Universidade Federal do Rio Grande do Sul

Faculdade de Medicina

Programa de Pós-Graduação em Ciências Médicas: Endocrinologia

Avaliação da Presença das Complicações Microvasculares do Diabetes em Pacientes com Diabetes Mellitus pós-transplante Renal

Dissertação de Mestrado

Thizá Massaia Londero

Porto Alegre, agosto de 2017.

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LISTA DE ABREVIATURAS E SIGLAS

ABTO	Associação Brasileira de Transplantes de Órgãos
ADA	*American Diabetes Association*
A1c	Hemoglobina glicada
CMV	Citomegalovírus
CID-9	Código Internacional de Doenças, 9ª versão
DM	Diabetes mellitus
DMPT	Diabetes mellitus pós-transplante
HLA	*Human leucocyte antigen*
HCV	Vírus da hepatite C
TOTG	Teste oral de tolerância à glicose

LISTA DE SÍMBOLOS

%	Porcentagem
>	Maior que
<	Menor que
≥	Maior ou igual que
≥	Menor ou igual que
mg	Miligrama
dl	Decilitro

LISTA DE TABELAS E FIGURAS

Capítulo 2

RESUMO

A ocorrência de hiperglicemia, transitória ou persistente, após um transplante de órgãos, é um evento bem documentado desde os primeiros transplantes realizados, na década de 60. Porém, só recentemente os critérios diagnósticos e a nomenclatura apropriada para esta condição foram definidos. O diabetes mellitus pós-transplante (DMPT) é a designação dada para a hiperglicemia persistente que ocorre após um transplante de órgão sólido ou hematopoiético em pacientes previamente não diabéticos. À medida que aumenta a sobrevida dos receptores e enxertos, fruto de melhor compreensão e manejo das complicações imunológicas e infecciosas pós-transplante, espera-se uma maior incidência do DMPT. O DMPT possui fatores de risco heterogêneos, sendo alguns deles específicos do período pós-transplante (principalmente os imunossupressores - glicocorticoides e inibidores da calcineurina), e outros comuns àqueles do diabetes mellitus (DM) tipo 2, como síndrome metabólica, obesidade e idade avançada. Apesar do seu caráter crônico, ainda é escasso o conhecimento acerca do apropriado manejo em longo prazo e das complicações associadas ao DMPT. A hiperglicemia crônica desempenha papel fundamental na patogênese das complicações microvasculares do diabetes. Retinopatia diabética, neuropatia diabética e doença renal do diabetes incidem frequentemente em pacientes com DM tipos 1 e 2, dependendo da duração do diabetes e do controle glicêmico obtido. Portanto, a triagem dessas complicações é recomendada na ocasião do diagnóstico do DM tipo 2 e no quinto ano do diagnóstico do DM tipo 1, e após anualmente, para ambos. Apenas um estudo de base de dados populacional descreveu o comportamento das complicações microvasculares no DMPT, propondo que teriam uma instalação acelerada, quando comparado ao DM tipos 1 e 2. Considerando que a ocorrência das complicações microvasculares permanece incerta em pacientes com DMPT, este estudo avaliou a ocorrência de retinopatia diabética, doença renal do diabetes e/ou neuropatia diabética em pacientes com DMPT renal com mais de 5 anos de evolução. Mais de 60% dos pacientes com DMPT apresentaram triagem positiva para polineuropatia distal, sendo o risco dobrado a cada 1% de incremento na hemoglobina glicada (A1c). Mais de 40% dos pacientes com DMPT apresentaram dois ou mais reflexos cardiovasculares alterados, achados compatíveis com neuropatia autonômica, assim como a maioria dos pacientes sem DMPT. Após tempo aproximado de 8 anos de diabetes, não se observou retinopatia diabética clínica por fotografia do fundo de olho. Entretanto, através de tomografia de coerência óptica, foram determinadas menores espessuras de segmentos das camadas internas da retina em pacientes com DMPT, comparados a pacientes transplantados renais não diabéticos, achado que pode sugerir

neuropatia diabética retiniana. Durante o primeiro ano e após 8,5 anos do transplante renal, a taxa de filtração glomerular e o índice proteinúria-creatininúria (IPC) foi semelhante entre pacientes com e sem DMPT. Este é o primeiro estudo a avaliar de modo longitudinal as complicações microvasculares do DMPT renal. Os achados são relevantes por suscitarem que a instalação dessas complicações difere do esperado em pacientes diabéticos tipos 1 e 2. O DMPT é uma patologia singular, com fatores de risco e consequências próprios.

ABSTRACT

Since the first transplants performed in the 1960s, transient or persistent hyperglycemia is a well-documented event following organ transplantation. However, only recently the diagnostic criteria and appropriate nomenclature for this condition has been defined. The persistent hyperglycemia after a solid or hematopoietic organ transplantation in previously non-diabetic patients is therefore denominated post-transplant diabetes mellitus (PTDM). As the survival of recipients and grafts increases, due to a better understanding and management of post-transplant immunological and infectious complications, a higher incidence of PTDM is expected. PTDM has heterogeneous risk factors, some are specific for the post-transplant period (mainly immunosuppressant medications such as glucocorticoids and calcineurin inhibitors), and others are common to those of type 2 diabetes mellitus (DM), such as metabolic syndrome, obesity, and older age. Despite its chronic nature, knowledge about long-term management and complications associated with PTDM is still missing. Chronic hyperglycemia plays a key role in the pathogenesis of diabetes microvascular complications. Diabetic retinopathy, diabetic neuropathy and diabetes kidney disease very often occur in patients with DM types 1 and 2, depending on the duration of diabetes and glycemic control. Therefore, screening for these complications is recommended at the time of diagnosis of type 2 DM and in the fifth year of diagnosis of type 1 DM, and annually thereafter. Only one population database study described the behavior of microvascular complications in PTDM, proposing that they would have an accelerated installation when compared to DM types 1 and 2. Considering that the course of microvascular complications remains uncertain in patients with PTDM, this study evaluated if recipients with more than 5 years of renal PTDM diagnosis presented diabetic retinopathy, diabetes kidney disease and/or diabetic neuropathy. More than 60% of PTDM patients presented positive screening for symmetric distal polyneuropathy and a 1%-point increase in glycated hemoglobin (A1c) doubled its odds. Forty-six percent of PTDM patients had at least two altered cardiovascular reflex tests, as most of NPTDM patients, without statistically significant difference between them. After approximately 8 years of diabetes, clinical diabetic retinopathy was not observed in color fundus photography. However, compared to non-diabetic recipients, inner retinal layers measured through optical coherence tomography were thinner in PTDM patients, a finding that may suggest retinal diabetic neuropathy. During the first year and after 8.5 years of renal transplantation, estimated glomerular filtration rate and protein to creatinine ratio were similar between patients with and without PTDM. This is the first longitudinal study to assess microvascular complications in renal PTDM. These findings are

relevant and suggest that installation of microvascular complications in PTDM differs from expected in type 1 and type 2 DM patients. DMPT is a unique pathology, with its own risk factors and consequences.

SUMÁRIO

Capítulo 1 - Introdução

O transplante renal é o tratamento de escolha para a maioria dos pacientes com doença renal crônica em estágio final (1). É a modalidade de transplante de órgão sólido mais frequentemente realizada no Brasil e no mundo (1). De janeiro de 2007 a março de 2017, foram realizados 50325 transplantes renais no Brasil, conforme dados da Associação Brasileira de Transplantes de Órgãos (ABTO) (2).

O transplante renal bem-sucedido melhora a qualidade de vida, é mais custo-efetivo e reduz o risco de mortalidade para a grande maioria dos pacientes, quando comparado à terapia de substituição renal dialítica (1, 3). Assim como é importante reconhecer os benefícios do transplante renal no momento de sua indicação, também devem estar claras as possíveis complicações decorrentes deste procedimento. Complicações infecciosas, virais e bacterianas, aumento do risco para desenvolvimento de neoplasias e diabetes mellitus pós-transplante são alguns exemplos de complicações decorrentes da exposição ao uso crônico de drogas imunossupressoras.

Nas eras iniciais do transplante renal (décadas de 1950 a 1970), o glicocorticoide, utilizado em altas doses, era a droga imunossupressora empregada para evitar e tratar os episódios de rejeição aguda. Como consequência, os receptores apresentavam altas taxas de hiperglicemia transitória e diabetes (4, 5). Com o advento da ciclosporina, um inibidor da calcineurina, no final da década de 70, e mais recentemente do tacrolimus (década de 90), as taxas de rejeição reduziram drasticamente, o que permitiu maior sobrevida ao binômio receptor-enxerto (6). A sobrevida do receptor, que não ultrapassava 77% após 1 ano do transplante nas eras iniciais, passou a alcançar 90% já no final da década de 90 (4, 5). Porém, mesmo com a redução da necessidade de altas doses de glicocorticoide com o advento dos inibidores da calcineurina, não foi observada redução na incidência da hiperglicemia e diabetes após o transplante, uma vez que estas drogas apresentam reconhecido efeito diabetogênico.

Com o aumento da sobrevida dos pacientes transplantados renais, que atualmente no Brasil é de 84% em 7 anos, a principal causa de perda do órgão transplantado é a morte do receptor devido a causas não relacionadas ao enxerto(4, 7). Deste modo, à medida que aumenta a sobrevida dos pacientes transplantados, aumenta o número de receptores que vão necessitar de cuidados crônicos e que ficarão expostos às patologias típicas do envelhecimento, como doenças cardiovasculares e metabólicas, especialmente o *diabetes mellitus* (DM) (7, 8).

