmark; §Department of Nephrology, Herlev Hospital, Copenhagen, Denmark; ‖Department of Nephrol
ogy Odense University Hospital, Odense, Denmark; and †Department of Biostatistics, Institute of Public Health, University of Copenhagen, Copenhagen, Denmark

Background and objectives: This study aimed to investigate the development of new-onset diabetes mellitus (NODM) in a prospective study of 97 nondiabetic uremic patients.

Design, setting, participants, & measurements: Included were 57 kidney recipients (Tx group, age 39 ± 13 years) and 40 uremic patients remaining on the waiting list for kidney transplantation (uremic controls, age 47 ± 11 years). All were examined at baseline before possible transplantation and after 12 months. The prevalence of diabetes, prediabetes, insulin sensitivity index (ISI), and insulin secretion index (Isecr) were estimated using an oral glucose tolerance test with measurements of plasma glucose and plasma insulin.

Results: One year after transplantation NODM was present in 14% (8 of 57) compared with 5% (2 of 40) in the uremic control group (P = 0.01). ISI in the Tx group deteriorated from 6.8 ± 3.9 before transplantation to 4.9 ± 2.8 at 12 months after transplantation (P = 0.005), and a slight increase in Isecr from 37 ± 19 to 46 ± 22 (P = 0.02) was seen. No significant changes occurred in the uremic controls (ISI was 7.9 ± 5 and 8.5 ± 5, and Isecr was 31 ± 17 and 28 ± 15). Using multivariate ordinal logistic regression, pre-Tx ISI and age predicted NODM (odds ratios: 0.82, P = 0.01 and 1.06, P = 0.02, respectively).

Conclusions: One year after kidney transplantation, NODM was present in 14% of patients. This was mainly caused by an increase in insulin resistance and was observed despite improvement in insulin secretion.


After successful solid organ transplantation, new-onset diabetes mellitus (NODM) develops in up to 50% of patients (1,2) and is associated with increased cardiovascular disease and decreased quality of life (3–6).

Whether NODM is related to the immunosuppressive treatment inducing insulin resistance or to impaired insulin production is a matter of debate. An impaired beta cell function has been suggested to play a major role in the development of NODM (7), and Hur et al. (8) found in Korean patients that the prevalence of NODM seen 1 year after transplantation remained high when reinvestigated after 7 years; it was mainly related to an impaired insulin secretion.

The majority of patients undergoing transplantation today receive a calcineurin inhibitor-based regimen which has been demonstrated to be toxic to the pancreatic beta cell in both pancreas- and kidney-transplanted patients (9). Thus, immunosuppressive regimens may play a major role in the development of NODM, either by reducing insulin sensitivity or secretion. This is interesting, especially because the pathophysiology behind insulin resistance also plays a role in the development of cardiovascular disease (2,10). In addition, cytomegalovirus (CMV) infection, HLA type, age, and gender (5,10–14) have also been associated with development of NODM.

The combination of triple immunosuppressive and preemptive CMV therapies has lowered the need for steroid and CMV treatment. This has been suggested as the reason for the reduction in the incidence of NODM in a recent study (15).

We prospectively studied the development of NODM by examining kidney graft recipients before and after transplantation. Furthermore, a control group of uremic patients was included to follow the natural history of diabetes in uremia. The presence of prediabetes, NODM, insulin resistance, and insulin secretion was determined by oral glucose tolerance tests (OGTTs). We hypothesized that both pretransplantation impaired glucose tolerance (IGT) and further posttransplantation insulin resistance were the main factors behind development of NODM.

