

EVALUATION OF POLINEUROPATHY MARKERS IN TYPE 1 DIABETIC KIDNEY-TRANSPLANT PATIENTS AND EFFECTS OF ISLET TRANSPLANTATION: NEUROPHYSIOLOGICAL AND SKIN BIOPSY LONGITUDINAL ANALYSIS

Ubaldo Del Carro MD^{1*}, Paolo Fiorina MD PhD^{2,4*}, Stefano Amadio MD¹, Luisa De Toni Franceschini MD¹, Alessandra Petrelli MD², Stefano Menini PhD⁵, Filippo Martinelli Boneschi MD PhD¹, Stefania Ferrari MD¹, Giuseppe Pugliese MD PhD⁵, Paola Maffi MD², Giancarlo Comi MD^{1,3} and Antonio Secchi MD^{2,3}

¹Department of Neurology and Clinical Neurophysiology and ²Medicine, San Raffaele Scientific Institute, Milan, Italy; ³Universita' Vita-Salute San Raffaele, Milan, Italy; ⁴Transplantation Research Center, Brigham and Women's Hospital/Children's Hospital/Harvard Medical School, Boston, USA; ⁵Department of Clinical Science, La Sapienza University Rome, Italy.

***Both authors contributed equally to the work.**

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Address for correspondence:

Paolo Fiorina, MD,
Department of Medicine,
San Raffaele Scientific Institute, Università Vita e Salute,
Via Olgettina 60, 20132 Milan, Italy.
Email: paolo.fiorina@hsr.it

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ABSTRACT

Objective: To evaluate whether islet transplantation may stabilize polyneuropathy in uremic type 1 diabetic patients (ESRD+T1DM) who received a successful islets after kidney transplantation (KI-s).

Research Design and Methods: 18 KI-s patients underwent electroneurographic tests of sural, peroneal, ulnar, and median nerves: the patient nerve conduction velocity (NCV) index and amplitudes of both sensory action potentials (SAPs) and compound motor action potentials (CMAPs) were analyzed longitudinally at 2, 4, and 6 years after islet transplantation. Skin content of advanced glycation end products (AGEs) and expression of their specific receptors (RAGE) were also studied at the 4-year follow-up. Nine ESRD+T1DM patients who received kidney transplantation alone (KD) served as controls.

Results: The NCV score improved in the KI-s group up to the 4-year time point ($p=0.01$ versus baseline), and stabilized 2 years later, whereas the same parameter did not significantly change in the KD group throughout the follow-up period or when a cross-sectional analysis between groups was performed. Either SAP or CMAP amplitudes recovered in the KI-s group, while they continued worsening in KD controls. AGE and RAGE-levels in perineurium and vasa nervorum of skin biopsies were lower in the KI-s than in the KD group ($P<0.01$ for RAGE).

Conclusions: Islet transplantation seems to prevent long-term worsening of polyneuropathy in ESRD+T1DM patients who receive islets after kidney transplantation. No statistical differences between the two groups were evident at cross-sectional analysis. Reduction in AGE/RAGE expression in the peripheral nervous system was shown in patients receiving islet transplantation.

Abbreviations: NCV, nerve conduction velocity; SAP, sensory action potential; CMAP, compound motor action potential.

INTRODUCTION

Chronic sensorimotor diabetic polyneuropathy (DPN) is a common long-term complication of type-1 diabetes mellitus, affecting more than 50% of patients (1). Electroneurographic studies, based on nerve conduction velocity (NCV) studies, represent an objective method for DPN assessment (2) and may also predict mortality (3). Among treatments that may prevent either DPN onset or progression by restoring normoglycemia, pancreas transplantation has been widely studied with NCV in the recent past (4-5). Until now, little has been known of the effect of islet transplantation on DPN.