Existe um subtipo específico de diabetes que ocorre em pacientes previamente não diabéticos, após um transplante de órgão sólido ou hematopoiético (9). Desde 2014, DM pós-transplante (DMPT) é a denominação oficial para esta condição (10). O DMPT foi descrito pela primeira vez em 1964 (11), por Starlz *et. al*, que observaram uma associação entre o transplante renal e surgimento pós-operatório de diabetes. Os critérios para diagnóstico de DMPT sugeridos por Sharif *et. al* (10) em um consenso são os mesmos empregados para diagnóstico de diabetes tipos 1 e 2, conforme a *American Diabetes Association* (ADA)(12): glicemia de jejum ≥126 mg/dl e/ou teste de tolerância oral a 75g de glicose (TTOG) em 2 horas ≥200 mg/dl e/ou hemoglobina glicada (A1c) >6,5% e/ou glicemia ao acaso ≥200 mg/dl com sintomas e sinais clássicos de diabete descompensado, sendo que os três primeiros critérios exigem a confirmação em uma segunda ocasião. Ainda, esses resultados devem ser confirmados após 45 dias do transplante renal, idealmente após a alta hospitalar do paciente e com o paciente clinicamente estável, quando as doses dos imunossupressores já foram ajustadas para aquelas de manutenção (10). O DMPT usualmente instala-se dentro dos primeiros meses após o transplante, porém a chance de desenvolver DMPT persiste ao longo da vida do receptor. Desse modo, recomenda-se a sua triagem periodicamente, com glicemia de jejum, TTOG e A1c. A A1c >6,5% permite diagnóstico de DMPT, porém o seu resultado negativo durante a triagem nos primeiros doze meses após o transplante não o afasta, de modo que não se recomenda sua utilização isoladamente(7, 13).

A incidência do DMPT é bastante variável na literatura, especialmente porque não havia, até recentemente, uma padronização da nomenclatura e dos critérios diagnósticos para este tipo específico de diabetes, dificultando uma análise apropriada dos estudos. O DMPT pode acometer de 10 a 75% dos pacientes transplantados renais, modalidade de transplante da qual provém a grande maioria dos estudos sobre DMPT (5, 7, 9). Sabe-se que sua ocorrência vem aumentando ao longo dos anos devido ao aumento e envelhecimento da população de receptores de órgãos. Ainda, a incidência de DMPT é variável visto estar associada a diferentes fatores de risco. Usualmente, os fatores de risco para DMPT são classificados em não-modificáveis, potencialmente modificáveis e modificáveis(14). Os principais fatores de risco não-modificáveis referem-se a idade (superior a 45 anos), etnia (negra), tipo de doador (cadáver), sexo (masculino), causa da doença renal crônica (doença renal policística), polimorfismos genéticos, história familiar de diabetes e número de *mismatches* entre os HLA do receptor e doador (5, 14). Os fatores que potencialmente podem ser modificados, através de triagem e manejo durante a avaliação pré-transplante são os estados de pré-diabetes, como

glicemia de jejum alterada e a tolerância diminuída à glicose e infecções por vírus da hepatite C (HCV) e citomegalovírus (CMV) (14). Já as condições que podem ser ativamente modificadas são uso de imunossupressores (inibidores da calcineurina e glicocorticoides), número de episódios de rejeição aguda, ganho de peso e obesidade após o transplante, além dos componentes da síndrome metabólica como hipertrigliceridemia e anormalidades bioquímicas (hipomagnesemia e hiperuricemia)(5, 14, 15). O regime de imunossupressão parece ser o principal responsável pela variabilidade na ocorrência do DMPT, podendo responder por até 74% da variação na sua incidência(16).

Por ser uma comorbidade do período pós-transplante recentemente determinada, de ocorrência modificável e crescente, a grande maioria dos estudos sobre DMPT deteve-se na determinação dos seus fatores de risco e do tratamento antihiperglicemiante(17). Contudo, considerando o previsto aumento na sobrevivência dos receptores de órgãos com diagnóstico de DMPT, é necessário precisar se eles também estarão sujeitos às típicas complicações crônicas do DM, tanto macro como microvasculares. Assim como na população geral, as doenças cardiovasculares são a maior causa de morte e de perda do enxerto dentre os pacientes transplantados renais, sendo que 40 a 60% das mortes pós-transplante são diretamente atribuíveis a causas cardiovasculares(18). A associação, tanto do DM pré-transplante como do DMPT, com o aumento do risco de complicações cardiovasculares, principalmente infarto agudo do miocárdio e insuficiência cardíaca, já é bem estabelecida na literatura (19, 20) justificando a necessidade de seu rastreio e apropriado manejo destas patologias. Todavia, ainda é pouco conhecido o comportamento das complicações microvasculares do DM (neuropatia, retinopatia e doença renal do diabetes) no âmbito do DMPT(8).

Em relação à retinopatia diabética, sabe-se que mais de 50% dos pacientes com DM tipos 1 e 2 desenvolvem algum grau de retinopatia ao longo da vida(21). Além disso, a retinopatia diabética é a principal causa de cegueira dentre adultos em idade produtiva em todo o mundo(22). A doença renal do diabetes é mundialmente responsável pela maior parte dos casos de doença renal crônica em estágio final(12). Tanto estágios iniciais como finais da doença renal crônica são associados à maior utilização dos sistemas de saúde e maior morbimortalidade, especialmente cardiovascular(23). Após 10 anos do diagnóstico de DM tipo 2, 25% dos pacientes apresentarão albuminúria e 1%, elevação dos níveis de creatinina sérica ($\geq$2.0 mg/dl). Esses últimos necessitarão de terapia renal substitutiva dentro de um período mediano de apenas 2,5 anos(24). A neuropatia diabética abrange um amplo espectro de manifestações, das quais as mais prevalentes são a neuropatia autonômica e polineuropatia

simétrica distal, que podem acometer mais de 50% dos pacientes com DM tipos 1 e 2(25, 26). Ambas são relacionadas a consequências debilitantes, como dor crônica, úlceras e amputações de membros inferiores(12, 25). Por essas razões, a triagem dessas complicações microvasculares é recomendada na ocasião do diagnóstico do DM tipo 2 e no quinto ano do diagnóstico do DM tipo 1, e após anualmente, para ambos os casos(12).

Quanto à avaliação dessas complicações em pacientes com DMPT, poucos estudos documentam sua ocorrência. A principal publicação sobre este tema é de 2007, quando Burroughs *et al.* (27) descreveram, em um estudo de base populacional usando dados do *United States Renal Data System* (USRDS), a incidência de complicações microvasculares (identificadas por códigos do Código Internacional de Doenças-9, CID-9) em pacientes receptores renais com DMPT. Neste estudo, aproximadamente 60% dos pacientes com DMTP desenvolveram pelo menos uma complicação microvascular durante um seguimento de 3 anos, sugerindo que a instalação dessas complicações poderia ser acelerada na população de pacientes transplantados, comparada à geral. Mais recentemente, em 2013, Prasad *et. al* (28) propuseram-se a avaliar o impacto da nefropatia diabética *de novo*, confirmada por diagnóstico histopatológico em biópsia do enxerto, em pacientes com DMPT. Os autores concluíram que nefropatia diabética *de novo* é uma importante causa de falência renal nessa população, porém somente 9 pacientes com DMPT foram incluídos, de um universo de 421 indivíduos transplantados. Observa-se, portanto, que ainda é limitado o conhecimento acerca das complicações microvasculares em receptores de órgãos que desenvolvem DMTP. Permanece incerto se a instalação e a progressão dessas complicações crônicas nessa população se comportam de maneira similar ao acometimento em pacientes com DM tipos 1 e 2(8).

Considerando o exposto, estudos que avaliem as complicações crônicas microvasculares em pacientes com DMPT se fazem necessários, sendo, portanto, este o escopo desta dissertação.

Assim, os objetivos desta dissertação são:

1. Determinar a prevalência de retinopatia diabética, doença renal do diabetes e neuropatia diabética em pacientes transplantados renais com diagnóstico de DMPT há pelo menos 5 anos;

2. Avaliar as associações possíveis entre a presença dessas complicações microvasculares e as características clínicas e laboratoriais da população estudada.

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Capítulo 2 – Artigo Original

Different Course of the Microvascular Complications of Diabetes Mellitus in Kidney Transplant Recipients with Posttransplant Diabetes: a Longitudinal Study

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ABSTRACT

Objective: to evaluate the occurrence of microvascular complications (diabetic retinopathy, diabetes kidney disease and diabetic neuropathy) in kidney transplant recipients with at least five years of posttransplant diabetes mellitus (PTDM).

Research Design and Methods: patients aged >18 years, with no history of diabetes before transplant and with at least five years of PTDM were included from a cohort of kidney transplant recipients from January 2000 to December 2011 (n = 895). Diabetic retinopathy was evaluated by fundus photographs and optical coherence tomography (OCT). The presence of diabetes kidney disease was evaluated by protein to creatinine ratio (PCR) and estimated glomerular filtration rate (eGFR). Distal symmetric polyneuropathy was assessed by Michigan Protocol and 10 g-monofilament foot exam. The Ewing protocol identified cardiovascular autonomic neuropathy. Controls were recipients transplanted in the same period, but without PTDM diagnosis (NPTDM).

