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Peripheral sensory and cardiovascular autonomic dysfunction in kidney transplant patients

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Abstract

Introduction: Kidney transplantation (KTx) is the preferred treatment for end-stage renal disease. Patients with chronic kidney disease (CKD) have an increased risk for arrhythmias and sudden cardiac death. The autonomic nervous system dysfunction seems to participate in the arrhythmogenic process. Limited information is available on peripheral sensory neural impairment, cardiovascular autonomic dysfunction and QT interval variability abnormalities following successful kidney transplantation. The present study aimed to assess the peripheral and autonomic neuronal functions in transplanted patients and to compare it to age- and sex-matched healthy controls.

Subjects and methods: Our study involved 23 KTx patients. The control group consisted of 19 age- and gender-matched healthy individuals. All KTx patients and healthy controls successfully underwent a 10-minute 12-leads ECG for repolarization parameter analysis, 5 standard Ewing tests for evaluation of the cardiovascular autonomic neuropathy and a complex peripheral neuronal testing.

Results: According to the Ewing tests, the heart rate response to deep breathing (controls vs. KTx patients: 21.21 ± 6.93 vs. 16.7 ± 5.89 beats/min., p = 0.045), the heart rate response to standing up (30/15 ratio controls vs. KTx patients: 1.20 ± 0.15 vs. 1.07 ± 0.16, p = 0.007), and the systolic blood pressure response to standing up (controls vs. KTx patients: 4.63 ± 6.07 vs. 12.26 ± 13.66 mmHg, p = 0.022) were significantly impaired in the KTx patients. Significant differences could be demonstrated in the peripheral sensory function of peroneal and median nerves at all three tested frequencies with Neurometer®. At the median nerve the sensory testing revealed increased perception thresholds compared to controls (controls versus KTx patients at 2 kHz: 157.8 ± 61.5 vs. 278.3 ± 84.35, p < 0.001, at 250 Hz 48.0 ± 42.56 vs. 112.2 ± 91.12, p = 0.005, at 5 Hz 28.95 ± 23.14 vs. 77.96 ± 71.24, p = 0.004, respectively). The parameters of the peroneal nerves were also significantly elevated (controls versus KTx patients at 2 kHz: 288.6 ± 98.22 vs. 453.4 ± 175.9, p < 0.001, at 250 Hz: 156.8 ± 82.89 vs. 262.3 ± 168.9, p = 0.013, on 5 Hz 82.0 ± 58.97 vs. 142.5 ± 129.4, p = 0.053, respectively). The application of Neuropad®-test, Tiptherm®, Monofilament®, and Rydel-Seiffer tuning fork did not reveal significant differences between KTx patients and controls. The repolarization parameters of KTx patients and controls were similar.

Conclusions: Cardiovascular autonomic dysfunction and peripheral sensory neural impairment were detected in KTx patients compared to healthy volunteers. A prospective follow up of this population and a cross-sectional study with controls suffering in CKD without kidney transplantation are planned to evaluate a possible protective function of the successful kidney transplantation.

Keywords: peripheral sensory dysfunction, cardiovascular autonomic dysfunction, kidney transplantation

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Background

Chronic kidney disease (CKD) has a high global prevalence [Hill 2016] and patients with CKD have an increased risk of mortality due to cardiovascular disease [Herzog 2011]. The risk of sudden cardiac death proportionally increases as kidney function deteriorates [Pun 2009]. Coronary artery disease-related risk factors do not explain this high risk in patients with CKD completely

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[Herzog 2011, Chonchol 2008]. KTx patients are regarded as a special subpopulation among patients with CKD. Peripheral sensory neural impairment, cardiovascular autonomic dysfunction and cardiac repolarization abnormalities might develop in CKD which all could predict or contribute to sudden cardiac arrest.

