

PREVALENCE AND RISK FACTORS FOR INSULIN RESISTANCE AND DYSGLYCEMIA AFTER KIDNEY TRANSPLANTATION IN PATIENTS ON CYCLOSPORINE-A BASED IMMUNOSUPPRESSION

Petkovska Lidija^{1,5}, Natasha Simonovska^{1,5}, Aleksandra Babulovska^{1,5}, Afrodita Berat-Huseini^{1,5}, Julijana Brezovska-Kavrakova^{2,5}, Zvezdana Petronijevik^{3,5}, Beti Zafirovska-Ivanova^{4,5}

¹University Clinic for Toxicology, ²Institute of Medical and Experimental Biochemistry, ³University Clinic for Nephrology, ⁴Institute of Epidemiology, ⁵Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, R. North Macedonia

Abstract

Glucose disorders and insulin resistance are major factors affecting cardiovascular morbidity after renal transplantation. We analyzed the prevalence of pre-diabetes, increased insulin resistance, the factors for their occurrence, as well as the consequences on graft function in kidney transplant patients who are on a cyclosporine-A based immunosuppressive protocol.

59 non-diabetic living donor kidney recipients were included in this cross-sectional and prospective study. All patients were on the same triple immunosuppressive therapy in maintenance doses. OGTT and indices of insulin resistance were analyzed at least 6 months after transplantation, as well as factors for their occurrence. According to the OGTT results, the patients were divided into two groups: a group with dysglycemia and a group of normoglycemic patients. Graft function was controlled after a period of follow-up.

The prevalence of dysglycemia and insulin resistance was 33.9% (20/59) and 86.44% (51/59), respectively. In the group with dysglycemia, insulin resistance was more prevalent 95% (19/20), than beta-cell hypofunction 40% (8/20). The insulin resistance index in the dysglycemic group was significantly higher (3.139 ± 1.11) versus the normoglycemic group (2.264 ± 1.00), $p < 0.01$. The most significant risk factors for increased insulin resistance in the dysglycemic group were: shorter transplant period, higher doses of cyclosporin-A, postload insulin, and insulin secretion index. In this group of patients, a significant decrease in e-GFR was observed after an average of 18 months of follow-up.

Insulin resistance is very prevalent after renal transplantation, and especially high in dysglycemic patients, and the associated risk factors are potentially modifiable. OGTT is an important diagnostic tool for assessing the prevalence of occult diabetes and insulin resistance, and its routine application may contribute to reducing their prevalence.

Key words: kidney transplantation, pre-diabetes, insulin resistance, immunosuppression

Introduction

Newer immunosuppressive protocols applied after renal transplantation provide a better control of immunological phenomena, and thus longer graft and patient survival. Mortality in these patients is still high, but the causes of fatal outcome are mostly not related to graft insufficiency [1].

Cardiovascular complications are the predominant cause of mortality [2,3]. Immunosuppressive therapy is associated with numerous non-immunological risk factors that increase cardiovascular morbidity, including several late metabolic risk factors for coronary artery disease (CAD). These include: hypertension, dyslipidemia, overweight/obesity, and disturbances in glucose metabolism, characteristic of the metabolic syndrome (MS). Although causes of MS are not sufficiently clearly established, it has long been known that insulin resistance (IR) is the main common denominator for its components [4].

On the other hand, IR is much more common in subjects after renal transplantation compared to the general population of similar age and BMI. Until now, factors that influence this excess insulin resistance have not been exactly discerned [5].

Even those patients who did not develop post-transplant diabetes were found to be more insulin resistant compared to healthy controls [6]. This suggests that other additional transplant-specific determinants contribute to the higher incidence of IR. These include primarily the use of

immunosuppressive drugs such as corticosteroids and calcineurin inhibitors (CNI), frequent viral infections and post-transplant glucose disorders, including pre-diabetes. Therefore, most often the analysis of the prevalence of IR after renal transplantation is derived from studies that simultaneously analyze pre-diabetes, as the condition most frequently associated with IR [7].

The aim of this study was to evaluate simultaneously the prevalence and potential modifiable and non-modifiable risk factors for the development of pre-diabetes and IR in living donor kidney transplant patients on triple CNI-based immunosuppression with Cyclosporine A (CsA) at maintenance doses, as well as their influence on graft function.

Subjects and methods

Patients: In this cross-sectional study 59 patients were enrolled, with mean age of 35.15 ± 8.75 (range 14-53) and satisfactory graft function (mean GFR $60,55 \pm 13,77$ ml/min) with an average time of 35.73 ± 27.03 months after successful living donor kidney transplantation. The same immunosuppressive protocol was used in all patients, including mycophenolate mofetil (MMF), cyclosporine A (CsA) and prednisone (Pred) at maintenance doses.