Results: After 578 weeks of follow-up, 135 (15%) patients developed PTDM, 64 of them with more than 5 years of PTDM diagnostic. Forty patients were included in the present analysis and were compared to 51 NPTDM controls. Most PTDM patients were white (80%) and female (60%), and aged 49.6±10.5 years upon DM diagnosis (median of 68 days after transplantation). Mean PTDM duration was 7.93±2.92 years and median glycated hemoglobin (A1c) was 7% at most recent evaluation. None of PTDM patients presented diabetic retinopathy at fundus photographs, but a thinning of inner retinal layers was observed with OCT in this group, a finding that may suggest retinal diabetic neuropathy. More than 60% of PTDM patients presented positive screening for distal polyneuropathy (OR 1.55; CI 1.26-1.91; $p<0.001$) and a 1%-point increase in A1c doubled its odds. Forty-six percent of PTDM patients had at least two altered cardiovascular reflex tests, as 65% of NPTDM patients, without statistically significant difference between them ($p=0.26$). During the 1st year and after 8.5±3.0 years of renal transplantation, eGFR and PCR were similar between patients with and without PTDM.

Conclusions: This is the first longitudinal study to assess microvascular complications in renal PTDM patients. A lower than expected prevalence was observed as well as a different clinical course of the complications. Interestingly, patients with PTDM had a thinning of internal retinal layers in comparison with non-PTDM subjects. Our findings suggest that installation of microvascular complications in PTDM differs from the expected in type 1 and type 2 DM

patients. PTDM seems to be a unique type of diabetes and its consequences may be milder than the reported for other types of DM.

INTRODUCTION

Hyperglycaemia is a well-documented event that may occur after organ transplantation and may be transient or persistent(1). Since 2014(2), posttransplant diabetes mellitus (PTDM) is the recommended denomination for persistent post-operative hyperglycaemia. As grafts and recipients survival rates have increased in recent decades due to a better understanding and management of post-transplant immunological and infections complications, the incidence of PTDM has also increased(3), leading to the need for a better knowledge on the behaviour of this disease in long term. The PTDM prevalence increases in proportion to the number of transplantations performed, varying from 10 to 74% in series of kidney transplants(4). Some of the risk factors for PTDM are the same as for type 2 diabetes, such as obesity and older age, but others are specific of the post-transplant period such as calcineurin inhibitors and corticosteroids use(5-9)

Although there is a good understanding of PTDM pathogenesis(10), there are still uncertainness about proper long-term management of this entity(11, 12). Retinopathy, chronic kidney disease and neuropathy are microvascular complications frequently seen in type 1 and 2 diabetes. Diabetic retinopathy is the leading cause of blindness among working aged adults around the world(13). Diabetic kidney disease is responsible for most cases of end-stage renal disease worldwide(14). Diabetic neuropathy encompasses a broad spectrum of manifestations, with autonomic neuropathy and distal symmetrical peripheral polyneuropathy being the most common. Both are related to debilitating complications such as foot ulcers, lower-extremity amputations, and chronic pain(15, 16). For these reasons, screening for microvascular complications are recommended at the time of type 2 diabetes diagnosis and, for type 1 diabetes, after five years of diagnosis, and annually thereafter(14). However, regarding these complications in PTDM patients, little data is available(17). If the progression of chronic diabetic complications in transplant recipients is similar to that of patients with other types of diabetes, it remains unclear(18). Therefore, the aim of the present study was to evaluate the clinical course of diabetic microvascular complications in kidney transplant recipients with more than 5 years of PTDM diagnosis.

RESEARCH DESIGN AND METHODS

Study design and population selection

A retrospective cohort study was conducted with kidney transplant recipients from a tertiary hospital in south of Brazil. All patients transplanted from January 1st, 2000 to December 28th, 2011 had their charts reviewed. Patients with the following criteria were included in the study: age >18 years old, no history of diabetes mellitus before transplant and with at least five years of post-transplant diabetes mellitus (PTDM). The PTDM was diagnosed according to American Diabetes Association (ADA) criteria as suggested by Sharif (14) which includes fasting plasma glucose (FPG) ≥126 mg/dl and/or 2-hour plasma glucose in oral glucose tolerance test (OGTT) ≥200 mg/dl and/or glycated haemoglobin (A1c) >6.5% and/or random blood glucose (RBG) ≥200 mg/dl with classic symptoms of decompensated diabetes mellitus. The three first criteria were confirmed in a second occasion. Glucose samples available in the first 45 days after transplant were not considered, as recommended (2). Episodes of transient hyperglycaemia related to the high doses of corticosteroid and/or tacrolimus early after transplantation were not considered PTDM. Patients starting insulin and/or antihyperglycemic medication while in the kidney transplant hospital stay and that have maintained it after discharge were also considered to have PTDM. Subjects receiving a kidney transplant in the same period as the cases, but without PTDM diagnosis, were consecutively included as controls. Exclusion criteria were: death, kidney graft loss or loss of follow-up.

The ethical committee from the research board of Hospital de Clínicas de Porto Alegre approved this study. All participants provided written, informed consent and this study complies with the Declaration of Helsinki and the principles of Good Clinical Practice.

Clinical and laboratory evaluation

Demographic, anthropometric and graft related data were obtained by patients interviews as well as by reviewing transplant charts and electronic medical records. Pre-transplant data included were age at transplantation, gender, ethnicity, type of renal replacement therapy, time in renal replacement therapy, family history of diabetes, height, dry weight, body mass index (BMI was calculated: weight/height^2) and hepatitis C status. Information related to the donor and the transplant process included type of donor (living or deceased), donor sex and age and cold ischemia time. Post-transplant information included immunosuppressive regimen,

occurrence of delayed graft function, occurrence of acute rejections and weight (BMI was calculated) at PTDM diagnosis time, for cases. Other relevant information, such as smoking habits, previous cardiovascular events, as well as current immunosuppressive regimen and antihyperglycemic treatment were also recorded.

A blood sample was collected to measure FPG (glucose-peroxidase colorimetric enzymatic method, Biodiagnóstica), A1C (high-performance liquid chromatography system; normal range 4–6%; Merck-Hitachi 9100), total-cholesterol, HDL-cholesterol and triglycerides (colorimetric method). Serum creatinine (Jaffé method, traceable), spot urinary creatinine (Jaffé colorimetric method) and protein (turbidimetric method) were periodically measured as part of the routine kidney transplant clinical care.

Diabetic microvascular complications assessment

Diabetic retinopathy (DR): all patients with at least one eye without refractive media opacities were included for fundus photographs. Pupils were dilated and color fundus photographs were captured digitally in both eyes. Images were graded by a trained endocrinologist and an ophthalmologist according to the International Clinical Diabetic Retinopathy and Diabetic Macular Edema Disease Severity Scales, published in 2003, based on the Early Treatment Diabetic Retinopathy Study (ETDRS)(19, 20): "no apparent retinopathy'', "mild non-proliferative DR'', "moderate non-proliferative DR'', "severe non-proliferative DR'' and ''proliferative DR''. Swept-source optical coherence tomography (SS-OCT) was also performed. SS-OCT is an imaging modality that enables the documentation of tissue structure in real time and in situ with an axial resolution of 5.3μm and an axial scan rate of 100.000 scans per second. The 12x9mm scans provides simultaneous measures of the retinal layers, including full retinal thickness (RT), retinal nerve fibre layer (RNFL) and ganglion cell layer (GCL)(21-23), as shown in figure 1. RT is measured along 9 regions centred at the fovea and divided at three main zones: perifoveal zone, parafoveal zone and central zone, which includes the fovea. RNFL is measured along a circle centred at the optic nerve head. GCL is a composite of the inner plexiform layer, ganglion cell layer and nerve fibre layer, covering a zone that is centred to the fovea. All patients submitted to the fundus photograph were evaluated through SS-OCT (Triton SS-OCT, Topcon, Tokyo, Japan) by an experienced ophthalmologist. Measures of RT, RNFL and GCL were obtained and mean thicknesses of each layer were compared between the groups with and without PTDM. We also compared these measurements between males and females to avoid the gender effect on layers thickness(24, 25).

Diabetic kidney disease (DKD): Presence of DKD was evaluated by means of serum creatinine and by protein to creatinine ratio (PCR). Estimated glomerular filtration rate (eGFR) was assessed by CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation(26, 27). DKD was evaluated at 2 different periods: 1°) in the first year of transplantation, by serum creatinine and PCR recorded at 3th, 6th and 12th months after transplantation; 2°) after at least five years of PTDM diagnosis (current), in the same year of the other microvascular complications evaluations, through a mean of three serum creatinine and PCR dosages, with interval of at least three months between them. Since it was not feasible to perform kidney biopsy to diagnose DKD, we compared the eGFR and the PCR variation (delta) between the two evaluated periods as well as the mean values between patients with and without PTDM as indicators of renal damage, according to previous studies(28-30).

Distal symmetric polyneuropathy (DSP): DSP was assessed through Michigan Neuropathy Screening Instrument (MSNI) and 10 g- Semmes-Weinstein monofilament examination (SWME). MSNI is a useful screening test for diabetic neuropathy with both high specificity (95%) and sensitivity (80%)(31, 32). This test consists of 15 questions on foot sensation, with 2 questions to record possible vascular symptoms and a brief clinical examination of both feet to check for deformities and ulceration, grading of ankle reflexes and determining the vibratory perception threshold at the lateral malleolus with a 128 Hz tuning fork. The maximum score for the questionnaire is 15 points and 8 for clinical examination. The cut off points suggested for positive screening are 7 and 2 points, respectively. The higher the scores, the greater is the neuropathy. SWME tests feet sensitivity in 10 locations with the patient's eyes closed. Insensitivity is defined as less than 8 correct responses and it is an independent predictor of amputation and ulceration in diabetic patients(33, 34).