Cardiovascular autonomic dysfunction due to resistant hypertension, orthostatic and intradialytic hypotension, reduced heart rate variability or impaired spontaneous baroreflex sensitivity are exceptionally important complications of CKD, and all might be responsible for the high incidence of cardiovascular mortality in this patient population [Salman 2015]. The impaired baroreceptor, cardiopulmonary and chemoreceptor reflex function, activation of the renal afferents, accelerated renin-angiotensin-aldosterone system activity [Salman 2015] and cardiovascular structural remodelling have been suspected in CKD as further fatal risk factors [Chou 2016]. Krishnan and Kiernan reviewed [Krishnan 2009] the potential neurologic dysfunctions induced by uremic toxins such as urea, creatinine, parathyroid hormone, myoinositol and β2-microglobulin [Meyer 2007]. Although uremic toxins could directly alter the small nerve fibers via hydroelectrolytic changes producing expansion or shrinkage of the endoneurial space, the exact pathogenesis of the neuronal damage is not completely elucidated [Krishnan 2009]. Several previous studies demonstrated a significant regression of some neuropathic symptoms and improvement of electrophysiological indices in pancreas and kidney recipients, mostly with advanced neuropathy [Solders 1992, Navarro 1997, Allen 1997]. There is limited information on cardiovascular autonomic and sensory peripheral neuropathy, as well as cardiac repolarization abnormalities after KTx, therefore the present study aimed to assess and to compare it to healthy controls.

Methods

Study population

The present study comprised 23 KTx patients (mean ± SD; age: 50.4 ± 6.46 years, 11 males) who have regular care at the Department of Surgery, University of Szeged, Hungary. Inclusion criteria for the KTx patients were: first cadaver kidney transplantation, age of men: 18 – 55, age of women: 18 – 60. The mean time that elapsed since the transplantation in the KTx group was 8.55 years. The control group consisted of 19 age- and gender-matched healthy volunteers (age: 49.3 ± 7.31 years, 9 males). All of the control subjects and patients with KTx were Caucasian. The relevant clinical data of KTx patients and control subjects are shown in **Table 1**.

The measurements were performed from May 2018 to April 2019. All KTx patients and healthy controls successfully underwent the following examinations: 10-minute 12-leads ECG via Cardiosys EXTRA software (CAR03-IA, MDE Heidelberg GmbH, Heidelberg, Germany) for repolarization parameter analysis expressed as short-term QT interval variability (STVQT), 5 standard Ewing tests for evaluation of the cardiovascular autonomic neuropathy and a complex peripheral neuronal testing with Neurometer®, Neuropad®-test, Tiptherm®, Monofilament® and Rydel-Seiffer tuning folk.

12-lead electrocardiogram (ECG)

Both patients with kidney transplantation and controls were examined in supine position after 5 minutes of rest. The 12-lead ECG was performed continuously for 10 minutes in the same position to avoid motion artefacts as much as possible. The ECG signal digitization was performed by a multichannel data acquisition system (CAR03-IA, Cardiosys EXTRA software, MDE Heidelberg GmbH, Heidelberg, Germany), the sampling rate was 2,000 Hz, and data were stored for later analysis.

All data acquired from patients with kidney transplantation (KTx) and control subjects were suitable for analysis, no data were excluded on the basis of exclusion criteria determining the parameters for unbiased ECG: several (> 5 %) ectopic atrial or ventricular beats, none-sinus rhythm, abnormal repolarization (i.e. early repolarization,