Enrollment/inclusion criteria were: minimum 14 years of age, absence of diabetes mellitus (DM) before transplantation, satisfactory graft function and follow-up period of at least 6 months after transplantation. Exclusion criteria were: DM diagnosed before glucose metabolism testing, steroid use due to other comorbidities, and previous episodes of acute graft rejection.

All patients gave an informed consent for participation in the study. All investigations were carried out in accordance with the WHO rules and the Declaration of Helsinki.

The oral glucose tolerance test (OGTT) was performed in order to diagnose pre-diabetes and occult diabetes in all enrolled patients. After twelve hours overnight fasting, patients were loaded with 75 grams of anhydrous glucose (according to WHO recommendations) and blood samples for determination of glucose and insulin concentrations were harvested at 0 and 120 minutes after glucose administration. The test results were classified according to the revised ADA criteria as: Normal - fasting blood glucose level < 5.6 mmol/l, Impaired fasting glucose (IFG) - fasting blood glucose level between 5.6-6.9 mmol/l, Impaired glucose tolerance (IGT) - glucose levels between 7.8-11.1 mmol/l 120 min. after the load, DM > 6.9 mmol/l fasting glucose and > 11.1 mmol/l - 120 min. after the load.

Patients were divided into two groups: dysglycemia group DG (IFG/IGT, DM), and normoglycemic group NG.

Laboratory: Glucose levels were determined using the glucose-oxidase method in an automatic analyzer (Beckman) and using venous blood samples. CsA trough levels were determined using FPIA technique (Fluorescence polarization immunoassay) in an automatic analyzer (Abbott). Insulin concentrations in plasma were measured with MEIA (Microparticle Enzyme Immunoassay) in an automatic analyzer of the Abbott type. To estimate the glomerular filtration rate (e-GFR), we used the Cockcroft-Gault formula $(140 - \text{age in years}) \times (\text{wt in kg}) \times 1.23 / (\text{serum creatinine in } \mu\text{mol/l})$. Estimated GRF was repeated after follow-up period of 18 months.

For a quantitative estimate of IR and β -cell function, homeostatic model assessment for insulin resistance (HOMA-IR) by Matthews DR, et al. was used, according to which the normal value of HOMA-IR is considered to be 1, and for insulin secretion (HOMA- β) 100 %. Calculations were made using the following formulas: $\text{HOMA-IR} = [(\text{Fasting Insulin } (\mu\text{U/mL})) \times (\text{Fasting Glucose (mmol/L)})] / 22.5$ and $\text{HOMA-}\beta = 20 \times I \text{ in } \mu\text{IU/ml} / (\text{G mg/dlx}0.05551) - 3.5$

In order to correlate multiple factors presumably associated with pre-diabetes, diabetes and IR we used data from patients' files and from the results of biochemical analyses performed at the same time as OGTT. All necessary data were entered in specially prepared questionnaires, which included: G_0 -fasting glucose, G_2 - after loading glucose, I_0 - fasting insulin, I_2 - after loading insulin, CsA trough levels, daily steroid doses, triglyceride levels, cholesterol levels, e-GFR, blood pressure, gender, age, BMI, HCV

infections, length of post-transplant period, family history of diabetes, major causes of renal failure, duration of dialysis and HCV seropositivity.

Immunosuppressive protocol. All patients were on CsA (Neoral) based immunosuppressive protocol plus mycophenolate mofetil and prednisone, at maintenance doses. CsA trough levels were monitored periodically and doses were adjusted as needed. Target therapeutic levels of CsA were 200–300 ng/ml in the first three months after transplantation, 100–250 ng/ml from 4–12 months, and 100–200 ng/ml after the first year.

Statistical analysis. Statistical software SPSS for Windows (version 13.0) was used for statistical data processing. Descriptive statistics methods were used for continuous data: mean value and standard deviation, and categorical data were expressed in percentages. Analytical methods were also used: Pearson's correlation coefficient for certain risk factors; multiple regression analysis for independent risk factors; Student's t-test was used to analyze continuous data for unpaired samples and to find between-group differences. Categorical data were analyzed with the chi-square test and Fisher's test for equivalent pairs of frequencies. P-value < 0.05 was defined as statistically significant.