Cardiovascular autonomic neuropathy (CAN): We performed the Ewing protocol for cardiovascular reflex tests (deep breath, Valsalva and orthostatic) as recommended by the American Diabetes Association and the American Academy of Neurology, based on its good reproducibility (35). We also performed the orthostatic hypotension test (16, 36, 37). Patients were instructed to refrain smoking and drinking coffee for two hours prior to tests. Although the interference of some medications on cardiovascular reflex test results might occur, we considered a wash-out of continuous-use medication not feasible in the context of post-

transplant patients. This same strategy was used in the Hoorn Study, which assessed CAN in diabetic patients with cardiovascular disease(38). Antihypertensive drugs and bother potential cofounding factors were recorded. Blood pressure (Omron HEM-742INT, Omron Health Care, Kyoto, Japan) was measured before the test session. The cardiac cycle and the heart rate were measured under four conditions: (a) during spontaneous breathing over 5 min in the supine position for resting heart rate, (b) during six deep breaths over 1 min in the supine position (deep breath test), (c) during Valsalva manoeuvre and (d) during an active change in position from lying to standing (orthostatic hypotension and orthostatic test). The RR variation was continuously obtained from an electrocardiogram recorded on a computer-based data-acquisition system. CAN testing was performed with Poly-Spectrum-8/E software (Neurosoft Inc., Ivanovo, Russia). Established CAN diagnosis requires at least two altered of the four Ewing tests. (16, 37)

Statistical Analysis

Patient's baseline characteristics are described using means (± standard deviations) or median (interquartile interval) for continuous variables and as absolute number (proportions) for categorical data. Shapiro-Wilk test assessed normality. For normally distributed continuous variables, comparisons between groups were done with Student`s 2-tailed t-test. For variables that did not follow Gaussian distribution, a Mann-Whitney U test was used, or logarithmic transformation was performed. The categorical variables were compared with the Chi-square test. Relative risks and their 95% confidence intervals were calculated. Variables investigated as risk factors for PTDM or microvascular complications development were examined with univariate and bivariate analyses. Variables that reached statistical significance $p < 0.25$ in bivariate analysis were included in a multivariable modelling technique. We used step-by-step logistic regression for evaluating the presence of possible confounding factors. Associations were considered significant if the p value was less than 0.05. Statistical calculations were done with PASW 20.0 Software (SPSS Inc., Chicago, IL, USA).

We considered that the minimum clinically relevant difference in eGFR between NPTM and PTDM patients would be 15ml/min. Using an eGFR standard deviation of 20ml/min according to previous studies(39, 40), we reached a sample size of at least 28 patients in each group. Based on the expected diabetic retinopathy prevalence of 6% in patients with type 1 diabetes after 4 years of diagnosis (Wisconsin Diabetes Registry Study)(41) and considering the 64 patients with PTDM > 5 years in our cohort, we calculate a sample size (with correction

for small populations) of 37 patients, plus 10% for losses and refusals, to detect diabetic retinopathy. We also estimate the sample size needed to evaluate diabetic neuropathy according to a study that evaluated the prevalence of peripheral neuropathy in patients with chronic kidney disease with or without diabetes(42). A sample size of 92 patients was calculated to detect an odds ratio of 3.3 between exposed (PTDM) and non-exposed (non-PTDM). We chose to use the largest sample size. Calculations were made through OpenEpi software (version 3.01, Emory University, Rollins School of Public Health), at 80% power and 95% confidence interval.

RESULTS

Cohort Characteristics

From January 2000 to December 2011, 895 patients received a kidney transplant at our institution. Most of recipients were male (n = 512, 57%) and white (n = 644, 72%). Also, the majority of the patients received organs from deceased donors (n = 655, 73%) and the recipients mean age was 43.7±12.8 years-old. During the 578 weeks of follow-up (144.5 months), 135 (15%) patients developed PTDM. Of those, 64 had PTDM for more than 5 years and were eligible for study entry. Forty patients with PTDM (62.5%) agree to participate in the study and 51 patients without PTDM were included as controls. The study flowchart is presented in Figure 2.

Participants Characteristics

The characteristics of patients with and without PTDM are described in Table 1. Most patients of the PTDM group were caucasian (n = 32, 80%) and female (n = 24, 60%) aged 49.6±10.5 years upon DM diagnosis, which occurred 68 days (median, minimum-maximum: 0 – 2036 days) after transplantation. Two patients initiated antihyperglycemic treatment at the first days after transplantation and remained on insulin therapy after discharge from kidney transplant hospitalization. In 75% (n = 31) of PTDM patients, the DM development occurred before 110 days of kidney transplantation. The most frequent method used to diagnose DM was FPG (164.2±70 mg/dl) and 60% of the patients had an A1c measurement with a mean value of 7.35±2.4%. At the time of DM detection, the main immunosuppressive regimen were prednisone (93.3%), tacrolimus (68.4%) or cyclosporine (20.5%), and mycophenolate mofetil (64.3%). The mean serum value of tacrolimus was 10.8±6.07 ng/dl and mean daily prednisone dosage was 14.4±5.5 mg. More than 30% of PTDM patients were obese (BMI ≥30 Kg/m²) at DM diagnosis. The mean weight and BMI for men were 75.9±11.6 kg and 26.3±3.5 kg/m² and for women were 69.7±13 kg and 27.9±6.9 kg/m², respectively. At the end of follow-up, mean diabetes duration was 7.93±2.92 years. Forty-five percent of PTDM patients reported insulin use (one or more doses/day) and 17.5% used two or more antihyperglycemic medications. The maintenance immunosuppressive regimen was prednisone (5 mg/daily, 97.5%), mycophenolate mofetil (67.5%), and a calcineurin inhibitor, tacrolimus (65%) or cyclosporine (20%). Few patients were on azathioprine (5%) or sirolimus (10%). Current serum values of tacrolimus and

cyclosporine are 6.09±2.58 ng/dl and 93.4±41.7 ng/dl, respectively, after 8.5±3.05 years of transplantation.

To ensure representativeness of our sample, we compared the characteristics of the PTDM included patients (n = 40) with those that did not agree to participate (n = 24). Diabetes duration (7.93 vs. 8.29 years, p=0.474) and A1c (median 7.0% in both groups, p=0.632) were similar. Other main variables were equally distributed among these groups and are presented in Table 2.

Fifty-one receptors with no post-transplant diabetes (NPTDM), transplanted at the same period as the PTDM patients (8.9±3.44 vs. 8.5±3.05 years of transplantation, p=0.525), were consecutively included as controls. Compared to NPTDM group, PTDM are older, more likely to be female, and had polycystic chronic disease as the cause of kidney failure (Table 1). PTDM patients had a higher BMI and weight at transplantation, but these differences were attenuated along follow-up. Groups did not differ significantly regarding ethnicity, family history of type 2 DM and parameters related to the donor and the transplant process. We also assessed gender and age distribution among all PTDM (n=135) and NPTDM (n=442) cohort patients. Older age at the time of transplantation persisted as a characteristic of PTDM patients (48±11.5 vs. 42±12.8 years, p<0.001). The frequency of women among the PTDM patients was numerically higher than in NPTDM patients (53% vs. 44%, p=0.09), but the difference did not reach statistical significance. This finding was different from the gender distribution in the study cohort, and was considered in the subsequent statistical analysis

Diabetic microvascular complications assessment

Diabetic Retinopathy: There were 176 eyes from 88 transplanted patients (40 PTDM and 48 NPTDM) included for fundus photographs. In three patients, retinopathy evaluation was not performed due to corneal opacity (n = 2) and refusals (n = 1). Although PTDM patients had average diabetes duration of approximately 8 years, none of them presented findings of diabetic retinopathy at fundus photograph, according to the International Clinical Diabetic Retinopathy and Diabetic Macular Edema Disease Severity Scales. Eighty-five patients (36 PTDM and 46 NPTDM, 5 refusals) were evaluated through SS-OCT. PTDM patients had reduced thickness of all segments of full retina, retinal nerve fibre layer and ganglion cell layer in right eye, and in most of the left retinal layers (Table 3). Age, gender, presence of PTDM and serum tacrolimus levels were associated with retinal layers thickness, as depicted in Table 4. After adjustment through multiple linear regression, presence of PTDM and serum tacrolimus levels

remained predictors of retinal thickness in right eyes. In left eyes, gender and age were the main predictors of retinal thickness (Table S1).

Distal symmetric polyneuropathy: The Michigan questionnaire and the SWME were applied to all patients including those with only one foot. The MSNI questionnaire median scores were significantly different between PTDM and NPTM (3 vs. 1, p = 0.022). Although the risk of DSP increases proportionally to the increase in questionnaire score, the analysis of this variable as continuous appears to be less informative. So, we compared the proportion of positive screening between groups. In both, few patients reached seven or more points (p=0.318) and were considered to have high risk of DSP. Results of MSNI clinical examination showed different scores between PTDM and NPTDM. Median scores were 2 and 0 (p=0.001), respectively, with more patients in the PTDM group scoring more than 2 points (64% vs. 20%, p <0.001), what is compatible with DSP diagnosis. Besides PTDM (OR 1.55; CI 1.26-1.91; p <0.001), positive screening on MSNI was associated also with age (OR 1.02; CI 1.01-1.03; p <0.001); A1c (OR 1.12; CI 1.03-1.22; p = 0.008), and dialysis duration (OR 1.005; CI 1.001-1.008; p = 0.005). No association was observed with gender, cholesterol and triglycerides values, smoking habit, eGFR or tacrolimus serum levels in univariate logistic regression. In multivariate analysis, PTDM (OR 6.11; CI 1.68-22.3; p=0.006), age (OR 1.09; CI 1.02-1.17; p=0.014) and dialysis duration (OR 1.02; CI 1.01-1.04; p=0.037), remained associated with positive MSNI. A separate model in which PTDM was replaced by A1c, a one-point increase in A1c doubled the odds ratio for positive polyneuropathy screening. PTDM patients also had cumulative higher number of errors in the SWME (p=0.036). The MSNI questionnaire and its clinical examination did not correlate and had a poor agreement (Kappa = 0.10).