	KTx patients (n=23)	Controls (n=19)	p
Clinical data			
Age (year)	50.4±6.5	49.3±7.3	0.633
Weight (kg)	79.5±14.0	78.8±18.8	0.913
Height (m)	170.5±9.7	173.1±11.0	0.258
Body mass index (kg/m²)	27.4±4.2	26.1±4.7	0.428
Male sex, n (%)	11 (48)	9 (47)	0.977
Systolic BP (mmHg)	144.4±16.7	133.3±19.1	0.095
Diastolic BP (mmHg)	85.0±9.1	82.2±11.3	0.519
Smoking history, n (%)	9 (39)	2 (11)	0.030
Alcohol consumption, n (%)	1 (4)	4 (21)	0.126
Hypertension, n (%)	19 (83)	4 (21)	<0.001
Hypercholesterolemia, n (%)	7 (30)	3 (16)	0.268
Diabetes mellitus, n (%)	8 (35)	0 (0)	0.002
Medication			
β-blocker, n (%)	9 (39)	2 (11)	0.030
ACE inhibitor or ARB, n (%)	14 (61)	2 (11)	0.0003
Ca-antagonist, n (%)	11 (48)	1 (5)	0.001
Antidiabetics but not insulin, n (%)	4 (17)	0 (0)	0.043
Insulin, n (%)	2 (9)	0 (0)	0.162
Statin, n (%)	4 (17)	2 (11)	0.530
Diuretics, n (%)	9 (39)	0 (0)	0.001
ASA/PAI, n (%)	4 (17)	3 (16)	0.893
Steroid, n (%)	13 (57)	0 (0)	<0.001
Tacrolimus, n (%)	20 (87)	0 (0)	<0.001
Everolimus, n (%)	2 (9)	0 (0)	0.162
Cyclosporin, n (%)	2 (9)	0 (0)	0.162
Mycophenolate mofetil, n (%)	18 (78)	0 (0)	<0.001
Laboratory data			
Haemoglobin (g/l)	129.7±19.2	142.4±12.7	0.015
Haematocrit (%)	39.2±4.6	41.9±3.5	0.042
Glucose (mmol/l)	5.7±2.1	5.0±0.5	0.136
Blood urea nitrogen (mmol/l)	11.4±5.7	5.4±2.4	<0.001
Creatinine (µmol/l)	162.2±93.9	79.3±17.3	<0.001
eGFR (ml/min/1.73 m²)	47.7±22.4	89.4±15.6	<0.001
Uric acid (µmol/l)	332.8±73.6	270.9±17.3	0.015
C-reactive protein (mg/l)	4.3±5.2	0.90±2.7	0.012
Albumin (g/l)	46.1±3.3	49.8±3.1	<0.001
Cholesterol (mmol/l)	5.2±1.1	5.6±0.8	0.123
Triglyceride (mmol/l)	2.0± 1.0	1.6± 1.1	0.215
HDL-cholesterol (mmol/l)	1.4±0.3	1.6±0.4	0.089
LDL-cholesterol (mmol/l)	2.8±0.8	3.4±0.70	0.032

Abbreviations: BP, blood pressure; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; Ca, calcium; ASA, acetylsalicylic acid; PAI, platelet aggregation inhibitor; eGFR, estimated glomerular filtration rate.

Table 1. Clinical data in the study groups

T-wave inversion, complete bundle branch block), permanent pacemaker activity, acute metabolic disorder, significant artefacts in the ECG signal recording, high amount of food intake during the last 3 hours, alcohol or caffeine consumption or smoking during the last 10 hours.

Repolarization was analysed by the following parameters: frequency corrected QT interval (QTc) using Bazett ($QTc = QT/\sqrt{RR}$), Fridericia ($QTc = QT/[RR/1{,}000]1/3$), Framingham ($QTc = QT + [0.154 \times \{1{,}000 - RR\}]$) and Hodges formulae ($QTc = QT + 1.75 \times [60{,}000/RR - 60]$), QT dispersion (QTd), terminal T-wave duration (Tpeak – Tend), frequency corrected Tpeak – Tend interval using Bazett and Fridericia formulae, and short-term variability of QT intervals (STVQT).

The RR and QT intervals as well as the duration of the T wave from the peak to the end (Tpeak – Tend) intervals were measured semi-automatically in 30 consecutive beats (minimum number of intervals needed for variability measurements) and were calculated as the average of 30 beats. The measurements of RR intervals, QT intervals and terminal T-wave duration (Tpeak – Tend) intervals were performed semi-automatically in 30 consecutive beats (minimum criterion for measuring variability) and then the averages were calculated. Conventional computerized QT measurement technique was used for the analysis of QT intervals; blinded QT interval checking and if necessary, manual repositioning of the automatically set fiducial cursor position were performed by the same investigator of the team [Baumert 2012]. The duration of QTc interval was determined as the average of the measured QTc intervals. PQ and QRS intervals were determined as the average of the measured intervals of 15 consecutive beats. The measurements were performed in lead II or lead V5, if significant noise were present in the former.

Poincaré plot analysis of the QT and RR intervals was performed to determine the temporal instability of the beat-to-beat heart rate and repolarization. Each QT and RR value was plotted against its former value. STVQT and STVRR were calculated using the

following formula: $STV = \Sigma|Dn+1 - Dn| (30x\sqrt{2}) - 1$, where D represents the duration of the QT and RR intervals. The calculation defines the STV as the mean distance of points perpendicular to the line of identity in the Poincaré plot and relies on previous mathematical analysis [Brennan 2001]. In order to reliably assess the duration of ventricular repolarization and to minimize the influence of changing heart rate on the Tpeak – Tend interval, frequency correction of the Tpeak – Tend was also performed by the Bazett, and Fridericia formulas.

