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*Original Article*

# Doppler ultrasound findings in kidney transplant recipients with and without of new onset diabetes mellitus beyond 5 years after transplantation

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**Abstract:** Objective: Renal Doppler Ultrasound (RDU) indices: resistive index (RI) and pulsatility index (PI) are frequently applied as a noninvasive method that measured possible causes of allograft dysfunction in kidney transplant patients. We aimed to compare long-term prognosis and associated risk factors including the RDU markers in recipients with and without new-onset diabetes after transplantation (NODAT) beyond 5 years after kidney transplantation. Methods: A prospectively maintained database of 137 kidney allograft recipients, transplanted in a single center, maintained on reduced tacrolimus-based immunosuppressive regimen and angiotensin receptor blocker (ARB) was retrospectively analyzed. The assessment including incidence of NODAT and associated risk factors including RI and PI was compared between 12 recipients with and 125 recipients without NODAT median 77.5 months and 74 months, respectively, after kidney transplantation. Results: NODAT was detected in 12 (9.6%) of the 137 kidney transplant recipients, without gender predilection. In univariate regression analysis recipient age ($P < 0.001$), recipients weight at the time of NODAT ≥ 65 kg ($P < 0.001$), as well as proteinuria ($P = 0.026$), tacrolimus trough levels ($P = 0.005$), PI ($P = 0.023$) were associated with the long-term risk of NODAT and multivariate regression analysis also revealed that recipients weight at the time of NODAT ≥ 65 kg ($P = 0.004$) was independent long-term risk factor for NODAT. Conclusions: Our study demonstrated that beyond 5 years after kidney transplantation the RDU indices: RI and PI are not long-term risk factors for NODAT and the correction of recipient's body weight, the treatment with ARB and maintained reduced TAC doses lowered the incidence of NODAT.

**Keywords:** Diabetes mellitus, doppler ultrasonography, kidney transplantation

## Introduction

New-onset diabetes after transplantation is a common potentially preventable complication that has adverse effects on patient and graft survival. The etiopathogenesis of NODAT is multi-factorial, with risk factors varying among different studies. In general, risk factors include older age, overweight and immunosuppressive regimens [1, 2]. Although a high resistive indices at 1, 3 and 12 months has been shown to be a long-term risk factor for NODAT [3], to our knowledge, the relationship between intrarenal RI and PI determined by Doppler ultrasound and long-term incidence of NODAT (beyond 5 years post-transplantation) has not been confirmed. However, resistive and pulsatility indices are not directly associated with renovascular resistance, and additional intra renal/extra renal hemodynamic factors have an impact on resistance indices in renal allografts [4, 5]. Additionally, the role of nutritional interventions on improving long-term outcomes of kidney transplantation is also unknown [6]. Identification of those potentially modifiable risk factors may help in the development of strategies to prevent NODAT. The aim of the present study is to analyze the incidence of NODAT after correction of body weight as preventive strategy and associated long-term risk factors including intra renal resistive and pulsatility indices.

## Materials and methods

The study group consisted of 137 kidney transplant recipients, 93 males and 44 females, who underwent transplantation in a single center and were treated with tacrolimus (TAC), mycophenolate mofetil (MMF), steroid, and angiotensin receptor blocker (ARB) during the follow-up period. Renal allograft function of recipients was stable during follow-up period and renal biopsy was not performed. This study was approved by local institutional review board and conducted in accordance with declaration of Helsinki. All subjects provided informed consents prior to entering the study.

*Resistance index measurements*

Doppler ultrasound measurements for all patients were performed using a single ultrasound system Logiq 7 (GE Healtcare, Tokyo, Japan) equipped with a 7-MHz linear transducer while the patients were lying in supine position. An anterior approach was utilized, and the procedure was performed by a single radiologist. In Doppler measurements, renal parenchyma was scanned in both longitudinal and transverse planes in B-mode and color Doppler. Pulsed Doppler velocity waveforms were obtained in segmental, interlobar, and arcuate arteries. Following these measurements, peak systolic (Vmax) and minimal diastolic (Vmin) velocities were determined (calculated by taking the average values of three to four measurements), and the segmental arterial RI and PI were calculated with the following formula [7]: RI = peak systolic frequency shift-minimum diastolic frequency shift/peak systolic frequency shift; PI = peak systolic frequency shift-minimum diastolic frequency shift/mean frequency shift. All analyses were performed between 8.00 and 10.00 a.m., or between 4.00 and 5.00 p.m., at least 6 hours (range 6-12 hours) following immunosuppressive medications.