Cardiovascular autonomic neuropathy: The results of cardiovascular reflex tests were similar in patients with and without PTDM. Rest heart rate (p=0.807) and proportion of abnormal results of respiratory index (p=0.416), 30:15 index (p=0.776), Valsalva index (p=0.215) and orthostatic hypotension test (p=0.669) were not different between groups. Forty-six percent of PTDM patients had at least two altered cardiovascular reflex tests, in comparison with 65% of NPTDM patients (p=0.26). Almost half of the evaluated recipients in both groups were on beta blockers (47.5% vs. 41%, p=0.696), which may have influence the results. In logistic regression, PTDM diagnosis (p=0.191), beta blocker use (p=0.23), number of

antihypertensive drugs (p=0.53), gender (p=0.24) and age (p=0.37) were not associated with positive CAN diagnosis.

Diabetic kidney disease: PCR, eGFR and delta eGFR were analysed to compare the postoperative renal function of patients with and without PTDM. There were no significant differences between these measurements, as presented in Table 5. Use of angiotensin-converting-enzyme inhibitor (AEC) or angiotensin receptor blockers (ARB) was the same between PTDM and NPTDM (17.6% vs. 18.7%, p=0.66) kidney receptors and did not influence eGFR (p=0.55) and protein/creatinine ratio (p=0.20). Mean systolic (137±21.6 vs. 132±19.9mmHg, p=0.32) and diastolic (82.1±19.9 vs. 83.6±12.5mmHg, p=0.57) blood pressure levels were similar between diabetic and non-diabetic patients.

DISCUSSION

In the current study of kidney transplant recipients with PTDM for at least 5 years of duration, we observed a lower than expected prevalence of classical diabetic microvascular complications. With a mean diabetes duration of 8 years and a median A1c of 7.0%, none of the evaluated patients presented clinical diabetic retinopathy, and renal function was comparable to non-diabetic recipients with similar time elapsed from transplant. Regarding diabetic neuropathy, more than 50% of PTDM patients had positive screening for distal symmetric polyneuropathy, which was higher than the observed in NPTDM subjects, but cardiovascular autonomic neuropathy parameters were similar in both groups. Interestingly, a higher frequency of retinal layer thinning through SS-OCT examination was observed in PTDM patients in comparison with controls.

Burroughs et. al(17), in a population database study from United States Renal Data System (USRDS), evaluated the incidence of chronic diabetic complications in PTDM patients. Fifty-eight percent of PTDM patients developed at least one diabetic complication over a 3-year-follow-up period, suggesting that development of complications might be more accelerated in transplant patients than in general DM population. Reasons for disagreement between these findings and ours include the period of the mentioned study (1995-2001), when immunosuppressive regimens used higher doses of corticosteroids and calcineurin inhibitors(43), the lack of uniformity in the PTDM definition, and, most importantly, the diagnosis of complications based on ICD-9 codes instead of the evaluation by specific clinical and laboratory evaluation. Most of the well-conducted studies evaluating PTDM addresses complications directly related to transplantation, such as graft failure. To date, only case reports presents data on microvascular complications of PTDM (44)

The absence of diabetic retinopathy in our sample of PTDM patients was unexpected. Comparing with the retinopathy prevalence in type 1 diabetic patients at Wisconsin Diabetes Registry Study(41), we would expect an occurrence of 6 to 23% of clinical retinopathy, after 4 and 7 years of DM diagnosis, respectively. Similar retinopathy frequency would be expected considering recently published data from the Diabetes Control and Complications Trial Research Group(45) in type 1 diabetes, and from Romero-Aroca et al study (46), which found an annual retinopathy incidence of 15% in type 1 and of 8% in type 2 diabetic patients. We are aware that both conditions are not ideal comparison models for PTDM. Although type 1 diabetes has a clear onset, probably it has also a worse glycemic control than the observed in

PTDM. In the case of type 2 diabetes, the glycemic control might be closer to the observed in our cohort, but the diabetes duration is uncertain. Notably, despite the absence of conventional vascular retinopathy, PTDM was associated with thinning of inner retinal layers. Considering the better glycemic control exhibited by our patients in relation to those of the previously mentioned studies and the evidence of retinal neuronal damage evaluated by SS-OCT, we may speculate that inner retinal layers thinning is an earlier manifestations of diabetic retinopathy, which has recently been named retinal neuropathy(47).

Diabetic retinopathy is considered a form of vasculopathy and classically manifests with microaneurysms, small haemorrhages or lipoprotein exudates(48). Over the past decade, a new pathophysiological model has become accepted, emphasizing that neurodegeneration is an important and early component of retinopathy. Retinal neuropathy is observed structurally, with neural apoptosis, ganglion cell loss, reactive gliosis and thinning of the inner retina, and is perceived functionally, both in complementary diagnostics exams, as electroretinogram, and clinically, as deficits in dark adaptation and color vision (49-51). In this sense, diabetic retinopathy seems to be a neurovascular rather than a solely microvascular disease(49). Perhaps diagnosis and intervention at the stage of retinal neuropathy would minimize the progression to severe retinal vascular complications.

The finding of similar renal function between PTDM and NPTDM patients is corroborated by other recent studies. Sheu *et al*.(40) compared outcomes 12 months after kidney transplantation between PTDM, NPTDM and pre-transplant diabetic recipients. As well as our results, at 1 year post-transplant, all patients had chronic kidney disease stage 3(52) with comparable eGFR (45±18ml/min/1.73m²). A study of 37448 subjects from Organ Procurement and Transplant Network/United Network for Organ Sharing (OPTN/UNOS) database(53) also assessed the impact of PTDM on post-transplant outcomes. Creatinine and creatinine category (≥2, 1.5-2 and ≤1.5 mg/dl) at 12 months after transplantation were equivalent between pre-transplant diabetes, PTDM and NPTDM patients without a history of acute rejection. In a cohort of two distinct transplant periods (1990-1995 and 1996-2011), Choi *et al* also observed that renal function was not different among pre-DM and PTDM patients nor among non-DM and PTDM patients regardless the period of follow-up (54). These results together with ours corroborate to reject the previous hypothesis that PTDM had a marked negative effect on the long-term kidney graft function.

We expected distal symmetric polyneuropathy and autonomic cardiovascular neuropathy to be present in both PTDM and NPTDM recipients, since the relationship between

chronic kidney disease and neuropathies is well documented (55). Surprisingly, in our sample, PTDM and glycemic control measured by A1c remained predictors of polyneuropathy occurrence. Whether peripheral neurological complications would be prevented by strict glycemic control is an object of future randomized clinical trials. We are very cautious in interpreting the cardiovascular autonomic neuropathy assessment due to the various interferences, as chronic kidney disease itself(56) and use of antihypertensive drugs. However, the substantial prevalence of altered tests suggests that further studies are needed to properly evaluate the impact of autonomic neuropathy on cardiovascular morbimortality in posttransplant population.

Our study has some limitations. The diabetic kidney disease evaluation should ideally be detected with histopathological diagnosis by renal biopsy, but this procedure was not feasible due to ethical considerations. So, we used laboratory tests that are suitable to biases: protein-to-creatinine ratio may be influenced by residual diuresis of native kidney and ARB/ACEI use, and renal function assessed by eGFR and serum creatinine may fluctuate according to serum levels of immunosuppressant medications and intercurrent infections.

Results from our study point to a paradigm shift on the significance of PTDM for kidney transplant patients. Studies published up to the first decade of the 2000s demonstrated a marked detrimental effect of PTDM on post transplantation outcomes and suggested the possibility of a more aggressive DM than types 1 and 2(17, 57). However, an attenuated impact of PTDM on early posttransplant mortality and graft loss was observed in two recent cohorts(58, 59), which, as ours, reflect current practice in terms of immunosuppression rationalization and management of diabetes. Despite almost 8 years of diabetes duration, patients enrolled in our cohort had a good glycemic control (median A1c 7%). In addition, patients had frequent medical appointments, a factor that is known to improve diabetes management (60). All these particular characteristics may be reasons to justify the low occurrence of microvascular complications in our cohort, but we also believe that PTDM is a unique type of diabetes, less insulinopenic than type 1 and less inflammatory than type 2 diabetes, and with milder target organ repercussions.

To date, this is the first longitudinal study evaluating diabetic microvascular complications in kidney transplant recipients with PTDM. Our results have important practical implications. Peripheral diabetic neuropathy may affect most patients with PTDM after 5 years of diagnosis. Despite the absence of long-term studies assessing foot ulcers and amputations in this population, screening with already validated methods for type 1 and 2 DM, as MSNI and

SWME, may be useful in the prevention of such outcomes. Thereafter, screening for peripheral neuropathy should be incorporated in PTDM management guidelines. On opposition, no conventional vascular retinopathy, autonomic cardiovascular neuropathy, or kidney disease was demonstrated in this sample of PTDM patients, indication that aggressive screening for these conditions may not be justified. Most importantly, we have added evidence regarding possible early manifestations of diabetic retinopathy, the retinal neuropathy, which can only be identified through OCT and may be a window of opportunity for prevention of more severe forms of retinopathy. Our results point to the need for long-term studies to determine the retinal thinning related to DM clinical course and its possible relation with hard outcomes, such as vision loss and lower limb amputations. Another unexpected finding that needs further studies is the detected association between serum tacrolimus level and thinning of some segments of the retinal layers. The clinical significance of this observation cannot be elucidated and was outside the scope of this study.

