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Side effects of the calcineurin inhibitor, such as new-onset diabetes after kidney transplantation

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New-onset diabetes after transplantation (NODAT) is one of the frequent complications following kidney transplantation. Patients were randomized to receive cyclosporine A- or tacrolimus-based immunosuppression. Fasting and oral glucose tolerance tests were performed, and the patients were assigned to one of the following three groups based on the results: normal, impaired fasting glucose/impaired glucose tolerance (IFG/IGT), or NODAT. NODAT developed in 14% of patients receiving cyclosporine A-based immunosuppression and in 26% of patients taking tacrolimus ($p = 0.0002$). Albumin levels were similar, but uric acid level ($p = 0.002$) and the age of the recipient ($p = 0.003$) were significantly different comparing the diabetic and the normal groups. Evaluation of tissue samples revealed that acute cellular rejection (ACR) and interstitial fibrosis/tubular atrophy (IF/TA) were significantly different in the NODAT group. The pathological effect of new-onset diabetes after kidney transplantation can be detected in the morphology of the renal allograft earlier, before the development of any sign of functional impairment.

Keywords: calcineurin inhibitors, new-onset diabetes mellitus after kidney transplantation, protocol biopsy

New-onset diabetes mellitus after transplantation (NODAT) is one of the most common complications following kidney transplantation. The diagnosis of NODAT is often late or missed; therefore, it impairs the implanted renal allograft. Not only does untreated NODAT negatively influence the allograft, but it increases the risk of cardiovascular diseases (ischemic heart disease, hypertension, and hyperlipidemia) and death (5, 9, 11, 13). NODAT is the same risk factor of cardiovascular diseases as diabetes diagnosed before the transplantation (5). Its etiopathogenesis is complex, endogenous factors, such as severe obesity, positive family history, age, hepatitis C- and cytomegalovirus infection, and immunosuppressive (IS) therapy play a role in the development of NODAT (9, 11, 13, 16). Several clinical studies have confirmed that NODAT induces tissue damage in the allograft shortly after transplantation which leads to functional impairment in the long term (3, 4, 8).

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The aim of our study is to compare the incidence of new-onset diabetes mellitus one year after kidney transplantation in patients taking cyclosporine-A (CsA) and tacrolimus (Tac) and to compare the effect of new-onset diabetes on the histopathology of the allograft.

Patients and Methods

Our studies were conducted in the Department of Surgery, University of Szeged, Szeged, Hungary. Zero-hour biopsy was performed in case of all cadaver kidneys before transplantation. During the study, kidney transplantation was performed in 98 cases. Patients who had no zero-hour biopsy did not agree to the biopsy in accordance with the protocol being performed, died during the study, had diabetes mellitus diagnosed before the transplantation, were younger than 18 years, or were living-donor recipients were excluded from the study. Two patients died during the study, and the kidney had to be removed in 4 cases; therefore, a total of 69 patients were enrolled in the study.

Patients were randomized in two groups, one group received CsA-based immunosuppression ($n = 35$), and the other group received Tac-based IS ($n = 34$). All patients received steroid free immunosuppressive therapy. Fasting glucose level was measured in all patients before the transplantation, and patients having diabetes mellitus were excluded from the study. In our study, according to the criteria of the American Diabetes Association (ADA), diabetes is present if fasting blood glucose level is ≥7 mmol/L, or the blood glucose level measured 2-h following the oral administration of 75 g glucose (Oral glucose tolerance test, OGTT) is ≥11.1 mmol/L. Impaired fasting glucose (IFG) is defined as fasting blood glucose level between 5.6 mmol/L and 6.9 mmol/L, whereas the normal value (N) for fasting blood glucose is <5.6 mmol/L or impaired glucose tolerance (IGT) (2-h values in the OGTT) between 7.8 mmol/L and 11.0 mmol/L (1).

One year after the transplantation, check-up laboratory tests and OGTT were performed in all patients, and patients were classified into one of the following three groups based on the glucose value: normal (N), increased fasting glucose/impaired glucose tolerance (IFG/IGT), and NODAT.