*Data assessment*

The renal transplants were divided into two groups according to presence or absence of NODAT and according to their weight at the time of NODAT ≥ 65 kg vs. < 65 kg. To assess risk factors predisposing NODAT, the following data was analyzed: the resistive and pulsatility indices, age and sex of recipients and donors, the type of donor (living or deceased), the number of HLA-mismatches, allograft and patients survival, degree of proteinuria rate, serum creatinine, estimated glomerular filtration rate (eGFR), cholesterol, triglyceride levels, and target TAC trough levels. Laboratory data, such as serum creatinine, cholesterol, triglyceride, and target TAC trough levels were determined just prior to ultrasound evaluation. Rates of urinary protein excretion for 24 hours and eGFR (in milliliters per minute per 1.73 $m^2$ of body surface area), were measured 24 hours prior to the Doppler ultrasound examination. All Doppler ultrasound measurements were obtained using a single ultrasound system and were performed by a single radiologist.

*Statistical methodology*

Continuous variables were expressed as mean (standard deviation; SD) or median (interquartile range [IQR]) and categorical variables are measured as count and percentage. Categorical variables were compared using chi-square or Fisher's exact tests and continuous variables were compared using the Mann Whitney U test. Univariate and multivariate logistic regression models were used to evaluate the effects of variables on the risk of NODAT development. Univariate linear regression analysis was used to evaluate the effects of variables on estimate graft survival and eGFR. Differences were considered significant at $P < 0.05$. Statistical analyses were performed using SPSS for Windows Version 15.0 (SPSS Inc., Chicago, IL, USA).

## Results

New-onset diabetes after transplantation occurred in 12 (8.8%) of the 137 renal transplants, without gender predilection. The demographic and clinical characteristics of the transplant pairs, and post-transplantation results are shown in **Table 1**. Donor age and percentage of males did not differ significantly between the two groups. Median age ($P < 0.001$), and recipient's weight at the time of NODAT ≥ 65 kg ($P < 0.001$) variables were significantly higher in the NODAT group. Median cholesterol and triglyceride concentrations were significantly higher in NODAT, than in non-NODAT recipients ($P = 0.02$), but there were no significant differences between groups in deceases/living donors, HLA mismatches, serum creatinine concentration, eGFR), resistive indices, graft and patient follow-up time. Median proteinuria

**Table 1.** Donor and recipient characteristics and outcomes in recipients with and without NODAT

| | NODAT Group | Non-NODAT Group | *P* value* |
|---|---|---|---|
| Recipient age/Donor age, years; median (IQR) | 43 (14)/42.5 (20) | 30 (17)/44 (11) | < 0.001/0.8* |
| Recipient gender/Donor gender, male; n (%) | 9 (75.0)/6 (50.0) | 84 (67.2)/51 (40.8) | 0.58/0.54** |
| Recipient's weight at the time of NODAT ≥ 65 kg vs. < 65 kg; n (%) | 11 (91.7 ) | 12 (9.6) | < 0.001*** |
| Living donor; n (%) | 9 (75.0) | 107 (85.6) | 0.39*** |
| HLA mismatches; mean (SD) | 3 (1.5) | 3 (0.0) | 0.82* |
| Serum creatinine, mg/dL; median (IQR) | 1.02 (0.19) | 1.07 (0.3) | 0.34* |
| Cholesterol, mg/dL; median (IQR) | 213.5 (40.0) | 188 (72) | 0.027* |
| Triglyceride, mg/dL; median (IQR) | 205 (71) | 135 (86) | 0.020* |
| Proteinuria, mg/day; median (IQR) | 75 (86.9) | 120 (147) | 0.005* |
| eGFR, ml/min/1.73 $m^2$; median (IQR) | 78.2 (23.6) | 70.4 (29.8) | 0.09* |
| Tacrolimus trough level, ng/ml; median (IQR) | 4.1 (0.7) | 5.1 (1.7) | 0.001* |
| Resistive index; median (IQR) | 0.66 (0.09) | 0.66 (0.07) | 0.41* |
| Pulsatility index; median (IQR) | 1.25 (0.29) | 1.10 (0.30) | 0.044* |
| Graft/patient follow-up, months; median (IQR) | 77.5 (39) | 74 (43) | 0.62* |

*Mann-Whitney U test, **Chi square, ***Fisher's exact tests, NODAT: new-onset diabetes after transplantation.

(*P* = 0.005) and median TAC trough level (*P* = 0.001) were significantly lower, whereas median intra-renal PI was significantly higher (*P* = 0.044) in NODAT group.

*Diabetes control*

During the first 3 months after transplantation all 12 patients who developed NODAT were started on insulin. After 6 months, 5 of the 12 (41.7%) patients were still treated with insulin, while diabetes treatment changed in seven patients: 3 (25%) patients were continued with oral anti-diabetic drugs, and in 4 (33.3%) patients treatment consisted of diet alone. Beyond 3 months of post-transplantation all recipients received nutritional orientation, with a low-calorie diet monitored by nutritionist and increased physical activity.