Materials and Methods

This is a prospective, observational, national multicenter study including 54 (67%) of 81 patients, mostly Caucasians, with a scheduled living donor kidney transplantation in the period between January 2006 and March 2008 at four Danish transplantation centers: Copenhagen, Skejby, Odense, and Herlev University Hospitals (Tx group). A control...
group consisted of patients from the transplantation waiting list at Copenhagen and Herlev University Hospital (uremic controls). All 81 patient charts were pre-screened, and the following were excluded from the study: diabetes present in the chart (n = 7), diabetes diagnosed with OGTT (n = 3), prednisolone treatment above 12.5 mg/d (n = 3), and newly initiated high-dose immunosuppressive treatment as in ABO-incompatible transplantation (n = 4). Ten patients did not want to participate. The control group was established by selecting 52 patients among all 510 patients listed in the waiting list for transplantation with a deceased donor, and they were included in the Tx group. Four patients died in the control group, and five patients did not want to participate in the 12-month examination, leaving us with a control group of 40 uremic patients without diabetes to be reexamined after 1 year. The main reason for nonattendance was long traveling time to the laboratory or inability to spend extra days for the examinations besides the time already used for dialysis. The patients were interviewed about their family history of diabetes, defined as a parent or siblings having diabetes type 1 or 2. The patients were interviewed about diabetes type 1 or 2.

A healthy control group consisting of 14 age-, body mass index (BMI-), and sex-matched subjects was recruited from public announ-

cation/American Diabetes Association 2007 criteria (17). We determined the prevalence of normal glucose tolerance (fasting plasma glucose <5.6 mmol/L and 2-hour postload glucose <7.8 mmol/L), impaired fasting glucose (IFG: fasting plasma glucose between 5.6 and 6.9 mmol/L and a 2-hour postload glucose <7.8 mmol/L), impaired glucose tolerance (IGT: fasting plasma glucose <6.9 mmol/L and 2-hour postload glucose between 7.8 and 11.1 mmol/L) as well as prediabetes (IFG plus IGT) and diabetes (fasting plasma glucose >7.0 mmol/L or 2-hour postload glucose >11.1 mmol/L). Plasma concentrations of glucose and insulin were measured at times −30, −15, 0, 30, 60, 90, and 120 minutes. According to Matsuda et al. (18), an insulin sensitivity index (ISI) was calculated as 10,000/square root of [fasting glucose × fasting insulin] [mean glucose × mean insulin during OGTT]. This index is highly correlated with the rate of whole-body glucose disposal during a euglycemic insulin clamp in patients with varying degrees of glucose tolerance.

The area under the curve (AUC) for insulin and glucose during the OGTT was calculated using the trapezoid rule. These variables were implemented in the insulin secretion index (IScr), Secr_AUC = AUCins/AUCGlu (19). The difference in ISI and IScr between baseline values and 1-year values was calculated as ΔISI and ΔIScr (Table 1).

**Immunosuppression**

Immunosuppression varied to some extent between the centers. Induction therapy included basiliximab (Simulect; Novartis), daclizumab (Zenapax; Roche), or antithymocyte globulin (Thymoglobulin; Genzyme B.V.).

**Corticosteroids.** The majority of patients received 100 to 500 mg of intravenous methylprednisolone preoperatively, and treatment with oral prednisolone was started by 20 to 100 mg/d and was tapered to a dose of 7.5 to 10 mg at 3 months and to 5 to 7.5 mg at 9 to 12 months. In the seven patients from Odense University Hospital, no prednisolone treatment was started as part of the routine treatment regimen (20). Rejection episodes, indicated by increased plasma creatinine of 20% or greater for 2 days, or biopsy proven, were treated with intra-venous methylprednisolone, 500 mg, for 3 to 5 days. For each patient the accumulated corticosteroid dose within the first 90 days was calculated and given in equivalents of prednisolone dose in grams.

**Calcineurin Inhibitors.** Forty-three patients started on cyclosporine (Sandimmune Neoral; Novartis) at a dose of 2.5 to 6 mg/kg twice daily tapered to a trough level of whole-blood concentration of 150 to 300 μg/L for the first 3 months and 100 to 150 μg/L thereafter. The remaining 14 patients started on tacrolimus (Prograf; Astellas) at a dose of 0.075 to 0.15 mg/kg twice daily tapered to a whole-blood concentration of 8 to 15 μg/L for the first 3 months and 5 to 10 μg/L thereafter.