In these groups, the effect of abnormal glucose metabolism on renal histopathology was evaluated. Morphological changes were confirmed with protocol biopsy one year after transplantation after giving informed consent. It has already been known that long term diabetes has an effect on the functional and morphological deterioration of the kidney, but it has not been described yet that 1 year after transplantation if diabetes has developed and fasting blood glucose level has increased, kidney function may be deteriorated or even histological changes may be detected in the kidney.

The ultrasound-guided protocol biopsy was performed (with prior consent) after the 1-year fasting laboratory testing. We used 16-G Tru-Cut needles and a biopsy gun to obtain tissue cylinders. Morphologic examinations included standard light microscopic staining (hematoxylin and eosin, periodic acid Schiff, trichrome, and methenamine silver), as well as immunofluorescence staining of frozen sections using antibodies to human leukocyte class II antigens, complement 4d (C4d), C3, immunoglobulin (Ig)G, IgA, and IgM. Embedding for electron microscopy was performed in all cases, some of which underwent ultrastructural evaluation. Renal lesions were graded and diagnosed according to the 2003 modification of the Banff ’97 classification. Histological changes were classified as acute cellular rejection (ACR), calcineurin inhibitor toxicity (CNI-tox) and IF/TA for grades II and III, compared

with grade I, which was considered to be normal we also sought changes associated with pyelonephritis (PN) and other diseases, e.g., acute tubular necrosis, glomerulonephritis, and BK polyomavirus nephritis (10).

Mechanism of action of the calcineurin inhibitors

Calcineurin inhibitors (CsA and Tac) differ from their predecessor immunosuppressive drugs by virtue of their selective inhibition of the immune response. Their immunosuppressive effect depends on the formation of a complex with their cytoplasmic receptor proteins, cyclophilin for cyclosporine A and tacrolimus binding protein (FKBP) for tacrolimus. This complex binds with calcineurin, the normal function of which is to act as a phosphatase that dephosphorylates certain nuclear regulatory proteins and hence facilitates their passage through the nuclear membrane. Inhibition of calcineurin thereby impairs the expression of several critical cytokine genes that promote T-cell activation including those for IL-2, IL-4, interferon-γ, and tumor necrosis factor-α. The transcription of other genes, such as CD40 ligand and the proto-oncogenes is also impaired. Calcineurin inhibitor agents may have a direct impact on the transcriptional regulation of insulin gene expression in the pancreatic β cells. The exact mechanism of calcineurin inhibitor induced toxicity to β cell is unknown. The diabetogenic effect of tacrolimus may be reversible, as evidenced by observations that impaired insulin secretion was reversed 3 days after tacrolimus discontinuation, and insulin secretion improved after the reduction of tacrolimus through blood concentration. With the above understanding, in the following sections, genes related to diabetes or to the mechanism of developing diabetes are compared with the genes associated with calcineurin inhibitor induced diabetes.

Our study was approved by the Regional Human Biomedical Research Ethics Committee of the Albert Szent-Györgyi Clinical Center, University of Szeged, Szeged, Hungary (Reg. No.: 17/2011). Each patient was provided with comprehensive information regarding the study.

Immunosuppressive protocol

The initial daily dose of *tacrolimus* (0.20 mg/kg in 2 portions) was adjusted to target blood trough levels of 10–15 ng/mL at 6 weeks, and 5–10 ng/mL thereafter. The initial *cyclosporine-A* dose was 8–10 ng/kg daily in 2 portions with target blood levels at two hours post-dose of 1,300–1,600 ng/mL at month 1; 900–1300 ng/mL at months 2 and 3; 750–950 ng/mL at months 4–6; and 700 ng/mL thereafter.

For the treatment of ACR, steroid in a daily dose of 500 mg was administered intravenously for 3 days, followed by 250 mg per day intravenously for 2 days.

Statistical analysis

Continuous variables were summarized with mean ± standard deviation, whereas categorical variables were presented with proportions and Student's *t*-test, or one-way analysis of variance (ANOVA) was used for group comparisons as appropriate. The normal distribution of samples was evaluated by the Kolmogorov–Smirnov test. Categorical data were analyzed using chi-square and Fisher's exact tests. The multivariable dependences of NODAT categorical and continuous data were analyzed using logistic regression. A p value less than 0.005 was considered to be significant. SPSS version 15.0 (2007 SPSS, Inc. Chicago, III.) was used for the statistical analysis.