Indications of allogenic pancreatic islet transplantation in type 1 diabetes mellitus have been expanding over the last few years (6), thanks to recent improvements in long-term graft survival rates (7) that depend on advances in islet isolation and purification and new immunosuppressive protocols (8). Compared with the whole-organ transplant, which has a 5% mortality rate 1 year after surgery and severe surgical complications (9), islet transplantation is a minimally invasive therapeutic approach, allowing for long-term insulin independence and metabolic control (7-8).

However, the question of whether long-term diabetes complications may be halted or even reversed by transplantation is still under investigation. Immunosuppressive treatment may interfere with renal function (10), yet the protective role of islet transplantation on both long-term graft survival and function of the transplanted kidney in type 1 diabetic patients has been demonstrated (11-12). Benefits for either macro/microangiopathy or cardiovascular function were reported in type 1 diabetic kidney-transplanted patients (13-14), as well as for retinal complications (15-16). Stabilization of peripheral neuropathy was reported following islet transplantation alone, i.e., not associated with kidney transplantation (15-16).

The aim of this study was to evaluate whether islet transplantation might stop the progression of neuropathy in type 1 diabetic patients with end-stage renal disease (ESRD+T1DM) bearing a kidney graft: thus, we performed longitudinal NCV studies in a group of uremic

type 1 diabetic, kidney-transplanted patients throughout the 6 years following islet transplantation. We further investigated cross-sectionally at the 4-year follow-up the skin expression of advanced glycation end products (AGEs) and their specific receptor (RAGE), which were reported to play a role in the pathogenesis of DPN (17).

RESEARCH DESIGN AND METHODS

Among all consecutive ESRD+T1DM patients who had received a successful islet after kidney transplantation from 1991 until January 2004, 18 (KI-s [successful islet after kidney] group: age=38.7±5.7 years; male/female ratio 8/10) with a sustained C-peptide secretion (>0.5 ng/ml) for longer than 6 months levels higher, were referred to our laboratory for electroneurographic assessment of their polyneuropathy, which had been previously diagnosed on the basis of clinical findings (18). Nine ESRD+T1DM patients were considered as a control group (KD [kidney only] group: age=39.1±2.2; male/female ratio 4/5). The efficacy of islet function in the KI-s group was assessed by fasting circulating C-peptide levels. KI-s patients were followed for an average of 53.4±7.1 months after transplantation. Any patients who have lost islet function early after transplant (within 6 months) were enrolled in the KD group. If the transplanted islets produced C-peptide (>0.5 ng/ml) the patient was considered in the KI-s group.

Sub-analysis of the KI-s group

Within the KI-s group, different patients appeared to have a different degree of metabolic control; therefore, a sub-analysis of the patients who reached full islet function (fasting C-peptide > 1 ng/ml) was performed.

Diabetes management after kidney-islet transplantation

Insulin therapy was used after transplantation to maintain strict glycometabolic control in patients. In some patients oral hypoglycemic agents were used to improve islet function, particularly when a clear insulin resistance was evident on the basis of very high doses of insulin requirement.

Laboratory assessment

Fasting levels of cyclosporine, creatinine, glycated hemoglobin (A1C), serum C-peptide, total cholesterol, and triglycerides were assayed

at baseline and 2, 4, and 6 years after transplantation (19). Serum C-peptide levels (intra-assay CV, 3.0%; interassay CV, 3.0%) were assayed by RIA using commercial kits (Medical System, Genova, Italy).

Islet transplantation

Both kidneys and islets came from cadaver donors; transplantation was performed according to HLA matching for kidney graft, while ABO compatibility was used for islet transplantation (13). The cross-match test was negative in all cases. The details of the procedure can be found in the online supplement (available at <http://care.diabetesjournals.org>).

Nerve conduction study

Nerve conduction study was performed following standard laboratory procedures by an operator who did not know what group the patient belonged to. The details of the procedure can be found in the online supplement.