Results

A total of 59 patients after successful living donor kidney transplantation were included in this study. The majority were males 37 (62.71%), while 22 (37.29%) were females, with a mean age of 35.15 ± 8.75 (14-53). All included patients had no previous history of diabetes and were followed for an average of 35.73 ± 27.03 months after transplantation. Only 10 (16.94%) patients were anti-HCV positive, while the majority 49 (83.05%) were anti-HCV seronegative. Mean CsA levels were 130.62 ± 70.64 ng/ml, while mean prednisone doses were 7.45 ± 2.68 mg per day. Most of the patients, 50 (84.74%), had a negative first-degree family history of diabetes. Fifty-five (93.22%) had hypertension.

After performing the OGTT, patients were divided into two groups: group with dysglycemia (DG) (n=20) and group with normal glucose levels (NG) (n=39). The overall incidence of glucose disorders, including diabetes and pre-diabetes was 33.9% (20/59), while the prevalence of post-transplant diabetes mellitus (PTDM), IGT and IFG was: 3.39% (2/59), 30.5% (18/59) and 11.87% (7/59), respectively.

The prevalence of increased IR was 86.44% (51/59) in all included patients. Values of HOMA-IR higher than 1.2 are considered to be pathological. HOMA- β index was increased in 54.24% of patients (32/59). Normal function of beta-cell is considered to be from 90-120%. In DG, 95% of patients had HOMA-IR above 1.2, and in only 5% they were within reference values.

Testing for proportions showed that the difference was highly significant ($p < 0.001$), pointing out that increased IR was the dominant mechanism for pre-diabetes and occult diabetes. There was no significant difference between the groups in the prevalence of increased IR and secretion ($p < 0.05$). Testing for proportions did not show a significant difference in the occurrence of IR in the two groups ($p < 0.05$).

Using univariate analysis, a correlation was made of all potential risk factors for the occurrence of glucose metabolism disorder and IR. The results showed that in all patients there was a significant correlation between postprandial glucose and the following factors: higher CsA levels, time since transplantation, increased total lipids (TL) and LDL-c.

The correlation of certain risk factors was repeated in the DG, and was found that CsA trough levels ($r=0.38$, $p < 0.05$), TL ($r=0.44$, $p < 0.05$) and LDL-c ($r=0.51$, $p < 0.05$) were significantly related to the occurrence of glucose disorders.

The main differences between the groups were as follows: male recipients dominated in DG (79% vs. 33.3%, $p < 0.05$), the time since transplantation was significantly shorter compared to NG (24.50 ± 81.21 vs. 41.48 ± 28.06 , $p < 0.05$), CsA level was higher (160.90 ± 81.21 vs. 115.10 ± 59.90 , $p < 0.05$), the IR index was higher (3.139 ± 1.11 , vs. 2.264 , $p < 0.01$). Also, e-GFR was significantly lower in DG after

18 months of follow-up (62.63 ± 16.51 ml/min vs. 52.76 ± 13.03 ml/min, $p < 0.05$), while in NG it was 59.49 ± 12.24 vs. 60.19 ± 10.57 , $p > 0.05$.

Table1. Comparison between patients with DG and NG - most significant intergroup differences

	DG N=20	NG N=39	p-value
Gender male (%)	(16) 79%	(13) 33%	$p < 0.05$
Time since transplantation	24.50 ± 81.21	41.48 ± 28.06	$p < 0.05$
CsA trough level	160.90 ± 81.21	115.10 ± 59.90	$p < 0.05$
HOMA-IR index	3.139 ± 1.11	2.264 ± 1.00	$P < 0.01$
e-GFR 1	62.63 ± 16.51	59.49 ± 12.24	$P > 0.05$
e-GFR 2	$52.76 \pm 13.03^*$	60.19 ± 10.57	$P > 0.05$

Values are expressed as mean \pm SD or numbers (percentages)

In order to evaluate the risk factors for the increased IR after renal transplantation, a multiple regression analysis was performed in all patients, and in DG separately.

The multiple correlation coefficient R with which we examined the association between the IR index and the risk factors (G_0 , G_2 , I_0 , I_2 , HOMA- β , BMI, CsA, daily dose of corticosteroids, e-GFR1, e-GFR2, and HTA) in entire cohort, showed that between HOMA-IR and the mentioned factors there was a real connection ($R=0.85$), which was highly statistically significant ($F=10.82$, $p < 0.01$).

The adjusted R^2 of 0.65 showed that 65% of the changes that occur in the value of the IR index can be attributed to the changes in the analyzed risk factors. At the same time, G_0 , I_0 , HOMA- β values and e-GFR value (at the first time point) appeared as significant risk factors on which HOMA-IR depended.