A few patients (n = 8) changed from one treatment modality to another during the course of the study, and two patients were changed to rapamycin (Rapamune; Wyeth) treatment.

**Other Immunosuppression**

Mycophenolate mofetil (Cellcept; Roche; or Myfortic; Novartis) was used for most patients, and azathioprine (Imurel; GlaxoSmithKline Pharma) was used as an alternative drug.

**Antihypertensive Treatment**

Antihypertensive treatment mainly included β-blockade, calcium channel blockade, angiotensin II blockade, and diuretics. All antihyper-tensive medication was stopped at the time of transplantation and thereafter titrated aiming for a BP below 130/80 mmHg.

**Statistical Analyses**

Data analyses were done using Statistical Analysis Software (SAS®) version 9.1. Unless specified otherwise, continuous data are described

**Evaluation of Glucose Tolerance**

A 75-g OGTT was done according to the World Health Organization/American Diabetes Association 2007 criteria (17). We determined...
Results

The patients in the two groups were well matched according to most clinical and demographic parameters. The patients in the Tx group were younger and had a shorter duration of ESRD (P = 0.03 and P = 0.001, respectively; Table 2). Two patients (4%) in the Tx group and four patients (10%) in the uremic control group had a family history of diabetes. The prevalence of prediabetes, mainly IGT, was high in both groups (33% in the Tx group and 50% in the uremic controls, respectively; Table 3). Furthermore, both patient groups were shown to be insulin resistant compared with a well-matched healthy control group (P < 0.001; Table 2), but insulin secretion was not impaired. Estimated GFR was 77 ± 20 and 78 ± 23 ml/min at 3 and 12 months after transplantation, respectively (Table 3). At 1 year, immunosuppressive treatment of the kidney recipients was: 37% received tacrolimus, 56% cyclosporine, 91% prednisolone (average 6 mg/d), and 96% mycophenolate mofetil. Accumulated prednisolone dose at 3 months after transplantation varied between centers, with an individual range from 0 to 6455 mg. The mean daily dose of prednisolone was 13 mg at 3 months and 7 mg at 12 months. Nine patients (16%) were diagnosed with and treated for a rejection, and five were treated for CMV disease with valganciclovir for 3 to 6 months. Estimated GFR was 77 ± 20 and 78 ± 23 ml/min at 3 and 12 months after transplantation, respectively (Table 3). At 1 year, immunosuppressive treatment of the kidney recipients was: 37% received tacrolimus, 56% cyclosporine, 91% prednisolone (average 6 mg/d), and 96% mycophenolate mofetil. Accumulated prednisolone dose at 3 months after transplantation varied between centers, with an individual range from 0 to 6455 mg. The mean daily dose of prednisolone was 13 mg at 3 months and 7 mg at 12 months. Nine patients (16%) were diagnosed with and treated for a rejection, and five were treated for CMV disease with valganciclovir for 3 to 6 months. BMI increased significantly in the Tx group compared with the uremic controls, but waist-hip ratio remained similar between the groups (Table 3).

Eight patients (14%) developed diabetes in the Tx group compared with two patients (5%) in the uremic controls (P < 0.01; Table 3). One patient required insulin treatment for a short period, whereas the others were treated with diet alone. The transplantation patients who developed diabetes (n = 8) had a significantly lower ISI at baseline and were slightly older compared with patients with normal glucose tolerance at follow-up.
Among the 14 patients receiving tacrolimus, three (21%) developed NODM compared with five (11%) of the 43 receiving cyclosporine; however, the ordinal logistic regression model could not demonstrate statistically significant difference between the two immunosuppressive regimens. The average accumulated prednisolone dose was 0.5 g (22%) higher in patients developing NODM compared with patients with normal glucose tolerance after transplantation, but these data did not reach statistical significance in the ordinal logistic regression model.