In conclusion, our findings contribute to a better understanding of PTDM. The initiation of microvascular complications does not seem to be accelerated as previously supposed. Even though, screening for distal peripheral polyneuropathy may be recommended in patients with PTDM for at least 5 years of duration. Longer prospective studies might elucidate the course of PTDM complications, in particular, the retinal neuropathy.

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FIGURES AND TABLES

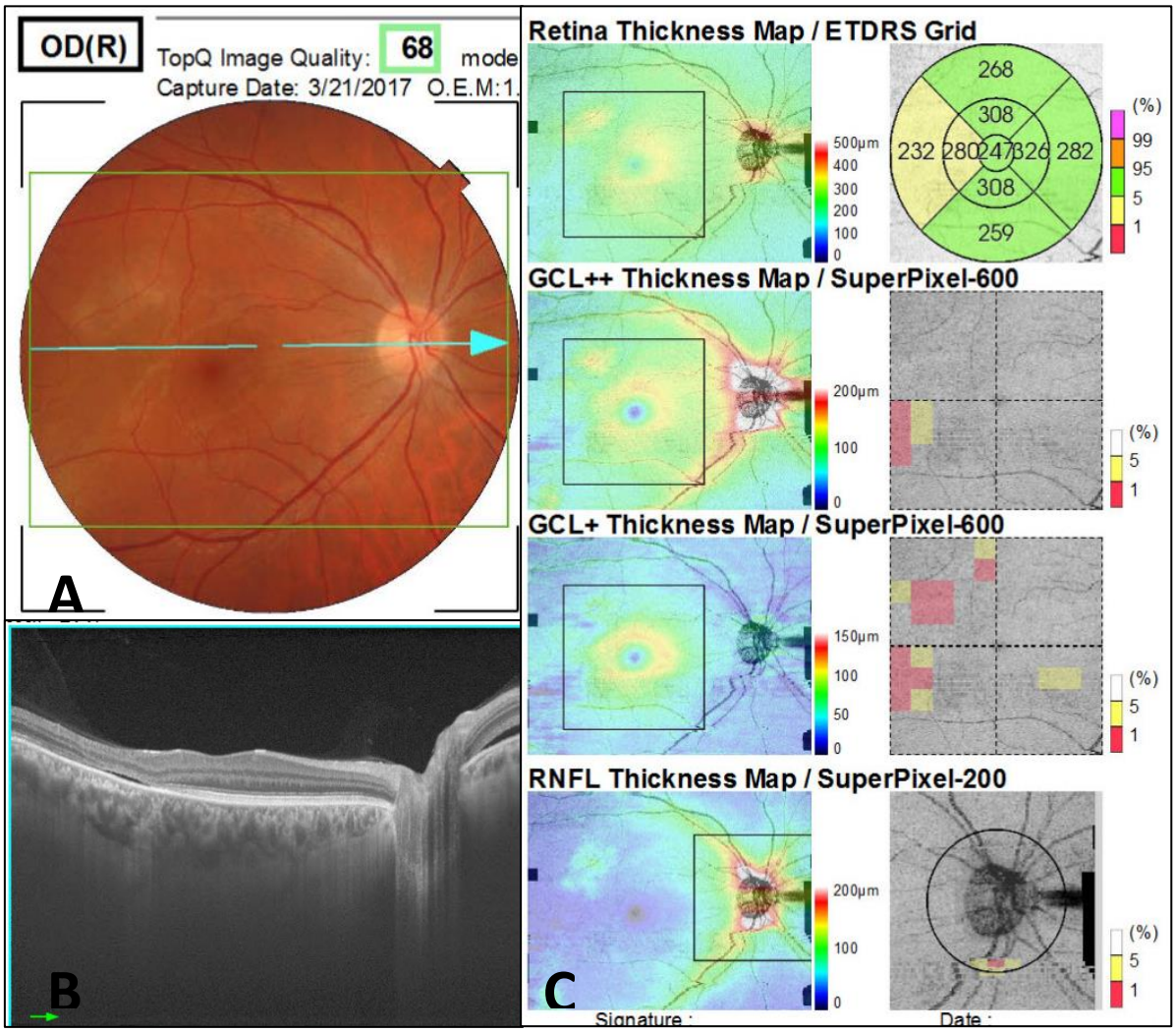

Figure 1. Swept source optical coherence tomography (SS-OCT) examination.

A) Color fundus photograph. B) Histological view of the retinal layers by SS-OCT high resolution. C) SS-OCT single scan shows automatic segmentation layer with thickness maps according to Early Treatment Diabetic Retinopathy Study (ETDRS).

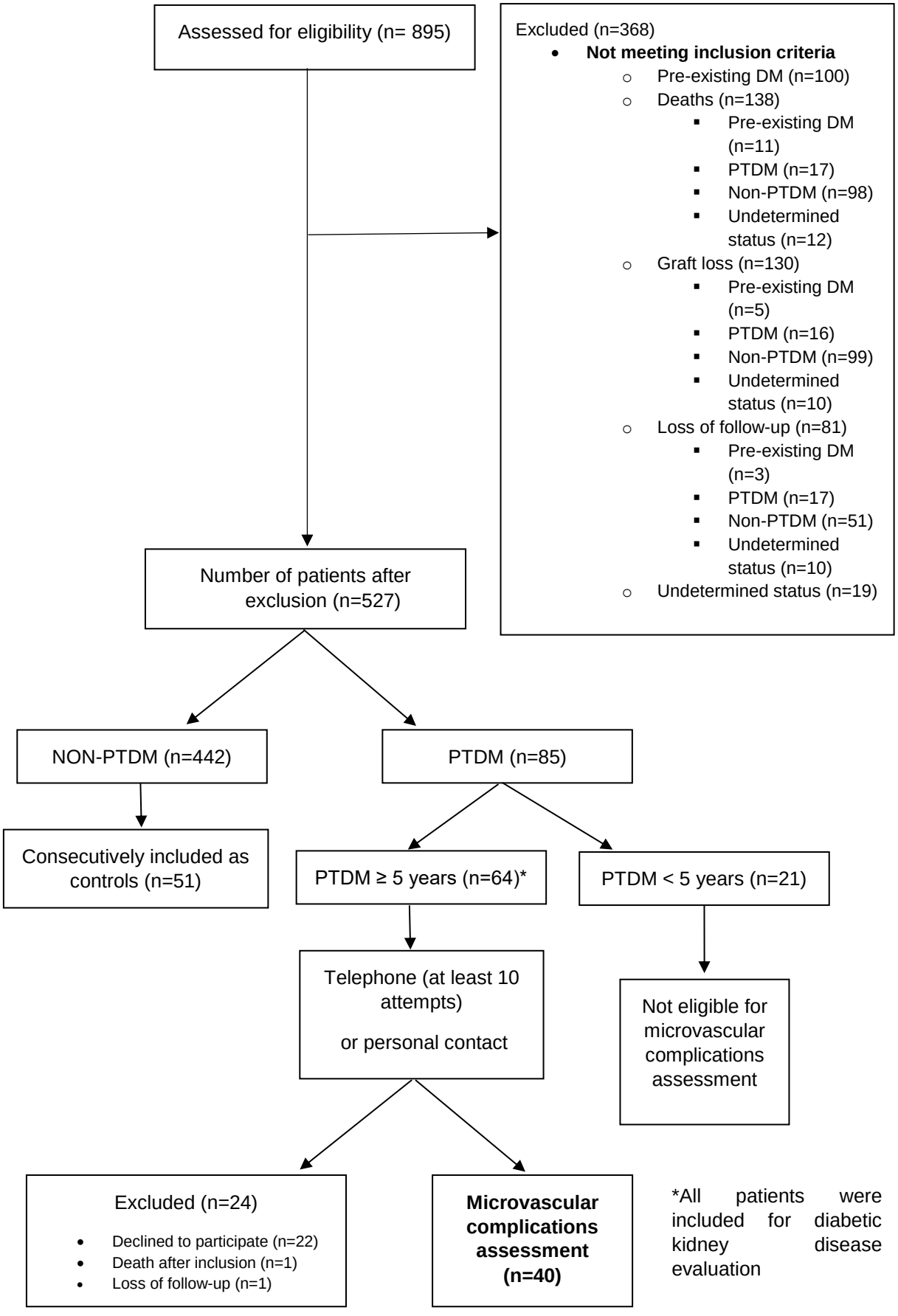

Figure 2. Study flowchart.