Nerve conduction velocity index (NCV index)

Besides standard conduction parameters, an NCV index was attributed to each patient, as already reported (20). Briefly, conduction velocities of each nerve segment (motor conduction velocities of deep peroneal and ulnar nerves; sensory conduction velocities of sural nerve and either wrist-finger or elbow-wrist segments of median nerve) were first considered to obtain 5 *nerve* NCV Z-scores [(patient's NCV value – mean NCV value in control healthy subjects)/standard deviation of the same nerve in control healthy subjects]. The *patient* NCV index was the mean of each nerve NCV Z-score. Age-related normative values (mean and SD) of our electromyography laboratory were considered for calculation. NCV index estimates to what extent individual NCV values deviate from the mean value of a reference population in terms of standard deviation, limiting the specific intraindividual variability for each nerve trunk and allowing for an easier longitudinal evaluation. Due to the method of NCV index calculation, most negative NCV values identify most severe polyneuropathies. In cases with complete nerve unexcitability, the last available NCV value was considered for NCV index calculation.

Skin biopsy

Patients underwent skin punch biopsy on the

internal surface of the arm 4.5±1.2 years after transplantation as described elsewhere (13). The procedure is easy, minimally invasive, and well tolerated by patients. Samples were taken with the patient's consent and Ethical Review Board approval.

Advanced glycation end-product (AGE) and receptor for AGE (RAGE) quantification

N^ε-(carboxymethyl) lysine (CML)-protein adducts content and RAGE expression were assessed in paraffin-embedded sections by immunohistochemistry. Immunoreactivity for CML and RAGE in nerves and perineural vessels was evaluated with a semiquantitative scale (-: absent; +: mild; ++: moderate; +++: strong staining). The details of the procedure can be found in the online supplement.

Statistical analysis

Due to the limited sample size, statistical analysis was performed by means of non parametric tests. We performed the following comparisons: 1) NCV indexes at the different follow-up examinations (2 years, 4 years and 6 years) were compared with basal values by means of Wilcoxon signed Rank sum test for paired samples, in the entire sample and in the KI-s and KD sub-groups; 2) change from baseline of NCV indexes at the different follow-up examinations (2-year, 4-year and 6-year) were compared between the KI-s and KD groups by means of the Wilcoxon Rank sum test. We also performed a repeated measures analysis of variance to simultaneously explore the role of the time of evaluation, defined as a within-subject factor and of the group of treatment (KI-s vs. KD), defined as a between-subject factor, and their interaction on the NCV index value. This type of analysis permits to take into account the correlation on measures performed on the same subject, and to explore the role of the time of evaluation and the treatment on the NCV index.

RESULTS

Population and metabolic variables

The 2 groups of recipients had similar pretransplant and peritransplant characteristics, particularly the pattern of rejection episodes, CMV infections, and kidney re-transplantation (data not shown). The mean number of HLA matches for the kidney and PRA levels were similar in the two groups (data not shown), as were immunosuppression, lipid profile, and

medications. After steroid withdrawal, no kidney or islet rejections were evident. At 6 months after islet transplantation, when almost all the patients had completed the steroid withdrawal, a significant reduction in A1C was evident (data not shown) in the whole group of patients.

No significant intergroup differences were found with regard to baseline body weight (KI-s=61.3±9.6 vs. KD=62.0±2.2), diabetes duration (KI-s=26.3±10.4 vs. KD=22.2±1.4), and dialysis duration (KI-s=42.5±6.2 vs. KD=27.6±4.1). Among follow-up data, the main result was the mean creatinine value in the KD group, which increased significantly over baseline at 6 –years' follow-up (p=0.004, see Table 1). These results confirm previous reports of a protective effect of transplanted islets on kidney grafts. C-peptide secretion was higher in the KI-s group and absent in the KD group (Table 1); insulin requirement was lower in the KI-s group than in the KD group (data not shown), with better glycometabolic control (Table 1).