The time course for changes in glucose tolerance categories for the individual patients from baseline to follow-up for both groups is shown in Figure 1, demonstrating that patients with NODM at 1 year did not necessarily have IGT before transplantation.

Data are presented as mean ± SD or median (range), unless otherwise noted. Wilcoxon rank sum test or two-sample \( t \) test was used where appropriate to test. HD, hemodialysis; CAPD, continuous ambulatory peritoneal dialysis; Tx, transplantation; PKD, polycystic kidney disease; DM, diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure.

\( ^a \)Uremic patients versus healthy controls \( P < 0.0005. \)

\( ^b \)Tx group versus uremic controls \( P < 0.05. \)

\( ^c \)Tx group versus uremic controls \( P < 0.005. \)
significant association with pre-Tx ISI ($P < 0.0001$) and pre-Tx Isecr ($P = 0.0001$). The two initial immunosuppressive regimens with tacrolimus or cyclosporine both reduced the ISI but with no difference between the drugs. However, when multivariate analysis was done, only high levels of pre-Tx ISI and high waist-hip ratio were associated with worsening of ISI ($R^2 = 0.61$).

When the change in Isecr from baseline to 1 year was considered as the dependent variable, univariate linear regression showed a significant association with pre-Tx Isecr ($P = 0.0002$) and pre-Tx ISI ($P = 0.004$). Multivariate regression analysis revealed only an association between (low) baseline Isecr and an increase in Isecr ($R^2 = 0.24$). The increase in Isecr in the patients receiving cyclosporine after transplantation was significant ($P = 0.003$), whereas no change was seen in the patients receiving tacrolimus ($P = 0.73$). The difference between the two drugs did not reach statistical significance.

Using multivariate ordinal logistic regression analysis, low pre-Tx ISI and high age predicted NODM (odds ratio (OR): 0.82, $P = 0.01$; and OR: 1.06, $P = 0.02$, respectively) but not CMV (OR: 1.19, $P = 0.75$) or the use of tacrolimus (OR: 1.54, $P = 0.47$).

### Table 3. Metabolic and immunosuppressive data before and after kidney transplantation in 97 uremic patients without known diabetes (DM) at baseline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tx Group</th>
<th>Uremic Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time 0</td>
<td>Time 3 mo</td>
</tr>
<tr>
<td>Number of patients</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td>Fasting p-glucose, mmol/L</td>
<td>5.1 ± 0.5</td>
<td>5.6 ± 0.7$^a$</td>
</tr>
<tr>
<td>p-glucose at 2 h, mmol/L</td>
<td>7.4 ± 1.6</td>
<td>8.7 ± 3.1</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.2 ± 0.4</td>
<td>5.6 ± 0.5$^a$</td>
</tr>
<tr>
<td>AUC insulin, pmol/L × 120 min</td>
<td>35,358 ± 19,884</td>
<td>36,977 ± 17,023</td>
</tr>
<tr>
<td>AUC glucose, mmol/L × 120 min</td>
<td>948 ± 157</td>
<td>1059 ± 287</td>
</tr>
<tr>
<td>ISI composite</td>
<td>6.8 ± 3.9</td>
<td>4.9 ± 3.9$^b$</td>
</tr>
<tr>
<td>Isecr</td>
<td>36.9 ± 18.5</td>
<td>35.8 ± 17.3</td>
</tr>
<tr>
<td>OGTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, n (%)</td>
<td>0</td>
<td>11 (19)</td>
</tr>
<tr>
<td>IFG/IGT, n (%)</td>
<td>0/19 (33)</td>
<td>3/17 (35)</td>
</tr>
<tr>
<td>NGT, n (%)</td>
<td>38 (67)</td>
<td>26 (46)</td>
</tr>
<tr>
<td>Estimated GRF, ml/min</td>
<td>&lt;15</td>
<td>73.6 ± 20.3$^a$</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>24.4 ± 3.9</td>
<td>25.4 ± 3.9</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.92 ± 0.09</td>
<td>0.94 ± 0.09</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>104 ± 14</td>
<td>97 ± 11</td>
</tr>
<tr>
<td>AH</td>
<td>2.5 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Beta-receptor blocker, n (%)</td>
<td>27 (47)</td>
<td>34 (71)</td>
</tr>
<tr>
<td>Number of acute rejection episodes (%)</td>
<td>0</td>
<td>8 (14)</td>
</tr>
<tr>
<td>CMV disease, n (%)</td>
<td>0</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Prednisolone, n (%)</td>
<td>12 (21)</td>
<td>52 (91)</td>
</tr>
<tr>
<td>Prednisolone, dose, mg</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Tacrolimus, n (%)</td>
<td>1 (2)</td>
<td>19 (33)</td>
</tr>
<tr>
<td>Tacrolimus, dose, mg</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cyclosporine, n (%)</td>
<td>1 (2)</td>
<td>36 (63)</td>
</tr>
<tr>
<td>Cyclosporine, dose, mg</td>
<td>0</td>
<td>214</td>
</tr>
<tr>
<td>Azathioprine/sirolimus, n (%)</td>
<td>0</td>
<td>2 (4)</td>
</tr>
<tr>
<td>MMF; Cellcept, n (%)</td>
<td>4 (7)</td>
<td>52 (91)</td>
</tr>
<tr>
<td>MMF; Myfortic, n (%)</td>
<td>0</td>
<td>2 (4)</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD unless otherwise noted. Wilcoxon rank sum test or unpaired t test was used where appropriate to test. NGT, normal glucose tolerance; AH, mean number of antihypertensive medications; CMV, cytomegalovirus; MMF, mycophenolate mofetil.