*Predictors of NODAT*

After univariate analysis we found that recipient age (*P* < 0.001; odds Ratio (OR) 1.1; 95% confidence interval (CI): 1-1.2), recipient's weight at the time of NODAT ≥ 65 kg (*P* < 0.001), proteinuria (*P* = 0.026, OR 0.99; 95% CI: 0.9-1.0), and pulsatility indices (*P* = 0.023, OR 0.97; 95% CI: 1.5-1.9) were risk factors for NODAT development. Multivariate regression analysis which included variables according to univariate analysis, revealed that recipient's weight at the time of NODAT ≥ 65 kg (*P* = 0.004) has significant effect on risk of NODAT with accuracy rate of 97.8%.

*Graft and patient survival*

The univariate analysis showed that no variable effected graft or patient survival in NODAT and non-NODAT recipients after a median 77.5 months and 74 months, respectively, after kidney transplantation. Serum creatinine (*P* < 0.001; OR-38.5; 95% CI: -46.5-(-30.5)), number of HLA mismatches (*P* = 0.019; OR -3.4; 95% CI: -6.3-(-0.6)) and proteinuria (*P* = 0.002; OR -0.03; 95% CI: -0.04-(-0.01)) were factors affecting eGFR in univariate analysis.

## Discussion

New-onset diabetes after transplantation is a frequent complication in renal transplants receiving steroid and especially TAC for immunosuppressive therapy [8]. However, it was reported that low-dose (5 mg/day) prednisone had no effect on the development of insulin resistance in liver transplant recipients [9]. The apparent discrepancy between studies [8, 10, 11] with high frequency of NODAT (26.7%, 18.3%, 12%, respectively) and our current finding (8.8%), may be explained by lifestyle intervention, including diet, increased physical activity, weight reduction, lower TAC trough doses and ARB used in the recipients. A biochemical events associated with nephrotoxic effects of CNIs include intrarenal overactivation of the renin-angiotensin system (RAS) that results in chronic allograft injury through activation of transforming growth factor-ß [12]. Thus, RAS blockade, in theory, may have a

nephroprotective effect on chronic vascular and tubulointerstitial lesions. In the present study, we assessed RI and PI long-term after kidney transplantation, and in the meantime, CNI doses were reduced and all of our recipients received ARB therapy. Our findings were also compatible with the thought that ARB promotes allograft improvement and reduces the risk of NODAT in renal recipients [13, 14]. The negative effect of NODAT on patient's survival was related with cardiovascular risk profile such as obesity, hypertension [15], and with a lower percentage of ARB therapy [1], which has useful effect on cardiovascular risk in non-transplanted patients [16]. In our study all the recipients were maintained on ARB therapy during the follow-up period and patient and graft survival in NODAT group was not different from those in non-NODAT group. Also our multivariate analysis revealed that intrarenal RI and PI were not associated with the long-term risk of NODAT after transplantation, median 77.5 and 74 months in NODAT and non-NODAT group, respectively. However, a multivariate regression analysis revealed that the patient's weight at the time of NODAT ≥ 65 kg ($P < 0.001$) have significant effect on risk of NODAT. Supporting our findings, the risk associated with NODAT has been found to increase between 4% and 9% per additional year of age [17, 18] and continuously for every increment of body weights above 60 kg [15]. Nearly all kidney recipients have experienced weight gain after transplantation, due to the absence of dietary restrictions and increased appetite due to the absence of uremia. Additionally, it has been shown, that obesity is associated with post-transplantation dyslipidemia [19]. In this study cholesterol ($P = 0.027$) and triglyceride ($P = 0.020$) levels were significantly higher in NODAT group. Weight gain is an important problem after transplantation, suggesting that in these recipients lifestyle intervention, including diet, increased physical activity, and weight reduction should be added to immunosuppressive drugs treatment. Various risk factors, such us older age of the donor or recipient, poorer kidney function in early post-transplantation period, presence of proteinuria, presence of uncontrolled hypertension, and 5 or 6 HLA mismatches, have all proposed a difference between recipients with excellent long-term survival of kidney allograft, and those with a poor survival [20-24]. However, none of these factors alone or in combination had a significant correlation with an increased resistive and pulsatility indices value [25]. To our knowledge, the comparison of detected RI and PI in recipients with and without of NODAT at beyond 60 months is unknown. Our study demonstrated that beyond 5 years after kidney transplantation the Renal Doppler ultrasound indices: RI and PI are not a long-term risk factors for NODAT. The limitation of our study is that the number of recipients with NODAT is relatively low which may be result of identification of those potentially modifiable risk factors and of strategies preventing NODAT.

In conclusion, the correction of recipient's body weight, the treatment with ARB and maintained reduced TAC doses lowered the incidence of NODAT. Beyond 5 years after kidney transplantation the renal Doppler ultrasound resistive and pulsatility indices are not long-term risk factors for NODAT.

## Disclosure of conflict of interest

None.

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