The tested correlation between HOMA-IR, on one hand, and gender of the recipient and donor, age of the recipient, time after transplantation, total lipids and their fractions, on the other hand in all patients, showed that this correlation was weak ($R=0.36$) and statistically insignificant ($F=0.75$; $p > 0.05$).

Of all these factors, only a shorter transplant period was found to significantly influence changes in the IR index.

The relationship between HOMA-IR and the following risk factors: G_0 , G_2 , I_0 , I_2 , HOMA- β , BMI, CsA, dose of corticosteroids, e-GFR₁, e-GFR₂ and HTA were analyzed by multiple correlation separately in the group of patients with dysglycemia. There was a strong correlation between HOMA-IR and the mentioned factors ($R=0.96$) which was highly statistically significant ($F=9.21$, $p < 0.001$).

The adjusted R^2 of 0.82 showed that up to 82% of the variations in the value of the HOMA-IR index can be explained by changes in the analyzed risk factors. At the same time, as significant factors on which HOMA-IR depended were: post-load insulin, HOMA - β and CsA level. These results are shown in Table 2.

Table2. The relationship between HOMA-IR and multiple risk factors in dysglycemia group.

R=0.96 Adjusted R²=0.82 F=9.21 p<0.001							
DG-patients with dysglycemia	Beta	B	Std.Err. of B	t (8)	p-level	Sig. / N.Sig	
		-3.625	1.350	-2.685	0.027	Sig.	
G ₀ -fasting glucose level	0.422	0.540	0.240	2.245	0.054	N.Sig.	
G ₂ -glucose post- load	-0.168	-0.080	0.092	-0.870	0.409	N.Sig.	
I ₀ –insulin basal	-0.477	-0.108	0.094	-1.158	0.280	N.Sig.	
I ₂ – insulin post-load	0.870	0.062	0.024	2.585	0.032	Sig.	
HOMA-β	0.882	0.013	0.003	3.541	0.007	Sig.	
BMI	-0.245	-0.067	0.040	-1.671	0.133	N.Sig.	
CsA level	0.409	0.005	0.002	2.454	0.039	Sig.	
Corticosteroid-dose	-0.072	-0.022	0.042	-0.538	0.604	N.Sig.	
e-GFR 1	0.119	0.008	0.018	0.434	0.675	N.Sig.	
e-GFR 2	0.047	0.004	0.027	0.147	0.886	N.Sig.	
HTA	0.415	2.073	1.107	1.871	0.098	N.Sig.	

The relationship between HOMA-IR, on one hand, and gender of the recipient and donor, age of the recipient, time after transplantation, total lipids and their fractions, on the other hand, in the group with glucose disorders showed that this tested relationship was weak to moderate and statistically insignificant (F=1.76, p>0.05).

Discussion

The occurrence of post-transplantation disorders in glucose metabolism, the increased IR, the most common risk factors for their occurrence, as well as the complications associated with them, have been extensively studied in recent years [4]. PTDM has been long recognized as a common complication of renal transplantation that promotes numerous cardiovascular diseases, death and graft failure. Its incidence varies from 2% to 50% in different studies, which is due to a lack of uniformity in the definition and diagnosis of the disease, different immunosuppressive protocols, as well as the difference in the patient population included in the study [8].

The evolution of PTDM and pre-diabetes after renal transplantation has a bimodal character. The largest percentage of newly discovered disorders in glucose metabolism develops 3-6 months after transplantation and is partially reversible, and in the remaining cases it develops after 12 months [9].

Pre-diabetes (IFG and IGT) is much more common in patients after renal transplantation compared to the general population, and most of these patients can be diagnosed with OGTT alone. Bergrem et al.

[10] found out that 37% of 889 patients had pre-diabetes (25.9% IGT and 11.2% IFG). Our results were similar and the prevalence of occult diabetes, IGT and IFG, were: 3.39% (2/59), 30.5% (18/59) and 11.87% (7/59), respectively. Hyperglycemia early after renal transplantation can be observed in 80% of patients, which is most often associated with perioperative stress [11].

It should not be diagnosed as diabetes, but should be monitored, because it is associated with further development of the disease. Therefore, the diagnosis of post-transplantation disorders in glucose metabolism (including PTDM) should be delayed until the patient is on stable maintenance doses of immunosuppressive drugs, with stable kidney graft function and in the absence of acute infections. Our study also included patients after a follow-up period of at least 6 months. OGTT is considered as the gold standard for diagnosis of PTDM, and is very useful in detecting occult glucose abnormalities that coincide with normal fasting blood glucose, and is therefore preferred as a diagnostic tool. Conversely, HbA1-c is not reliable as the sole diabetes screening method in the first year after transplantation [4, 12,].