$^a$Tx patients before versus after Tx $P < 0.0005$.

$^b$Tx patients before versus after Tx $P < 0.005$.

$^c$Tx patients before versus after Tx $P < 0.05$. 
The incidence of NODM has been reported in the range between 2% and 50% (1,2). Our study is the first to include a uremic control group and at the same time use strict diagnostic criteria based on an OGTT done before and after transplantation. In this setting, we found a 12-month incidence of NODM of 14% in the transplantation patients compared with 5% in the uremic control group, despite a higher age and longer duration of ESRD in the control group. This confirms that NODM is a significant clinical problem after kidney transplantation. The high prevalence of prediabetes before transplantation (21) also underlines the fact that NODM occurs in a population of patients with an a priori high risk of diabetes.

The incidence of NODM is similar to the relatively few studies also applying strict diagnostic criteria of diabetes before and after transplantation, with incidences ranging from 18% to 24% (7,13,14). We confirmed the earlier documented high prevalence of glucometabolic changes in nondiabetic patients with stage 5 chronic kidney disease (22). In our hands, the prevalence of prediabetes (IGT plus IFG) was 41% in the combined Tx and uremic control group before transplantation. It is noteworthy that presence of prediabetes did not necessarily precede development of NODM. The independent factors predicting deterioration of glucose tolerance were low ISI and high age at baseline.

At baseline, the insulin secretion was relatively high, which may have compensated for the insulin resistance and helped to maintain a normal OGTT in the majority of patients. Our estimations of Isecr and ISI are to some extent biased, because insulin is metabolized differently before and after transplantation, mainly because the insulin excretion is significantly decreased with decreasing GFR (23). The estimated Isecr was based on direct measurements of plasma insulin (p-insulin; AUC of p-insulin during the OGTT) and p-glucose (AUC of p-glucose during the OGTT). In the algorithm used for calculating the Isecr, the AUC insulin is in the nominator and AUC glucose in the denominator. Thus, the estimated Isecr is probably overestimated before transplantation. Our observation of a reduced ISI after transplantation corresponding to an increased insulin resistance was, again, made despite this negative bias, supporting our conclusion that the Isecr is increased after transplantation.