Table 1. Demographic and clinical characteristics of the cohort stratified between NPTDM and PTDM patients

	PTDM (n=40)	**NPTDM (n=51)**	**p value**
Female sex, n (%)	24 (60)	15 (29.4)	**0.017**
Age, years, mean (±SD)			
At transplantation	49 (10.9)	40 (11.8)	**0.001**
Current	58 (10.6)	50 (11.1)	**0.002**
Transplantation time, years, mean (±SD)	8.5 (3.05)	8.94 (3.44)	0.525
Weight, Kg, mean (±SD)			
At transplantation	75.2 (13.4)	66.3 (14.2)	**0.005**
Current	75.09 (13.7)	74.54 (10.8)	0.834
BMI, Kg/m², (±SD)			
At transplantation	28.1 (4.3)	24.2 (4.9)	**0.001**
Current	28.38 (4.81)	27.08 (4.06)	0.222
Caucasian, n (%)	32 (80)	40 (78.4)	1.00
Polycystic kidney disease, n (%)	11 (27.5)	4 (7.8)	**0.026**
Hypertension *Preoperative history, n (%)*	19 (47.5)	19 (37.2)	0.442
Current smoking, n (%)	4 (10)	6 (11.7)	1.00
Parental DM, n (%)	16 (40)	22 (43.2)	0.931
Pre-transplant dialysis, n (%)	39 (97.5)	48 (94.2)	0.628
Dialysis time, months, median (IQR)	35 (53)	23 (47)	0.162
Positive HCV status, n (%)	4 (10)	6 (12)	1.00
Deceased donor, n (%)	31 (77)	35 (66.5)	0.367
Donor age, years, mean (±SD)	37.11 (13.84)	41.33 (13.54)	0.173
Donor gender, male, n (%)	18 (45)	23 (51.1)	0.730
Cold ischemic time, minutes, median (IQR)	1095 (1399)	840 (1260)	0.138
Delayed graft function, n (%)[#]	24 (29)	14 (20.7)	0.122
Acute rejection, n (%)[##]	8 (9.6)	12 (14.5)	0.559
Current use of antihypertensive drug, n (%)	33 (82.5)	39 (76.5)	0.658
Number of antihypertensive, median (IQR)	2 (2)	1 (2)	0.148
Use of ACE, n (%)	11 (27.5)	15 (29.4)	1.000
Use of ARB, n (%)	5 (12.5)	3 (5.9)	0.463

Blood pressure			
Systolic	134.4 (15.2)	138.6 (18.8)	0.389
Diastolic	85.7 (17.5)	82.2 (10.3)	0.397
Current immunosuppressants, n (%)			
Tacrolimus	26 (65)	29 (56.8)	0.567
Cyclosporine	8 (20)	16 (31.3)	0.326
Prednisone	39 (97.5)	51 (100)	0.440
Mycophenolate mofetil	27 (67.5)	44 (86.3)	0.059
Azathioprine	2 (5)	2 (3.9)	1.00
Sirolimus	4 (10)	5 (9.8)	1.00
Tacrolimus, serum level, mg/dl, mean (±SD)			
10^{th} day after transplant	10.9 (3.5)	11.5 (4.5)	0.630
30^{th} day after transplant	11.8 (4.6)	10.6 (3.6)	0.284
Higher (first 90 days)	20.4 (5.4)	22.2 (5.8)	0.269
Fasting plasma glucose, mg/dl, median (IQR)			
At transplantation	91.5 (27)	89 (11)	0.123
Current	118 (41)	92 (20)	**<0.001**
HbA1c (%), median (IQR)			
Current	7.0 (1.7)	5.60 (0.6)	**<0.001**
Total cholesterol, mg/dl, mean (±SD)			
At transplantation	202.1 (50.8)	181.6 (38.1)	0.085
Current	193.4 (43.4)	192.2 (49.1)	0.905
Triglycerides, mg/dl, median (IQR)			
At transplantation	177 (101)	151 (128)	0.089
Current	166 (136)	140.5 (68)	0.114

NPTDM, non post-transplant diabetes mellitus; PTDM, post-transplant diabetes mellitus; SD, standard deviation; BMI, body mass index; IQR, interquartile range; DM, diabetes mellitus; HCV, hepatitis C virus; ACE, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; HbA1c, glycated haemoglobin A1c

n=82; ## n= 83. Significant p values in **bold**.

Table 2. Main characteristics of included and excluded patients with post-transplant diabetes mellitus longer than 5 years

	Participant (n=40)	**Non Participant (n=24)**	**p value**
Female sex, n (%)	24 (60)	10 (47.6)	0.513
Age, years, mean (±SD)			
At transplantation	49 (10.9)	43 (10.9)	**0.044**
Current	58 (10.6)	53 (11.8)	0.101
Transplantation time, years, mean (±SD)	8.5 (3.05)	9.3 (3.02)	0.341
Weight, Kg, mean (±SD)	75.1 (13.7)	72.5 (17.9)	0.575
Caucasian, n (%)	32 (80)	16 (76)	0.453
Diabetes duration, years, mean (±SD)	7.93 (2.9)	8.29 (2.9)	0.474
Deceased donor, n (%)	31 (77)	14 (66.7)	0.543
Tacrolimus serum value, mean (±SD)	6.42 (2.4)	5.63 (1.6)	0.261
Fasting plasma glucose, mg/dl, mean (±SD)	118 (41)	117 (43)	0.412
HbA1c (%), median (IQR)	7.00 (1.72)	7.0 (2)	0.632

SD, standard deviation; HbA1c, glycated hemoglobin A1c; IQR, interquartile range. Significant p values in **bold.**

Table 3. Ophthalmic measures by SS-OCT in PTDM and NPTDM patients.

Ophthalmic Measures (mean, ±SD, μm)	PTDM	NPTDM	Mean difference (PTDM-NPTDM, 95% CI)	p value
Full retinal thickness	n = 36	n = 46		
Central (fovea)	214.9 (33.9)	239.0 (28.4)	-24.1 (-37.7 -10.4)	**0.001**
Superior perifovea	255.9 (22.1)	270.6 (26.2)	-14.7 (-25.5 -3.9)	**0.008**
Inferior perifovea	242.2 (19.9)	266.1 (24.5)	-23.8 (-33.8 -13.9)	**<0.001**
Nasal perifovea	265.3 (24.9)	287.2 (25.0)	-21.8 (-32.8 -10.8)	**<0.001**
Temporal perifovea	241.2 (31.7)	263.1 (23.2)	-21.8 (-33.8 -9.87)	**<0.001**
Superior parafovea	282.7 (27.7)	309.4 (29.3)	-29.7 (-39.3 -14.1)	**<0.001**
Inferior parafovea	282.6 (31.6)	307.5 (30.3)	-24.9 (-38.6 -11.2)	**0.001**
Nasal parafovea	279.1 (37.3)	308.3 (31.5)	-29.2 (-44.2 -14.2)	**<0.001**
Temporal parafovea	272.1 (33.3)	298.9 (28.7)	-26.7 (-40.3 -13.2)	**<0.001**
Average Thickness	256.4 (20.2)	278.6 (23.3)	-22.1 (-31.8 -12.4)	**<0.001**
Retinal nerve fibre layer thickness	n = 36	n = 46		
Superior	105.7 (34.9)	123.8 (28.3)	-18.1 (-31.9 -4.33)	**0.01**
Inferior	111.6 (37.1)	133.5 (22.2)	-21.9 (-35.9 -7.96)	**0.003**
Nasal	72.1 (20.7)	83.6 (17.4)	-11.6 (-19.9 -3.27)	**0.007**
Temporal	66.6 (25.9)	77.7 (14.5)	-11.1 (-18.5 -3.77)	**0.004**
Total	88.9 (25.9)	104.8 (16.2)	-15.8 (-25.7 -5.98)	**0.002**
Ganglionar cell layer thickness	n = 36	n = 46		
Superior	59.9(13.9)	68.3 (12.9)	-8.46 (-14.3 -2.65)	**0.005**
Temporal superior	60.4(15.1)	70.2 (12.8)	-9.86 (-15.9 -3.84)	**0.002**
Nasal superior	64.3 (14.5)	73.5 (11.8)	-9.17 (-14.8 -3.47)	**0.002**
Inferior	58.1 (11.4)	68.2 (9.39)	-10.1 (-14.6 -5.64)	**<0.001**
Temporal inferior	60.1 (15.7)	73.2 (12.1)	-13.1 (-19.1 -7.07)	**<0.001**
Nasal inferior	62.7 (14.2)	72.8 (10.8)	-10.1 (-15.5 -4.68)	**<0.001**
Total	60.9 (12.1)	71.1 (10.3)	-10.2 (-15.1 -5.34)	**<0.001**

SS-OCT, swept source optical coherence tomography, PTDM, post-transplant diabetes mellitus; NPTDM, non post-transplant diabetes mellitus; SD, standard deviation; CI, confidence interval. Significant p values in **bold**.

Table 4. Multiple linear regression analysis for variables predicting full retinal thickness in both eyes.

	β	Confidence interval	p value
Full retina, average thickness			
Right Eye			
PTDM	-20.9	-30.7 to -11.3	**<0.001**
Age	-0.576	-28.2 to -7.93	**0.001**
Serum tacrolimus	-3.10	-5.30 to -0.90	**0.007**
Gender	-18.1	-28.2 to -7.92	**0.001**
Model 1			
Age	-0.257	-0.714 to 0.201	0.27
PTDM	-15.6	-28.8 to -5.36	**0.003**
Gender	-13.1	-23.9 to -3.27	**0.01**
Model 2			
Serum tacrolimus	-2.83	-4.72 to -0.95	**0.004**
PTDM	-15.3	-23.9 to -4.19	**0.008**
Gender	-9.88	20.9 to -1.17	0.08
Model 3			
Serum tacrolimus	-2.97	-4.97 to -0.97	**0.005**
Age	-0.33	-0.84 to -1.71	0.19
Gender	-15.4	-26.1 to -4.66	**0.006**
Model 4			
Age	-0.138	-0.65 to 0.37	0.59
Serum tacrolimus	-2.80	-4.71 to -0.91	**0.005**
Gender	-9.80	-20.9 to 1.36	0.08
PTDM	-14.2	-26.1 to -2.41	**0.02**
Left Eye			
PTDM	-5.91	7.36 to -19.2	0.38
Age	0.05	-0.56 to 0.65	0.88
Serum tacrolimus	-1.45	-3.61 to 0.70	0.18
Gender	-20.6	-33.3 to -7.95	**0.002**
Model 1			
Serum tacrolimus	-1.25	-3.29 to 0.78	0.22
Gender	-14.4	-24.7 to -3.33	**0.01**

PTDM: post-transplant diabetes mellitus. Significant p values in **bold**.