The reasons for this are numerous limitations, such as: anemia, treatment with erythropoietin or transfusion of erythrocytes, which are common after renal transplantation [13, 14]. PTDM is a multifactorial disease in which development genetic, environmental and physiological factors are involved, and according to its characteristics, it has the greatest similarity with DM type 2 in which increased IR plays a key role [4]. The incidence of IR is higher than that of PTDM after renal transplantation and it is also present in patients with normal glucose metabolism.

Therefore, it is most advantageous to determine the prevalence of IR simultaneously with an analysis of the occurrence of prediabetes. Besides being involved in the pathogenesis of diabetes, IR is a common denominator for many other conditions often present after renal transplantation such as: dyslipidemia (increases Tg and LDLc, decreasing good HDLc), blood hypercoagulability, pro-inflammatory effect (increases many pro-inflammatory markers such as CRP, fibrinogen, IL-6, TNF-I) and promotes hypertension (by increasing Na retention) [15].

Therefore, the consequences of IR are associated with numerous macrovascular and microvascular complications, including nephropathy [15]. The measurement of IR can be done in different ways. The gold standard remains the hyperinsulinemic-euglycemic glucose clamp technique.

The complexity and invasiveness of this method limits its clinical use. Consequently, multiple surrogate markers for IR, more favorable for application in clinical studies, have been developed and applied. The most widely used are the HOMA-IR and HOMA- β indices, which are based on measurements of fasting glucose and insulin levels. Indices derived from those measurements have been shown to correlate well with results from more invasive studies [16, 17]. Risk factors, that is, causes of dysglycemia and PTDM can be classified as pre-transplantation and post-transplantation, modifying and non-modifying. Of the pre-transplant risk factors, most of which are common to those of type 2 diabetes, the most significant are: age, non-Caucasian ethnicity, family history of diabetes, and the presence of pre-transplant MS or any of its individual components [18].

Despite the fact that metabolic syndrome and pre-diabetes are potentially modifiable risk factors that can be acted upon and select those patients who are at an increased risk for PTDM and are on the transplant waiting list, this is still rarely done because OGTT is required for assessment of pre-diabetes[4]. Such selection of patients was not done in our study due to the lack of consensus on the use of OGTT before transplantation.

Age over 55 years and BMI over 30 have been shown to be major risk factors for post-transplant diabetes [19]. A similar association of age with the development of abnormal glucose metabolism was found in another study, but patients included were older at the time of transplant [20].

In another study examining the frequency and risk factors for IR (using the homeostasis model) in 106 renal transplant patients without glucose disturbances, a significant correlation was found between HOMA-IR and age, BMI, and waist-to-hip ratio, and the waist-to-hip ratio proved to be the most significant using a regression analysis [21].

Although we previously reported that BMI was an independent risk factor for increased IR in 36 living donor kidney recipients, we could not confirm it in this study [22].

Our results did not show a significant association between the age and BMI and the development of pre-diabetes, diabetes and HOMA-IR. We believe that the possible explanation is that our patients were much (mTOR) inhibitors. Corticosteroids are most often incriminated for the occurrence of dysglycemia and increased IR.

In the large study by Oterdoom et al.[5], apart from age and BMI, the dose of corticosteroids was determined to be a risk factor for IR. Other authors have demonstrated that the reduction and withdrawal of steroids has led to a decrease in the incidence of PTDM [19].

Importantly, steroid reduction and withdrawal are associated also with an improvement in IR [3]. Contrary to previous reports, in the study by de Lucena et al. [18], which analyzed risk factors for diabetes after transplantation using univariate and multivariate analysis, found that the cumulative doses of corticosteroids in the first 6 months after transplantation were not a risk factor for the occurrence of PTDM, but it was transient hyperglycemia. Another group of authors found that daily steroid doses of 7.5 mg/day and less did not correlate with pre-diabetes [20].

We also did not find a significant correlation of daily doses of corticosteroids with postprandial glucose levels, nor did we prove that it was a risk factor for increased insulin resistance. We could interpret this with the fact that our patients were on similar daily prednisone doses of 7.45 ± 2.68 . In addition, all patients with acute episodes of graft rejection were excluded from our study, due to the need for higher doses of immunosuppressive therapy and consecutive exposure to higher cumulative doses of corticosteroids.