Cardiovascular autonomic function testing

Autonomic neuropathy (AN), neuronal dysfunction and consequent cardiovascular changes were characterized by Ewing's five standard cardiovascular reflex tests (CRT) [Ewing 1982]. These tests are the gold-standards of the determination of autonomic dysfunction; they provide non-invasive, clinically relevant, standardized and reproducible data of autonomic functions. Reflex tests were performed by measuring the blood pressure and obtaining continuous 6-lead ECG signals. Then the signals were digitized by a multichannel data acquisition system (Cardiosys-A01 software, MDE Heidelberg GMBH, Heidelberg, Germany), sampling rate was 2 kHz and data were stored for later analysis.

Three out of five tests record heart rate changes when performing specific activities, while the rest of them measures the changes of blood pressure. Tests which record heart rate changes predominantly reflect the changes of parasympathetic function, while those based on blood pressure responses describe primarily sympathetic function disturbances [Ewing 1985]. Heart rate changes were measured during deep inhalation and exhalation, in lying and standing positions with 30/15 ratio, and during and after Valsalva manoeuvre [Spallone 2011]. Systolic blood pressure changes were measured after standing up from a lying position, while diastolic changes were recorded during gripping with the hand for 3 minutes. CRT-s were scored separately: 0 (normal), 1 (borderline), 2 (abnormal). The overall autonomic score (0 – 10) was counted from the sum of each test results to characterize the severity of AN.

Heart rate response to deep breathing

Physiologically, the heart rate increases on inhalation and decreases on exhalation. Patients were instructed to take deep breathes with six breaths per minute rate (inhale for five seconds and exhale for five seconds). The difference between the measured maximum and minimum heart rates (beats/min) were calculated during six cycles of breathing.

Heart rate response to standing up (30/15 ratio)

Normally, the heart rate increases promptly after standing up from a lying position and reaches the peak at about the 15th heartbeat after standing up. After that, a relative bradycardia presents in healthy individuals with the lowest rate at around the 30th beat. Patients were lying supine at the beginning of the test, then they were asked to stand up while the ECG was recorded continuously. The ratio of the longest R-R interval (around beat 30) and the shortest R-R interval (around beat 15) was calculated, and recorded as the 30/15 ratio.

Heart rate response to Valsalva manoeuvre (Valsalva ratio)

In healthy individuals during the holding period of the Valsalva manoeuvre blood pressure is decreased while heart rate is increased. After the manoeuvre, blood pressure increases and the heart rate decreases. Patients were asked to exhale into a specific manometer through a mouthpiece and hold their breath at 40 mmHg for 15 seconds. During that, ECG was continuously recorded. The ratio of the longest R-R interval following the test, and the shortest R-R interval during the manoeuvre was calculated and recorded as the Valsalva ratio.

Systolic blood pressure response from lying to standing up

Normally, in a standing position, the realignment of blood to the lower limbs is immediately compensated by vasoconstriction in the peripheral vessels. The test is performed by measuring the blood pressure in a lying position and after standing up. The orthostatic drop of blood pressure is determined by systolic blood pressure measured 10 minutes after lying supine, and those measured at 1, 5 and 10 minutes after standing up. The difference is calculated between the values measured at the lying position and those of standing up. The largest difference is recorded as the response to standing up.

Diastolic blood pressure response during a sustained handgrip

Changes of diastolic blood pressure were measured during sustained handgrip. At first, patients were asked to clamp a hand-held dynamometer with their dominant hand exerting full force to determine the maximal clamping force, and then a sustained clamping at 30 % force was instructed to hold for 3 minutes. Blood pressure was measured once in every minute on the contralateral, relaxed upper limb and the maximally increased diastolic blood pressure was recorded as the response to sustained handgrip.

Sensory nerve testing

Sensory function of the peripheral nerves was examined by a Neurometer device (NM-01/CPT Neurometer, MDE Heidelberg GmbH, Heidelberg, Germany). The equipment provides a non-invasive, simple testing technique and a possibility for quantitative analysis of the sensory nerve function and different types of nerve fibres [Lv 2015]. Transcutaneous, low voltage, sine-wave electrical stimulation was applied and the current perception threshold (CPT) was determined. This study tested the median and the peroneal nerves. The 1 cm diameter surface electrodes were positioned on the distal phalanx of the index finger and that of the hallux. The electrodes were fastened to intact skin surfaces to avoid disturbances of peripheral sensation by scars and wounds. The amplitude range of the applied stimuli was 0.01 to 9.99 mA. At the beginning of the test, current intensity was gradually increased until

the patient indicated sensation. Then short (2–5 sec) stimulations were applied at progressively lower intensities until the minimal intensity of consistent sensation was determined. CPT intensities were determined at three different stimulation frequencies (2000 Hz, 250 Hz, 5 Hz) for both the upper and the lower limbs.