Results

The results of fasting glucose measurement and OGTT performed one year after the transplantation were the following (n = 69): NODAT n = 5 (14%), IFG/IGT n = 10 (29%), N n = 20 (57%) in patients receiving CsA-based IS; NODAT n = 9 (26%), IFG/IGT n = 15 (44%), N n = 11 (30%) in patients taking Tac. The incidence of diabetes was significantly different in the CsA group compared to the Tac group (14% vs. 26%, p = 0.0002). Examination of all patients revealed the following distribution regarding glucose metabolism: NODAT 21%, IFG/IGT 37%, and N 42% (Table I). Logistic regression showed that Tac increased the risk of the development of diabetes by 25% (Table II). Laboratory tests revealed no significant difference in the albumin level but showed that uric acid was significantly different in NODAT patients compared to patients having normal glucose metabolism (462.2 ± 137.3 versus 348.4 ± 97, p = 0.002). The age of the recipient was significantly different in diabetic patients compared to the normal group (55.7 ± 10.2 versus 41.3 ± 9.3; p = 0.003) (Table III). Histological assessment of protocol biopsies performed in patients with different glucose metabolism detected ACR in 3 (22%), IF/TA in 5 (36%), and normal histology in 3 (21%) samples in the NODAT group. ACR (p = 0.003), IF/TA (p = 0.0001), and N (p = 0.0002) were significantly different between the normal and the NODAT group (Table IV).

Table I. Glucose metabolism changes between cyclosporine-A and tacrolimus

One year after kidney transplantation					
	CsA (n = 35)		**Tac (n = 34)**		**(n = 69)**
	OGTT		**OGTT**		
	O.min	**120.min**	**O.min**	**120.min**	
NODAT	4 (11%)	5(14%)	7 (21%)	9 (26%)	14 (21%)
IFG/IGT	8 (23%)	10 (29%)	11 (32%)	15 (44%)	26 (37%)
N	23 (66%)	20 (57%)	16 (47%)	10 (30%)	(42%)

CsA – cyclosporine-A; Tac – tacrolimus; NODAT – new-onset diabetes after transplantation; IFG/IGT – increased fasting glucose / impaired glucose tolerance; N – normal; OGTT – oral glucose tolerance test

Table II. Results of Logistic Regression Analysis in the NODM group

	NODAT	
	***p* value**	**OR**
Cyclosporine-A	0.077	0.317
Tacrolimus	0.05	1.258

NODAT – new-onset diabetes after transplantation; OR – odds ratio

Table III. Laboratorial results of the other glucose metabolism groups

	NODAT (*n* = 14) mean±SD	**IFG/IGT (*n* = 26) mean±SD**	**N (*n* = 29) mean±SD**	***p* value (N vs. NODAT)**
Age (year)	55.7 ± 10.2	43 ± 8.1	41.3 ± 9.3	0.003
Gender (female/male)	9/5	12/14	16/13	
Uric acid (μmol/L)	462.2 ± 137.3	372.5 ± 86.3	348.4 ± 97.5	0.002
Albumin (g/L)	46.6 ± 3.2	46.27 ± 4.49	47.15 ± 3.65	0.568
Urea (mmol/L)	14.2 ± 5.8	12.51 ± 6.17	11.81 ± 7.58	0.134

NODAT – new-onset diabetes after transplantation; IFG/IGT – increased fasting glucose / impaired glucose tolerance; N – normal; SD – standard deviation

Table IV. Protocol biopsy results

	ACR	**IF/TA (grades II and III)**	**CNI-tox**	**PN**	**Other**	**Normal**
NODAT (*n* = 14)	3 (22%)	5 (36%)	1 (7%)	1 (7%)	1 (7%)	3 (21%)
IFG/IGT (*n* = 26)	2 (8%)	4 (15%)	2 (8%)	2 (8%)	3 (11%)	13 (50%)
N (*n* = 29)	2 (7%)	3 (10%)	3 (10%)	2 (7%)	4 (14%)	15 (52%)
p value (N vs. NODAT)	0.003	0.0001	0.143	0.063	0.056	0.0002