Despite numerous investigations in recent decades, the pathogenesis of post-transplantation dysglycemia is not fully understood. It has been demonstrated that normal fasting glucose levels combined with rising levels during the day are typical features in transplant patients; most presumably due to the metabolic effects of immunosuppressive drugs [23].

Apart from glucocorticoids, the use of CNIs (tacrolimus and cyclosporine A) is a well-known transplant specific risk factor. The diabetogenic effects of CsA were described in the early 1980s. There is some evidence that dysglycemia after transplantation is closely related to the degree of CNI exposure, that is, a lower prevalence of dysglycemia is observed in patients treated with lower therapeutic doses of CsA [24, 25]. Some authors have not confirmed a significant correlation between CNI and post-transplant DM probably due to lower doses of CNI used in their centers [20].

Our results showed a significant correlation between CsA levels and postprandial glucose. Also, DG had a shorter time since transplantation and higher CsA trough levels than NG, confirming a dose-dependent response in terms of disturbances in glucose metabolism. A more recent study examining the incidence of PTDM, insulin secretion, and resistance at 3, 6, 9, and 12 months after transplantation in patients treated with tacrolimus-based immunosuppressive protocol found a higher incidence of PTDM at 3 months (56.5%) and 12 months (26.4%).

IR indices were increased by the end of the trial in both patient groups, but more significantly increased in PTDM group. Increased insulin secretion in patients without glucose disturbances likely occurs as a compensatory mechanism to overcome IR [26].

The higher incidence of patients with DM could be explained by the greater diabetogenic effect of tacrolimus compared to CsA, as well as the inclusion of patients with episodes of acute graft rejection in the study, who were exposed to higher cumulative doses of immunosuppressive drugs. Our results were consistent with previous ones regarding the prevalence of IR after renal transplantation, and the IR index was significantly higher in DG, which we explain with the higher CsA levels in these patients.

Metabolic syndrome and IR contribute to chronic graft failure. The prevalence of IR and its consequences in terms of renal dysfunction is not the same in different components of the metabolic syndrome. Thus, for example, 50% of patients with HTA and 25% of obese patients have insulin resistance. This is why more accurate and still practical approaches to detect IR in the setting of renal transplantation are needed [6].

Application of the homeostasis model to assess insulin resistance may predict future development of chronic kidney disease [27].

The ways in which IR contributes to the development of chronic nephropathy are numerous, and the key ones are hyperinsulinemia and hyperglycemia. Hyperinsulinemia can contribute to renal dysfunction in several ways: proliferation of mesangial cells and matrix, synthesis of growth factors and collagen that promotes interstitial fibrosis, interferes with the renin-angiotensin system, increases oxidative stress and endothelial dysfunction [6].

Finally, hyperglycemia, advanced glycation end products, and dyslipidemia, which are common in IR, may induce nephrotoxicity by various mechanisms [6].

Glomerular and hemodynamic mechanisms are responsible for increased glomerular filtration which is considered to be an early consequence of IR and MS [28]. A group of authors demonstrated that increased glomerular filtration 1 year after transplantation was an independent risk factor for graft failure [29].

Our results are consistent with previously stated, as we found that increased e-GFR (along with fasting glycemia, basal insulin, and beta cell hyperfunction) at the first time point was an independent risk factor for increased IR in the entire cohort. Moreover, we found that e-GFR was significantly decreased in patients with dysglycemia who had a higher IR index at 18-month follow-up, confirming that IR was a marker of renal graft dysfunction. Elevated IR and pre-diabetes after renal transplantation are dynamic categories and can be modified. Many authors believe that understanding the dynamics and risks of impaired glucose metabolism after transplantation is crucial for targeted intervention [30].

In our study, IR and prediabetes were associated with a shorter post-transplantation period. The most significant risk factors for prediabetes in our study were: higher CsA levels, increased total lipids and LDL-c. We also found an association of G_0 , I_0 , HOMA- β and e-GFR at the first time point with IR in the entire cohort, but in patients with pre-diabetes independent factors for IR were I_2 , HOMA- β and CsA levels. A shorter period after transplantation and higher therapeutic levels of CsA were shown to be common risk factors for both pre-diabetes and increased IR. We also determined deterioration of renal function in patients with dysglycemia and a higher IR index.

A limitation of our study might be the underestimation of patients with pre-diabetes and increased IR before transplantation, because the diagnosis of glucose disturbances was based only on fasting glucose levels, and IR indices were not determined routinely.

Due to the lack of mandatory use of OGTT we may have included patients with dysglycemia in our study and overestimated the prevalence of pre-diabetes after transplantation. Although all included patients were on an immunosuppressive protocol at maintenance doses, the time point of testing was not predetermined in the protocol, resulting in significant differences at the time from transplantation to OGTT between patients in the two groups.