Sudomotor dysfunction, which frequently occurs in autonomic neuropathy, was examined by Neuropad® screening tests for all patients and controls [Zick 2003]. The test can detect neuropathy with very high sensitivity [Papanas 2005] on the basis of the fact that nerve fibre impairment in the distal extremities not only affects sensation, but perspiration as well and thus extreme dryness of the feet may occur. The adhesive pad of the kit contains anhydrous cobalt II chloride salt with blue colour, which reacts and change to pink when it absorbs water.

The tests were performed in room temperature (23 °C) following 10 minutes of rest after patients took off their shoes and socks. The pads were placed to the soles on both sides between the heads of the first and second metatarsi. The colour change was read at 10 minutes after the adhesion. Total decolouration to pink was considered normal, mixed pink and blue colour was evaluated as pending, while total blue colour was pathologic.

The 128-Hz Rydel-Seiffer graduated tuning fork was used to evaluate the vibration perception threshold at the distal end of the radius and at the level of the halluces. Results of the tuning fork examination were compared to age-dependent normal values published by Martina et al. in 1998 [Martina 1998]. On a scale of 1–8, the normal range was 7–8, borderline was 6, and pathologic was 1–5 reflecting impaired sense of vibration.

The Semmes-Weinstein Monofilament Test® using a 10 g monofilament is a simple device for objective screening of a diabetic foot for protective sensation loss [Abbott 2002]. Proper calibration is essential for the instrument, which was applied in calm and quiet circumstances, and the tested individuals were blinded for the place and way of application of the filament. Five regions of the sole were examined in all candidates: hallux, first metatarsus, second metatarsus, heads of the third and the fifth metatarsus. Unaffected sensation in at least 4 regions was considered normal, while 0–3 was defined as abnormal.

The Tiptherm® (Tip-Therm GmbH, Düsseldorf, Germany) device can be used for the early diagnosis of polyneuropathy with symmetrical pattern. It is a pen-shape instrument with flat sides, which tests temperature sensitivity of the skin [Viswanathan 2002]. It contains a 14 mm diameter plastic cylinder and a 14 mm diameter metal cylinder on each end separately. The examiner touches the skin of the patient randomly with one end for 1 seconds on both hands and feet. The patient has to report which touch was colder [Ziegler2005]. In case of normal sensation of temperature (< 10 °C), the individual can differentiate between the two subjective sensations of the flat surfaces of the Tiptherm®.

	KTx patients (n = 23)	Controls (n = 19)	p
Heart rate (HR) variation during deep breathing (beats/min)	16.7 ± 5.9	21.21 ± 6.9	0.031
Valsalva ratio	1.6 ± 0.2	1.6 ± 0.3	0.338
30/15 ratio	1.1 ± 0.2	1.20 ± 0.2	0.007
Systolic BP fall after standing up (mmHg)	12.3 ± 13.7	4.6 ± 6.1	0.022
Diastolic BP increase after sustained handgrip (mmHg)	18.2 ± 8.5	17.1 ± 14.7	0.763
AN score	2.4 ± 1.6	1.4 ± 1.2	0.020

Values are represented as mean ± SD. 30/15 ratio, immediate HR response to standing; BP, blood pressure.

Table 2. Autonomic neuropathy (AN) parameters of KTx patients and age-matched control subjects

	KTx patients (n = 23)	Controls (n = 19)	p
NM2000	278.3 ± 84.4	157.8 ± 61.5	< 0.001
NM250	112.2 ± 91.1	48.0 ± 42.6	0.005
NM5	78.0 ± 71.2	29.0 ± 23.1	0.004
NP2000	453.4 ± 175.9	288.6 ± 98.2	< 0.001
NP250	262.3 ± 168.9	156.8 ± 82.9	0.013
NP5	142.5 ± 129.4	82.0 ± 59.0	0.053

Values are represented as mean ± SD. NM2000, CPT value of the median nerve at stimulating frequency of 2000 Hz; NM250, CPT value of the median nerve at stimulating frequency of 250 Hz; NM5, CPT value of the median nerve at stimulating frequency of 5 Hz; NP2000, CPT value of the peroneal nerve at stimulating frequency of 2000 Hz; NP250, CPT value of the peroneal nerve at stimulating frequency of 250 Hz; NP5, CPT value of the peroneal nerve at stimulating frequency of 5 Hz.