ACR – acute cellular rejection; IF/TA – interstitial fibrosis / tubular atrophy; CNI-tox – calcineurininhibitor-toxicity; PN – pyelonephritis; NODAT – new-onset diabetes after transplantation; IFG/IGT – increased fasting glucose / impaired glucose tolerance; N – normal

Discussion

It is known that the risk of diabetes is increased following kidney transplantation. The incidence of NODAT may be increased as a consequence of the combination of multiple risk factors. Valderhang et al. have found that the incidence of NODAT was 14% (14). Several authors have shown that the most important risk factors of NODAT are immunosuppressive drugs, but family history, weight, and BMI of the recipient are also essential (3, 4, 6, 15, 16). In our study, it was shown that uric acid level was higher in the NODAT group. Although both CNIs are associated with hyperuricemia. The incidence of NODAT following kidney transplantation was 21% in the patients enrolled in our study. In our clinical study, the diabetogenic effect of the immunosuppressive drugs cyclosporine-A and tacrolimus were evaluated. Significant difference was found in the diabetogenic effect of the IS drugs tacrolimus and cyclosporine-A (26% versus 14%). Our results may confirm that tacrolimus exerts a stronger diabetogenic effect.

Cyclosporine-A appears to be less diabetogenic than tacrolimus, but both agents may have a direct impact on the transcriptional regulation of insulin gene expression in the pancreatic β cells. The exact mechanism of calcineurin inhibitor induced toxicity to β cell is unknown. The diabetogenic effect of tacrolimus may be reversible, as evidenced

by observations that impaired insulin secretion was reversed 3 days after tacrolimus discontinuation, and insulin secretion improved after the reduction of tacrolimus through blood concentration. With the above understanding, in the following sections, genes related to diabetes or to the mechanism of developing diabetes are compared with the genes associated with calcineurin inhibitor induced diabetes (6). The incidence of diabetes is 33.6% in case of tacrolimus and 26% in case of cyclosporine in the study of Vincenti et al. (16).

In our clinical study, there were no significant differences in case of urea between the N and the NODAT groups, however, the age of the recipient was significantly different between the two groups. Morphological abnormalities showed that ACR and IF/TA were significantly increased in the NODAT group compared to patients having normal carbohydrate metabolism.

One year after kidney transplantation, diabetic nephropathy (especially mesangial matrix increase and arteriola hyalinosis) does not develop; however, permanent hyperglycemia may result in morphological changes in the allograft. In IF/TA, hyperglycemia may lead to fibrogenesis by decreasing the number of functioning nephrons.

Arif et al. have found that IF/TA is significantly different comparing diabetic and normal patients ($p < 0.001$) (2). Diabetes not diagnosed and treated in time not only damages the graft but increases the cardiovascular risk as well. In case of kidney recipients, long term survival of the graft may be increased and the cardiovascular risk may be decreased with diagnosing and treating NODAT in time (12). Based on the studies of Gerő and Földes, micro- and macroangiopathy developed as a complication of NODAT significantly decrease the survival of the renal allograft: microangiopathy leads to early renal failure while macroangiopathy increases the risk of coronary thrombosis and stroke (7). Proper evaluation of the risk status should be emphasized in patient care and when selecting immunosuppressive therapy. In case high risk of diabetes (obesity, family history or old age) had been established even before the transplantation was performed, the administration of tacrolimus should be avoided. Carbohydrate metabolism should be checked regularly, especially in high risk patients. If deterioration in carbohydrate metabolism is noticed (IFG or IGT is developed), first the dose of calcineurin inhibitor should be decreased, and if it is proved to be ineffective, combinations without calcineurin inhibitor may also be administered. Regular control of carbohydrate metabolism is essential especially in high risk patients. In case of impaired carbohydrate metabolism (development of IFG or IGT), regular diabetological care is crucial. Interdisciplinary care of kidney recipients should be centralized and performed by a health care team or – if not possible – at least one check-up should be performed every year. Harmonized care may be essential in preserving the function of the allograft, as well as in preventing macrovascular complications and in decreasing mortality in the long term. By means of the protocol biopsy, an individually tailored immunosuppressive therapy can be set up for each patient, which may not only promote allograft function but might also increase the survival of the patient.

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