Conclusion

We could conclude that IR is highly prevalent after renal transplantation, including patients without disorders in glucose metabolism, and is especially high in patients with dysglycemia. The use of OGTT is crucial for determination of patients with pre-diabetes and occult diabetes. Post-transplant risk factors associated with the occurrence of IR and dysglycemia are often mutual, connected, and potentially modifying.

A predominant mechanism for the occurrence of pre-diabetes is reduced insulin action, which leads to compensatory hyperinsulinemia, resulting in beta-cell dysfunction. IR alone and/or associated with other non-immunological-related risk factors such as glucose disorders has a significant role in the development of atherosclerosis, which contributes to chronic renal allograft dysfunction.

Therefore, it might be considered as a marker for chronic graft dysfunction, and late graft loss. A new screening strategy for early detection of these conditions could contribute to the reduction of total cardiovascular morbidity and mortality among these patients by modifying or reducing the therapeutic levels of immunosuppressive therapy to a lower therapeutic rank, lifestyle changes, physical activity and adequate diet.

References

1. Morales JM, Marcén R, del Castillo D, Andres A, Gonzalez-Molina M, Oppenheimer F, et al. Risk factors for graft loss and mortality after renal transplantation according to recipient age: a prospective multicentre study. *Nephrol Dial Transplant*. 2012; 27(suppl_4): iv39-46.
2. Jardine A. Assessing cardiovascular risk profile of immunosuppressive agents. *Transplantation*. 2001; 72 (12): SS81-8.
3. Midtvedt K, Hjeltnes J, Hartmann A, Lund K, Paulsen D, Egeland T, Jenssen T. Insulin resistance after renal transplantation: the effect of steroid dose reduction and withdrawal. *J Am Soc Nephrol*. 2004; 15(12): 3233-9.
4. Rodriguez-Rodriguez AE, Porrini E, Hornum M, Donate-Correa J, Morales-Febles R, Ramchand SK et al. Post-Transplant Diabetes mellitus and Prediabetes in Renal Transplant recipients: An Update. *Nephron*. 2021; 145: 317-29.
5. Oterdoom LH, de Vries AP, Gansevoort RT, van Son WJ, van der Heide JJ, Ploeg RJ, et al. Determinants of insulin resistance in renal transplant recipients. *Transplantation*. 2007; 83(1): 29-35.
6. Porrini E, Delgado P, Torres A. Metabolic syndrome, insulin resistance, and chronic allograft dysfunction. *Kidney Int*. 2010 ; 78: S42-6.
7. Meyer C, Pimenta W, Woerle HJ, Van Haefen T, Szoke E, Mitrakou A, et al. Different mechanisms for impaired fasting glucose and impaired postprandial glucose tolerance in humans. *Diabetes care*. 2006; 29(8): 1909-14.
8. Montori VM, Basu A, Erwin PJ, Velosa A, Gabriel SE, Kudva YC. Posttransplantation diabetes: a systematic review of the literature. *Diabetes Care*. 2002; 25(3): 583-92.
9. Porrini EL, Diaz JM, Moreso F, Delgado Mallen PI, SilvaTores I, Ibernón M, et al. Clinical evolution of post-transplant diabetes mellitus. *Nephrol Dial Transplant*. 2016; 31(3): 495-505.
10. Bergrem HA, Valderhaug TG, Hartmann A, Hjeltnes J, Leivestad T, Bergrem H, et al. Undiagnosed diabetes in kidney transplant candidates: a case – finding strategy. *Clin J Am Soc Nephrol*. 2010; 5(4): 616-22.
11. Chakkerla HA, Weil EJ, Castro J, Heilman RL, Reddy KS, Mazur MJ, et al. Hyperglycemia during the immediate period after kidney transplantation. *Clin J Am Soc Nephrol*. 2009; 4(4):853-9.
12. Shivaswamy V, Boerner B, Larsen J. Post-transplant diabetes mellitus: causes, treatment, and impact on outcomes. *Endocr Rev*. 2016; 37(1): 37-61.
13. Fishbane S, Spinowitz B. Update on anemia in ESRD and earlier stage of CKD: core curriculum 2018. *Am J Kidney Dis*. 2018; 71(3): 423-35.
14. EL Okle AZ, El-Arbagy AR, Yassein YS, Khodir SZ, El Sayed Kasem H. Effect of erythropoietin treatment on hemoglobin A1c levels in diabetic patients with chronic kidney disease. *J Egypt Soc Nephrol Transplant*. 2019; 19: 86-94.
15. Freeman AM, Pennings N. Insulin resistance. In *StatPearls* [Internet] 2022 Jul 4. StatPearls Publishing.
16. Rabasa-Lhoret R, Bastard JP, Jan V, Ducluzeau PH, Andreelli F, Guebre F, et al. Modified quantitative insulin sensitivity check index is better correlated to hyperinsulinemic glucose clamp than other fasting-based index of insulin sensitivity in different insulin-resistant states. *J Clin Endocrinol Metab*. 2003; 88(10): 4917-23.
17. Muniyappa R, Madan R, Varghese RT. Assessing insulin sensitivity and resistance in humans. *Endotext* [Internet]. 2021 Aug 9.
18. de Lucena DD, de Sá JR, Medina-Pestana JO, Rangel ÉB. Modifiable variables are major risk factors for posttransplant diabetes mellitus in a time-dependent manner in kidney transplant: an observational cohort study. *J Diabetes Res*. 2020 Mar 18; 2020. <https://doi.org/10.1155/2020/1938703>
19. Axelrod DA, Cheungpasitporn W, Bunnapradist S, Schnitzler MA, Xiao H, McAdams-DeMarco M, et al. Posttransplant Diabetes Mellitus and Immunosuppression Selection in Older and Obese Kidney Recipients). *Kidney med*. 2022; 4(1): 100377.