Table 3. CPT values of the upper and lower limbs at three different stimulating frequencies (2 kHz, 250 Hz, 5 Hz) in KTx patients and age-matched control subjects

Laboratory data

Venous blood samples were collected for the determination of serum glucose, HbA_{1c} levels, blood count, haemoglobin, blood urea nitrogen, creatinine, eGFR, uric acid, albumin, cholesterol, triglyceride, HDL- and LDL-cholesterol.

Statistical analysis

Statistical data were reported as the mean ± SD; with numbers (n) and percentages (%), when appropriate. Pearson's chi-squared test or Fisher's exact test was used to analyze categorical data, whereas independent samples t-test was used in case of continuous data. The connections between the continuous or ordinal variables were examined by Pearson's and Spearman's correlation analysis. Statistical tests were performed using R statistical software (R version 3.6.0, https://www.r-project.org/), values of $p < 0.05$ were considered significant.

Results

The frequency of smoking, hypertension and diabetes mellitus was higher in the KTx group compared to controls (**Table 1**). The patients in the KTx group took more kinds of drugs than the subjects in the control group (**Table 1**).

The analysis of the laboratory parameters of the KTx group revealed anaemia and hypalbuminaemia (**Table 1**). The kidney function described with creatinine and urea was significantly impaired in KTx patients (**Table 1**). LDL-cholesterol was higher in the KTx group as well compared to controls.

A significant impairment was found in the KTx patients at the heart rate response to deep breathing, at the heart rate response to standing up, and at the systolic blood pressure response to standing up. The AN score was also significantly higher among KTx patients vs. controls (**Table 2**).

In the KTx group the heart rate response to deep breathing showed a positive correlation with the eGFR in the ($p = 0.007$, $r = 0.549$). A significant negative correlation between the serum creatinine level and the Valsalva-ratio was detected also by the separate KTx group analysis ($p = 0.04$, $r = -0.432$). The time duration since transplantation correlated positively only with the systolic blood pressure ($p = 0.024$, $r = 0.468$) in the KTx group.

Significant differences were demonstrated in the peripheral sensory function of peroneal and median nerves in all three tested frequency with Neurometer®. At the median nerve the testing revealed increased thresholds (**Table 3**) in KTx patients versus controls at all tested frequencies (2 kHz: 157.8 ± 61.5 vs. 278.3 ± 84.35, $p < 0.001$, at 250 Hz: 48.0 ± 42.56 vs. 112.2 ± 91.12, $p = 0.005$, at 5 Hz: 28.95 ± 23.14 vs. 77.96 ± 71.24, $p = 0.004$, respectively). The peroneal nerve parameters were also significantly elevated (**Table 3**) in KTx patients (at 2 kHz 288.6 ± 98.22 vs. 453.4 ± 175.9, $p < 0.001$, at 250 Hz 156.8 ± 82.89 vs. 262.3 ± 168.9, $p = 0.013$, at 5 Hz 82.0 ± 58.97 vs. 142.5 ± 129.4, $p = 0.053$, respectively). At the application of Neuropad®-test, Tiptherm®, Monofilament® and Rydel-Seiffer tuning folk no further significant difference was identified.

A significant positive correlation ($p = 0.025$, $r = 0.46$) was found between plasma glucose and the CPT value of the peroneal nerve at stimulating frequency of 5 Hz in KTx patients. There was no correlation between the plasma glucose and the remaining measured parameters.