NCV index

No significant differences were observed in pretransplant NCV scores between KI-s and KD patients (p=0.6). Both KI-s and KD groups were neuropathic at baseline according to electroneurographic findings, showing NCV index > 2 (e.g., with an NCV score exceeding mean normal value by more than 2 standard deviations). The longitudinal NCV index study showed that, at the 2-year follow-up, both KI-s and KD groups scored a little better than at baseline; at the following time-point (4 years after transplant), however, NCV index continued to improve only in the KI-s group, reaching statistical significance in comparison with pretransplant values at 4 years of follow-up (p=0.01), whereas NCV worsened toward baseline values in the KD group (p=ns). At the latest follow-up (6 years), NCV score improvement in comparison to baseline values was maintained in the KI-s group, while it further worsened in the KD group (Table 1). When we compared the NCV changes from baseline across the two groups, despite the evidence of a statistically significant difference at the 4-year follow-ups in the KI-s group, results were not statistically significant. In particular, median change from baseline at each time-point showed differences between KI-s and KD group which did not reach statistical

significance (0.41 versus -0.08, 0.55 versus 0.17 and 0.11 versus -0.03 at 2-, 4- and 6-year follow-up respectively).

Compound Motor Action Potential (CMAP)

In the KI-s group, longitudinal trend of CMAP mean amplitudes showed slight, though not significant, improvements of either peroneal or ulnar nerves at the 6-year follow-up compared with baseline values. On the contrary, CMAP amplitudes of both nerves progressively declined in KD patients over time; the worsening was statistically significant for ulnar CMAP mean amplitude 6 years after kidney transplantation (9.7±3.0 vs. 13.7±4.6 mV; p=0.03; see Table 1).

Sensory Action Potential (SAP)

A slightly improving trend of SAP amplitudes through the different time points up to the 6-year follow-up was also recognized in patients of the KI-s group, even though statistical significance was lacking. In general, this was true for both median and sural nerves, but only sural SAP amplitudes changed from neuropathic to normal values, whereas median SAP values started within the normal range at baseline. In the KD group both median and sural SAP amplitudes fluctuated slightly through the different time-points, but values always remained in a neuropathic spectrum (Table 1).

Regression model

Repeated measures analysis of variance showed that the type of treatment (between-subject factor) did not influence the NCV index (p: 0.5), and also the time of evaluation (within-subject factor), and their interaction were not statistically significant (p: 0.4 and 0.4). It is also worthwhile to notice that the 2-year response to treatment was maintained at the 4-year follow-up, since all of the patients with an improving 2-year NCV index had a further increase 2 years later.

Skin biopsy analysis and AGE/RAGE expression

An overall morphological analysis of skin biopsies showed dramatically the effect of diabetes and uremia on skin innervation (Figure 1). Compared with healthy controls (Figure 1A), the skin of uremic type 1 diabetic patients appeared to be completely denervated. Even the sweat glands showed no evidence of residual innervation (Figure 1B, insert).

Paraffin-embedded sections of skin biopsy specimens were analyzed for CML and RAGE content by immunoperoxidase technique. In the KD group (Figure 1C), the perineurium of peripheral nerves (asterisk) and vasa nervorum (arrows) in dermis showed strong staining for CML, while the KI-s group (Figure 1D) showed only mild staining. The score of CML expression (Figure 1E) differed between the two groups, though not significantly ($p=0.07$). Likewise, strong RAGE expression was observed in the bundles of axons and vasa nervorum in the KD group (Figure 1F), whereas only mild staining of the same tissue structures was evident in the KI-s group (Figure 1G). The score of RAGE expression (Figure 1H) confirmed the higher expression of RAGE in the KD than in the KI-s group ($p<0.01$).