20. Chan HW, Cheung CY, Liu YL, Chan YH, Wong HS, Chak WL, Choi KS, Chau KF, Li CS. Prevalence of abnormal glucose metabolism in Chinese renal transplant recipients: a single centre study. *Nephrol Dial Transplant*. 2008; 23(10): 3337-42.
21. Şaşak G, Sezer S, Colak T, Acar FN, Haberal M. Factors associated with insulin resistance after long-term renal transplantation. *Transplant proc*. 2011; 43(2): 575-7. Elsevier.
22. Petkovska L, Ivanovski N, Dimitrovski C, Serafimovski V. Clinical importance of insulin resistance after renal transplantation in patients on triple immunosuppressive therapy with cyclosporine, corticosteroids and mycophenolat mofetil. *Contributions, Sec.Biol. Med. Sci., MASA*. 2008; (29):129-39.
23. Trovati M, Ponziani MC, Massucco P, Anfossi G, Mularoni EM, Burzacca S, et al. Blood glucose pre-prandial baseline decreases from morning to evening in type 2 diabetes: role of fasting blood glucose and influence on post-prandial excursions. *Eur J Clin Invest*. 2002; 32(3): 179-86.
24. Cole EH, Prasad GV, Cardella CJ, Kim JS, Tinckam KJ, Cattran DC, Schiff JR, Landsberg DN, Zaltzman JS, Gill JS. A pilot study of reduced dose cyclosporine and corticosteroids to reduce new onset diabetes mellitus and acute rejection in kidney transplant recipients. *Transplantation research*. 2013; 2(1): 1-7.
25. Shah T, Kasravi A, Huang E, Hayashi R, Young B, Cho YW, Bunnapradist S. Risk factors for development of new-onset diabetes mellitus after kidney transplantation. *Transplantation*. 2006; 82(12): 1673-6.
26. Bang JB, Oh CK, Kim YS, Kim SH, Yu HC, Kim CD, et al. Insulin secretion and insulin resistance trajectories over 1 year after kidney transplantation: a multicenter prospective cohort study. *Endocrinol Metab*. 2020; 35(4): 820-9.
27. Kurella M, Lo J, Chertow G. Metabolic syndrome and the risk for chronic kidney disease among nondiabetic adults. *J Am Soc Nephrol*. 2005; 16: 2134-40.
28. Tomaszewski M, Charchar FJ, Maric C, McClure J, Crawford L, Grzeszczak W, et al. Glomerular hyperfiltration: a new marker of metabolic risk. *Kidney Int*. 2007; 71(8): 816-21.
29. Bosma RJ, Kwakernaak AJ, Homan Van Der Heide JJ, De Jong PE, Navis GJ. Body mass index and glomerular hyperfiltration in renal transplant recipients: cross-sectional analysis and long-term impact. *Am J Transplant*. 2007; 7(3): 645-52.
30. Guthoff M, Wagner R, Weichbrodt K, Nadalin S, Königsrainer A, Häring HU, et al. Dynamics of glucose metabolism after kidney transplantation. *Kidney Blood Press Res*. 2017; 42(3): 598-607.