Comparison of the two groups (KTx patients vs. control) revealed no significant differences in heart rate, the PQ, QRS and QT intervals and the QT dispersion. Furthermore, significant differences in the QTc values calculated with the Bazett, Fridericia, Framingham and Hodges formulas and the beat-to-beat short-term temporal variability of the QT interval) were not identified. However, a significant decrease of the beat-to beat short-term temporal variability of the RR interval (STVRR) in KTx patients compared to healthy controls was detected (9.5 ± 8.9 vs 15.1 ± 8.6 ms $p = 0.044$). The duration of the T wave from the peak to the end (Tpeak – Tend) was also significantly decreased (93 ± 16 vs 102 ± 11 ms, $p = 0.032$) in the KTx group compared to the control group. The frequency corrected Tpeak – Tend intervals (Bazett: 101 ± 17 vs. 108 ± 11 ms; $p = 0.133$; Fridericia: 99 ± 16 vs 106 ± 11 ms; $p = 0.075$) showed no significant differences between KTx patients and controls.

Discussion

A definitive impairment of cardiovascular autonomic and peripheral sensory neuronal function was detected in patients after kidney transplantation in comparison to healthy volunteers in our study.

The leading cause of death in renal transplant recipients is a cardiovascular event [Lindholm 1995]. Cardiovascular autonomic neuropathy in diabetic patients is a risk factor of earlier mortality, as the impairment of the parasympathetic and sympathetic function leads to ventricular repolarization disturbances [Spallone 2011]. Chronic renal failure is also associated with an increased risk of cardiac arrhythmias and sudden cardiac death [Zochodne 2000]. In our study 3 of the 5 cardiovascular reflex tests were abnormal in KTx patients in comparison to healthy controls, reflecting a common alteration of the parasympathetic and sympathetic functions. Beside the impaired function, the imbalance between the two systems is also responsible for an elevated risk of cardiac events in this patient population. The fact that both functions became abnormal demonstrates the presence of a late autonomic damage in KTx patients due to a long-standing pathogenetic process. The significant effect of the renal morbidity on the cardiovascular autonomic function might be explained by our observations as the heart rate response to deep breathing was more altered if the eGFR was lower and heart rate response to Valsalva manouevre was more abnormal if the serum creatinine level was higher. The role of long-standing exposure is shown by the positive association between the systolic blood pressure and the time that elapsed since transplantations. In case of longer duration, higher systolic blood pressure was found.

Instability of cardiac repolarization, which manifests in QT interval dispersion, accompanied with an increased risk for sudden cardiac arrest, has not been found during the analysis of 12 parameters in KTx patients compared to controls.

The peripheral sensory tests with Neurometer® point towards hypaesthesia in the KTx patients compared to controls both on upper and on lower limbs. Hypesthesthetic condition is usually found on both the median and peroneal nerves in advanced stages of diabetic neuropathy [Apfel 2001]. This finding points to the possible alterations of limb sensations in KTx patients that explains several symptoms and impaired protective mechanisms against different injuries. The aetiology of the detected autonomic and peripheral sensory neuropathy in the KTx subjects is considered to be a complex pathogenetic process. Uraemic neuropathy as a peripheral neuronal dysfunction is a common complication of uraemia in patients with CKD, especially with a GFR below 12 ml/min/1.73m^2. The estimated prevalence of peripheral neuropathy in this case is 50 – 70 % [Vaziri 1981, Kiernan 2002, Krishnan 2006]. Uraemia-specific pathogenic mechanisms of peripheral neuropathy include accumulation of uremic toxins [Heidbreder 1985, Wittmann 2015], hyperkalaemia [Wittmann 2015, Kiernan 2002], thiamine deficiency, accumulation of slowly dialyzed neurotoxic molecules, such as methylguanidine, myoinositol, phenol derivatives, guanidinosuccinic acid, and parathyroid hormone [Baumgaertel 2014]. The accumulation of the advanced glycation end-products contributes to diabetic, but also to non-diabetic renal diseases [Wittmann 2015] and also might be responsible for neuropathy.

Kidney replacement therapy seemed to be effective in prevention of the uraemic neuropathy's progression. It was previously documented in the literature that early uraemic peripheral neuropathy may improve with increasing adequacy of dialysis therapy [Bolton 1976]. Following the kidney transplantation clinical recovery of the neuropathy is documented [Orbach 2004, Hamed 2019].

The prevalence of autonomic neuropathy in uraemic patients has been estimated more than 60 % [Vita 1999]. The pathogenic mechanisms of uraemic autonomic disorders [Hamed 2019] are the accumulated toxins [Heidbreder 1985], the lack of a neurotrophic factors [Zochodne 2000], anaemia due to erythropoietin deficiency [Wittmann 2015, Biaggioni 1994, Kim 2009], and the advanced arterial calcification [Sato 2001]. Our KTx patients had lower haemoglobin and haematocrit levels than the controls.