Sub-analysis of the KI-s group according to the degree of metabolic control

Nine patients of the KI-s group experienced a full function (average= 52.0 ± 11.8 months) of transplanted islets, and 5 of them remained off of insulin for more than 2 years. Interestingly, an improvement in NCV index was evident in the group of patients who achieved the better glycometabolic control and the withdrawal of insulin therapy. Particularly, the NCV index improved in the full function group from a baseline value of -2.9 ± 0.3 to -2.0 ± 0.4 at 4 years after islet transplantation. This tendency was confirmed for sural SAP amplitude too, which improved from 16.2 ± 13.3 at baseline in the full function group to 20.1 ± 17.1 4 years after islet transplantation. This was not evident in the group which did not experience full function of the transplanted islets (data not shown).

Limitations of the study

We acknowledge that a randomized trial would be the only way to determine if islet transplantation can clearly improve diabetic neuropathy and be superior to intensive insulin treatment. The persistence of islet allograft function contributed to improved glycemic control and therefore to less severe diabetic complications. Similar results most probably will be obtained with better glycemic control in the control group as demonstrated by DCTT (5).

DISCUSSION

This study provided several evidences, coming from both physiological and pathological sources, that islet transplantation may induce long-lasting stabilisation of DPN. At a further cross-sectional analysis, however no statistical differences were evident between the two groups. It is likely that the reason for the lack of significance is the small number of patients and the low statistical power for NCV differences over time.

This evidence is first supported by an objective method, electroneurography, which is the most sensitive method of assessing DPN (21). In fact, the NCV index increased in the KI-s group from the first control (2 years), reaching its maximum improvement at the 4-year follow-up, before stabilizing at the latest time-point (6 years). On the other hand, the NCV index showed some improvement in the KD group as well, but it was short-lived (2 years), as previously experienced (20); then, the NCV index declined toward the pretransplant values after the 4-year follow-up and further worsened by 6 years. Since pretransplant variables of age, gender, laboratory values, and electroneurographic findings did not differ at baseline between groups, we can conclude that this result is not affected by a bias in the groups' selection, but it is likely due to the efficiency of islet function.

There are several reasons for this favourable trend of DPN in our KI-s patients. First, the islet function may prevent the well-known nephropathy of the transplanted kidney (11-12), as suggested by longitudinal behaviour of creatinine levels (stable in KI-s group and significantly worsening in KD group). Thus, islet transplantation would eliminate an important neuropathic noxa such as chronic renal disease (22). An additional benefit may be due to higher levels in KI-s patients of the C-peptide, which was reported to improve nerve function in both experimental and clinical settings (23). The feature we would like to highlight is that, despite wide and dramatic skin denervation at baseline, a significant reduction in vasa nervorum RAGE expression was found in patients with islet function at the 4-year follow-up, which clearly demonstrates that islet function reverses a primary pathogenetic mechanism specifically related to DPN (2, 17).

Intensive insulin treatment was in fact shown to significantly improve peripheral nerve function, both autonomic and sensorimotor, in type 1 diabetic patients, compared with conventional therapy; in the secondary-intervention cohort patients, i.e., those who had neuropathy at baseline as in our population, though less severe, intensive therapy reduced the appearance of clinical neuropathy at 5 years by 57% (5). The injurious effect of chronic hyperglycemia on vessels and nerves has been attributed to various biochemical consequences of intracellular metabolism of excess glucose, including nonenzymatic glycation with formation of AGEs (24). AGEs are heterogeneous compounds originating from precursors formed both nonoxidatively and oxidatively, the latter including the monolysyl adduct CML, which has been detected in peripheral nerves from diabetic patients (25). In addition to direct, physicochemical effects, such as trapping and cross-linking of macromolecules, AGEs exert indirect, biological effects, mediated by cell surface receptors. RAGE, whose expression is positively regulated by AGEs (26), is the prototypic AGE receptor mediating AGE-induced tissue injury via induction of reactive oxygen species formation and activation of redox-sensitive signalling pathways, and CML is a major RAGE ligand (27). The finding that, despite a comparable, dramatic degree of skin denervation, KI-s patients showed a significant reduction in RAGE expression (associated with a nonsignificant decrease in CML content) in nerves and vasa nervorum, compared with KD patients, supports a role for the down-regulation of the AGE-RAGE pathway as a molecular mechanism underlying the improvement of neuropathy observed after successful islet transplantation.