Table 5. Post-transplant renal function in PTDM and NPTDM patients

	PTDM	**NPTM**	**p value**
eGFR, ml/min, mean (±SD)			
3 mo	52.3 (22.1)	49.4 (16.9)	0.443
6 mo	52.6 (20.2)	48.2 (16.4)	0.210
12 mo	56.9 (22.2)	54.3 (17.6)	0.513
Current	57.8 (26.8)	53.1 (20.9)	0.301
Delta eGFR, median (IQR)			
3 mo[#]	8.05 (27.8)	6.83 (23.4)	0.757
First year after transplantation[##]	10.4 (54.1)	5.18 (24.7)	0.605
Protein-to-creatinine ratio, median (IQR)			
3 mo	0.20 (0.19)	0.10 (0.11)	0.260
6 mo	0.20 (0.36)	0.09 (0.13)	0.209
12 mo	0.25 (0.37)	0.11 (0.09)	0.489
Current	0.16 (0.87)	0.15 (0.36)	0.960

PTDM: post-transplant diabetes mellitus, NPTDM: non post-transplant diabetes mellitus, eGFR: estimated glomerular filtration, SD: standard deviation, MO: months; IQR: interquartile range, #: difference between current eGFR and at 3 months after transplantation, ## difference between current eGFR and mean eGFR of the first year after transplantation

SUPPLEMENTARY DATA

Table S1. Multiple linear regression analysis for variables predicting inner retinal layers thickness in both eyes.

	β	Confidence interval	p value
Full retina, average thickness			
Right Eye			
PTDM	-20.9	-30.7 to -11.3	**<0.001**
Age	-0.576	-28.2 to -7.93	**0.001**
Serum tacrolimus	-3.10	-5.30 to -0.90	**0.007**
Gender	-18.1	-28.2 to -7.92	**0.001**
Model 1			
Age	-0.257	-0.714 to 0.201	0.27
PTDM	-15.6	-28.8 to -5.36	**0.003**
Gender	-13.1	-23.9 to -3.27	**0.01**
Model 2			
Serum tacrolimus	-2.83	-4.72 to -0.95	**0.004**
PTDM	-15.3	-23.9 to -4.19	**0.008**
Gender	-9.88	20.9 to -1.17	0.08
Model 3			
Serum tacrolimus	-2.97	-4.97 to -0.97	**0.005**
Age	-0.33	-0.84 to -1.71	0.19
Gender	-15.4	-26.1 to -4.66	**0.006**
Model 4			
Age	-0.138	-0.65 to 0.37	0.59
Serum tacrolimus	-2.80	-4.71 to -0.91	**0.005**
Gender	-9.80	-20.9 to 1.36	0.08
PTDM	-14.2	-26.1 to -2.41	**0.02**
Left Eye			
PTDM	-5.91	7.36 to -19.2	0.38
Age	0.05	-0.56 to 0.65	0.88
Serum tacrolimus	-1.45	-3.61 to 0.70	0.18
Gender	-20.6	-33.3 to -7.95	**0.002**
Model 1			
Serum tacrolimus	-1.25	-3.29 to 0.78	0.22
Gender	-14.4	-24.7 to -3.33	0.01
Ganglion cell layer, total thickness			
Right Eye			
PTDM	-8.80	-3.80 to -13.8	**0.001**
Age	-0.35	-0.58 to -0.19	**0.005**
Serum tacrolimus	-2.24	-3.21 to -1.28	**<0.001**
Gender	-6.85	-12.1 to -1.63	**0.01**
Model 1			

Age	-0.22	-0.46 to 0.02	0.07
PTDM	-5.97	-0.66 to -11.3	**0.03**
Gender	-4.71	-9.77 to 0.36	0.07
Model 2			
Serum tacrolimus	-2.07	-2.95 to -1.19	**<0.001**
PTDM	-6.04	-10.8 to -1.24	**0.015**
Gender	-3.04	-7.80 to 1.72	0.205
Model 3			
Serum tacrolimus	-2.10	-2.96 to -1.25	**<0.001**
Age	-0.20	-0.41 to 0.12	0.06
Gender	-4.94	-9.41 to -0.48	**0.027**
Model 4			
Age	-0.13	-0.35 to 0.08	0.22
Serum tacrolimus	-2.03	-2.85 to -1.21	**<0.001**
Gender	-2.99	-7.66 to 1.69	0.20
PTDM	-5.09	-10.1 to -0.13	**0.045**
Left Eye			
PTDM	-5.67	-10.8 to -0.50	0.03
Age	-0.16	-0.39 to 0.08	0.20
Serum tacrolimus	-0.52	-1.48 to 0.46	0.29
Gender	-7.59	-12.7 to -2.47	**0.004**
Model 1			
PTDM	-3.90	-9.01 to 1.22	0.13
Gender	-6.50	-11.6 to -1.32	**0.014**
Retinal nerve fibre layer, total thickness			
Right Eye			
PTDM	-15.3	-24.5 to -6.04	**0.001**
Age	-0.54	-0.98 to -0.11	**0.015**
Serum tacrolimus	-3.51	-5.7 to -1.32	**0.002**
Gender	-11.5	-21.1 to -1.09	**0.02**
Model 1			
PTDM	-10.8	-20.6 to -0.97	**0.03**
Age	-0.33	-0.77 to 0.11	0.14
Gender	-7.65	-17.1 to 1.77	0.11
Model 2			
PTDM	-8.83	-21.2 to 3.52	0.16
Gender	-5.34	-17.6 to 6.95	0.39
Serum tacrolimus	-3.35	-5.45 to -1.26	**0.002**
Model 3			
Serum tacrolimus	-3.35	-5.42 to -1.28	**0.002**
Age	-0.47	-0.98 to 0.06	0.08
Gender	-7.34	-18.5 to 3.63	0.18
Model 4			
PTDM	-5.95	-18.7 to 6.84	0.35
Serum tacrolimus	-3.28	-5.33 to -1.22	**0.002**
Age	-0.38	-0.93 to 0.17	0.17
Gender	-5.11	-17.2 to 6.95	0.40
Left Eye			
PTDM	-15.3	-24.5 to -6.04	**0.001**
Age	-0.55	-0.98 to -0.11	**0.015**
Serum tacrolimus	-3.51	-5.70 to -1.32	**0.002**

Gender	-11.5	-21.1 to -1.91	**0.02**
Model 1			
PTDM	-9.09	-16.9 to -1.29	**0.02**
Age	-0.12	-0.47 to 0.22	0.48
Gender	-3.72	-11.2 to 3.79	0.33
Model 2			
PTDM	-9.37	-18.2 to -0.62	**0.04**
Gender	-2.17	-10.8 to 6.49	0.62
Serum tacrolimus	-1.53	-3.02 to -0.04	**0.04**
Model 3			
Serum tacrolimus	-1.69	-3.21 to -0.18	**0.03**
Age	-0.27	-0.63 to 0.10	0.15
Gender	-5.58	-13.6 to 2.44	0.17
Model 4			
PTDM	-8.03	-17.2 to 0.81	0.07
Serum tacrolimus	-1.52	-3.03 to -0.05	**0.04**
Age	-0.17	-0.54 to 0.19	0.35
Gender	-2.23	-10.8 to 6.35	0.60

PTDM: post-transplant diabetes mellitus. Significant p values in **bold**.

Capítulo 3 – Considerações finais e perspectivas futuras

Os resultados deste estudo, que avaliou pacientes transplantados renais com diabetes mellitus pós transplante (DMPT) com pelo menos 5 anos de diagnóstico, trouxeram novas perspectivas ao entendimento desta doença. Até pouco tempo atrás, acreditava-se que o DMPT impunha ao paciente transplantado um risco acelerado de desenvolver complicações microvasculares(17), e que esta condição teria um impacto negativo importante na evolução da função do enxerto, no caso do transplante renal(57). Nossos resultados sugerem que a instalação das complicações microvasculares do diabetes nesses pacientes difere do que se espera em pacientes com diabetes mellitus tipos 1 e 2, possivelmente porque o DMPT é, também, uma entidade diferente dessas. A diferença dos nossos achados em relação aos previamente publicados provavelmente deve-se a dois fatores: 1) a nossa coorte ser mais contemporânea do que as previamente estudadas, refletindo melhorias na imunossupressão empregada com necessidade de doses menores de glicocorticoide e melhor controle glicêmico e, 2) por termos realizado a pesquisa das complicações crônicas por métodos adequados e validados na literatura, e não somente pela pesquisa de citação do CID-9, como ocorreu na publicação prévia(17).

Este estudo abre perspectivas para a realização de estudos mais detalhados sobre o achado de afinamento da camada interna da retina nos pacientes com DMPT. Esta alteração pode indicar uma neuropatia da retina relacionada à hiperglicemia, visto que a camada mais acometida é a das células ganglionares. Portanto, planejamos avaliar mais detalhadamente esta anormalidade através da realização de testes de acuidade visual e potencial evocado visual, além do seguimento destes pacientes ao longo do tempo para determinar se existe progressão e prejuízo da visão durante o seguimento.

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