Immunosuppressive drugs applied in KTX patients might also induce neuropathy. Tacrolimus is a calcineurin-inhibiting drug, which is frequently and widely used for immunosuppression following kidney transplantation. There are just small series of patients or case reports that have described tacrolimus-related development of peripheral neuropathy: chronic sensorimotor polyneuropathy was found in 2 cases after tacrolimus use following renal transplantation [Bhagavati 2007]. Patients on tacrolimus suggested to be carefully monitored for symptoms of peripheral neuropathy [Bhagavati 2007]. Incidence rates for paresthesia and peripheral neuropathy have been reported as high as 40 % [U.S. Multicenter FK506 Liver Study Group 1994]. Several further case reports were published according to a tacrolimus-associated neuropa-

thy after other organs', not exclusively kidney transplantation [Weerdt 2008, Zhang 2019, Ayres 1994]. Tacrolimus-triggered optic neuropathy has been appeared in the literature in the last decade [Rasool 2018]. 87 % of our KTx patients have received immunosuppressive treatment with tacrolimus. 78 % of the KTx patients were on an immunosuppressive treatment with the reversible inhibitor of inosine monophosphate dehydrogenase, mycophenolate mofetil. Mycophenolate mofetil-induced peripheral neuropathy has also been detected recently according to the treatment of membranous glomerulonephropathy [Moghimi 2021].

9 % of the KTx patients received the mammalian target of rapamycin (mTOR) inhibitor everolimus, which has not well-known neuropathogenic effect. One case report was published so far about an everolimus-induced posterior reversible encephalopathy syndrome and bilateral optic neuropathy after kidney transplantation [Touhami 2014].

Preexisting/post-transplant diabetes mellitus (PTDM) induced neuropathy also might be an explanation of our findings. In our patients, 35 % of the KTx subjects had diabetes. Post-transplant diabetes is a common complication of the immunosuppressive therapy. The incidence rate of PTDM is considered in the literature above 10 % to 30 % [Markell 2001]. PTDM increases the risk of graft loss and patient death in renal transplant patients with diabetes [Ojo 2000]. 57 % of the KTx subjects had steroid therapy. In steroid-induced diabetes the potential induction of neuropathy must be considered. A transient, steroid induced carbohydrate metabolism disturbance may also contribute to the neuropathological findings in this special patient group. A significant positive correlation was observed between plasma glucose and the CPT value of the peroneal nerve at stimulating frequency of 5 Hz in our KTx patients. This association reveals a possible relationship between lower limb small fibre dysfunction and high glucose levels in this group. Further correlations were not found between the metabolic and neuronal abnormalities supporting the fact that other pathogenetic factors than hyperglycemia play important roles in the development of neuropathy. Cardiovascular autonomic and peripheral neuropathy is frequent in type 1 and type 2 diabetic patients with smoking, high blood pressure and hypertriglyceridaemia [Fleischer 2014, Kempler 2002]. The prevalence of smoking and hypertension was significantly higher in our KTx patients supporting the possible role of these factors as well.

The main limitation of our study is the cross-sectional data acquisition of the KTx patients and controls, a follow-up design could provide additional data on the progressive nature of neuropathy in chronic kidney diseases. The observed findings of KTx patients were compared to healthy controls and not to non-KTx subjects with kidney insufficiency. This kind of study would be an aim of our further research activity.

In conclusion extended cardiovascular autonomic dysfunction and upper as well as lower limb sensory neuropathy were found in chronic renal disease following kidney transplantation. The etiology seems to be multifactorial including the disease duration, the kidney function and several cardiovascular risk factors. Further exploration of the etiology and the possible role of transplantation in the pathogenesis is required in clinical studies.

Ethics statement

This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and was approved by the institutional Human Research Ethical Committee (approval No. 128/2018-SZTE). All subjects have given written informed consent of the study.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Autor contributions

AV, BB, IB, PK, AO, AN, GL, TV and CL had substantial contributions to the conception of the work and design of the paper, read and approved the final manuscript. AV, BB, AO and MS contributed to the measurements and analyses of data. AV, IB, PK, TV, AO and CL drafted the paper or revised it critically for important intellectual content.

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