Previous works have already reported beneficial effects of islet transplantation on diabetic polyneuropathy (24, 25), even though there are relevant differences from our study. First, both the above-mentioned studies included T1DM and not ERSD+T1DM patients. Hence, neuropathy was supposed to be even more severe in our study, and the role of islet transplant is a great deal strengthened by our study. Second, the peripheral nerve function was previously assessed with nerve conduction velocities only by Lee and co-

workers (25), even though the follow-up period lasted no longer than 2 years; in the paper by Várkonyi and colleagues (24), the follow-up was as long as 9.5 years on average, but they evaluated DPN only with a perceptive test of sensory threshold. Therefore, this is the first study based on both nerve conduction study and skin biopsy demonstrating that islet transplant may induce long-lasting stabilization or even improvement of polyneuropathy in type 1 kidney-transplanted diabetic patients.

Looking at the other electroneurographic variables such as the amplitude of both SAPs and CMAPs, which are currently recognized as indicator of axon integrity in peripheral neuropathies (27), we found that CMAP amplitude remained stable throughout the follow-up period in the KI-s group. On the contrary, the amplitude of both peroneal and ulnar nerve CMAPs declined progressively through the follow-up period in the KD group (with significant difference for the 6-year ulnar nerve CMAP from baseline: see Table 1), as though axonal damage were still progressing after isolated kidney transplant. The amplitude of both sural and median nerve SAPs increased slightly through the different time-points in the KI-s group, while the same parameter showed a fluctuating pattern in KD patients, suggesting that islet transplant positively affected even sensory nerve fibers; however, this conclusion must be taken with caution, due to the lack of statistical significance and somewhat heterogeneous baseline values.

Hence, changes of each electroneurographic variable depict distinct aspects of DPN influenced by islet transplant. In fact, since NCV changes are related to glycemic control (20), improving NCV index means an overall improvement of patient nerve function. As NCV mainly reflects pathological processes of large-diameter axon (27), we also included, among electroneurographic variables, the longitudinal analysis of either SAP or CMAP amplitudes, which were suggested as a means of assessing the contribution of smaller, slowing conduction nerve fibers (2). These latter data are in keeping with evidence of RAGE expression in vasa nervorum and small skin nerve terminals in our patients. Taken together, electroneurographic and skin biopsy studies allowed for longitudinal, long-term assessment of DPN patients and served as

surrogate markers of the restored glycaemic control.

In light of the advantages suggested in our paper, islet transplantation could become an option for improving quality of life for diabetic patients with brittle diabetes, life-threatening hypoglycaemia unawareness, and even severe, often painful forms of polyneuropathy. More studies with larger number of patients are required to definitely clarify if the positive trend observed in our preliminary study can ultimately result in a strong positive association.

In our small group of patients there were no differences with the kidney only transplantation. Islet transplantation in addition to kidney transplantation makes no difference to nerve function when compared with a kidney only.

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Table 1. NCV index and other electroneurographic findings (baseline and longitudinal). NCV: nerve conduction velocity; SAP_{ampl}: sensory action potential amplitude (expressed as μV = microvolt); CMAP_{ampl}: compound motor action potential amplitude (expressed as mV = millivolt); Values are shown as mean \pm standard deviation. * $p < 0.05$ versus basal values.