The increase in Isecr after transplantation was not sufficient to overcome the decline in ISI, resulting in deterioration of the glucose tolerance present 12 months after transplantation.
deterioration in ISI was apparent both at 3 and 12 months, whereas the increase in Isecr took place between 3 and 12 months after transplantation. This increase in Isecr was possibly related to decreased levels of CNIs. The observations made from 3 to 12 months are in keeping with the results of three previous studies (24–26), none of which included baseline data from before transplantation.

Among other risk factors to be discussed were the medications taken. The dosing of steroids did vary among centers. The average accumulated steroid dose was comparable to previous studies, and we observed an association between accumulated steroid dose and development of NODM (12,27). The average accumulated prednisolone dose was 537 mg (22%) higher in patients developing NODM compared with patients with normal glucose tolerance after transplantation, but these data did not reach statistical significance. Deterioration in insulin resistance was seen with both calcineurin inhibitors used, but the increase in insulin secretion during treatment with cyclosporine was not seen with tacrolimus. The prevalence of NODM in patients receiving tacrolimus was 21% compared with 11% in patients receiving cyclosporine. Although these findings did not reach statistical significance, they support the previous findings of an increased prevalence of NODM in patients receiving tacrolimus (12). Other drugs, like mycophenolate mofetil and azathioprine, were not associated with NODM in our study. The mode of dialysis before transplantation did not have a significant impact on the changes in ISI and Isecr. Multivariate analysis revealed that pre-Tx ISI and high WHR were significant predictors of deterioration in insulin resistance. Only pre-Tx Isecr predicted an increase in insulin secretion. Familial history of diabetes, CMV disease, acute rejection, BMI, HLA-B27 phenotype, previous transplantation, hepatitis C virus infection, and change in BMI and WHR could not be detected as risk factors for the development of insulin resistance and impaired insulin secretion in our cohort, but numbers were too small to exclude clinical significant associations.

The design of the present study was different from previous studies. We included a randomly selected uremic control group of patients from the waiting list. The transplantation patients were younger than the uremic control group but otherwise well matched and comparable by diagnoses, medication, and baseline glucometabolic status. Age was found to be a risk factor for NODM, and this was in agreement with previous findings (13,28).

Previous studies by Nam et al. (7) in 114 Korean kidney transplantation patients with normal glucose tolerance revealed that 23.7% had NODM and 44.7% had IGT 9 to 12 months after transplantation. In this study, with no control group, Nam et al. (7) found that low insulin secretion capacity was one of the reasons for the development of NODM. In another study by Hjelmesaeth et al. (13) of 167 kidney transplantation patients examined 10 weeks after transplantation, 19% of patients developed NODM, but in this study only 50% had a pretransplantation OGTT done, and there was no control group. National registry analysis suggests that 15% to 20% of renal transplant patients who do not have diabetes and receive a calcineurin inhibitor-based regimen develop NODM within 1 year of transplantation (5,29).

Our study indicates that attempts to diagnose diabetes before and after transplantation according to strict diagnostic criteria are important to identify patients who are likely to benefit from implementation of the multifactorial intervention so well documented in the treatment of diabetes. Additional studies are needed to further investigate the clinical impact of the development of prediabetes and diabetes in patients who undergo a kidney transplantation.

In conclusion, we found that at 1 year after kidney transplantation, NODM was present in 14% compared with 5% in the uremic controls. This was mainly because of a further increase in insulin resistance and was observed despite improvement in insulin secretion.

Acknowledgments

The study was supported by an unrestricted grant from the Danish Kidney Foundation, A. P. Møller Foundation for the Advancement of Medical Science, Eva and Henry Frankel Foundation, and the Helen Bjørnøv Foundation. M.H. was supported by a fellowship from Rigshospitalet. We thank the Research Laboratory, Department of Renal Medicine C, Århus University Hospital, Skejby, and the laboratory technicians Mette Svendsen, Annette Vinding, Helle Christensen, and Andreas Haltorp for their skillful contribution to the data.

Disclosures

None.

References