Groups	Variables	Basal	2 years	4 years	6 years
KI-s	Pts number	18	18	18	9
	Age (years)	41.8 \pm 6.2			
	A1C (%)	8.0 \pm 1.1	7.7 \pm 1.8	7.4 \pm 1.8	7.5 \pm 0.4
	Creatinine (mg/dl)	1.6 \pm 1.5	1.3 \pm 0.5	1.4 \pm 0.7	1.1 \pm 0.2
	C-peptide (ng/ml)	0.1 \pm 0.1	1.8 \pm 1.0	1.1 \pm 0.5	1.4 \pm 1.1
	NCV index	- 2.9 \pm 0.9	- 2.8 \pm 1.1	- 2.6 \pm 1.0*	- 2.7 \pm 0.9
	Sural SAP_{ampl}	7.9 \pm 3.2	9.0 \pm 8.1	16.4 \pm 25.3	13.7 \pm 19.0
	Median SAP_{ampl}	16.7 \pm 9.0	19.6 \pm 10.1	19.4 \pm 11.1	20.0 \pm 15.6
	Peroneal CMAP_{ampl}	3.0 \pm 4.2	2.5 \pm 2.6	2.8 \pm 3.9	4.4 \pm 4.5
	Ulnar CMAP_{ampl}	10.2 \pm 4.0	9.5 \pm 3.3	10.3 \pm 3.0	11.6 \pm 2.2
KD	Pts number	9	9	9	9
	Age (years)	39.1 \pm 2.2			
	A1C (%)	11.1 \pm 2.3	8.0 \pm 0.4	8.6 \pm 0.4	8.1 \pm 0.4
	Creatinine (mg/dl)	1.7 \pm 0.1	1.9 \pm 0.2	2.0 \pm 0.3	2.5 \pm 0.7*
	C-peptide (ng/ml)	0.1 \pm 0.1			
	NCV index	-2.7 \pm 1.2	-2.5 \pm 0.9	-2.6 \pm 1.0	-2.8 \pm 1.1
	Sural SAP_{ampl}	4.4 \pm 2.8	3.6 \pm 0.8	6.5 \pm 0.7	6.3 \pm 7.3
	Median SAP_{ampl}	13.3 \pm 6.8	15.7 \pm 9.0	17.8 \pm 5.0	11.8 \pm 6.6
	Peroneal CMAP_{ampl}	3.9 \pm 3.2	3.5 \pm 2.5	2.0 \pm 1.8	3.2 \pm 3.6
	Ulnar CMAP_{ampl}	13.7 \pm 4.6	10.6 \pm 5.1	12.4 \pm 3.3	9.7 \pm 3.0*

A1C, Glycated Hemoglobin:

Figure Legend

Figure 1. Comparison with skin biopsies of control patients (Panel A) showed dramatically the skin denervation in uremic type 1 diabetic patients (ESRD+T1DM) (Panel B). Even sweat glands appeared to be completely denervated (Panel B insert). **Immunohistochemistry for N ϵ -(carboxymethyl) lysine (CML).** Paraffin-embedded sections of skin biopsy specimens stained with immunoperoxidase technique. CML was detected by binding to anti-CML monoclonal antibody, biotin-conjugated. KD group (Panel C): Peripheral nerve (asterisk) with adjoining blood vessels (vasa nervorum, arrows) in dermis. There is a strong (+++) staining of the perineurium and vasa nervorum. KI-s group (Panel D): another skin biopsy section with only mild (+) staining of the same tissue structures. Original magnification, 400X. The score of CML expression (Panel E) showed a difference between the two groups despite no statistical significance ($p=0.07$). **Immunohistochemistry for RAGE.** Paraffin-embedded sections of skin biopsy specimens stained with immunoperoxidase technique. RAGE was detected by binding to anti-human RAGE goat polyclonal antibody followed by a biotinylated anti-goat IgG antibody. KD group (Panel F): Peripheral nerve (asterisk) with adjoining blood vessels (vasa nervorum, arrows) in dermis. There is a strong (+++) staining of the bundles of axons and vasa nervorum. KI-s group (Panel G): Another skin biopsy section with only mild (+) staining of the same tissue structures. Original magnification, 400X. The score of RAGE expression (Panel H) showed a higher expression of RAGE in the KD group ($p<0.01$).

