THE IMPACT OF PRE-EXISTING AND POST-TRANSPLANT DIABETES MELLITUS ON OUTCOMES FOLLOWING LIVER TRANSPLANTATION

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ABSTRACT

Background: Diabetes mellitus (DM) is said to adversely affect transplant outcomes. The aim of this study was to investigate the impact of pre-existing and post-transplant DM on liver transplant (LT) recipients.

Method: A single centre retrospective analysis of prospectively collected data of LT recipients (1990–2015) was undertaken.

Results: Of the 2,209 patients, 13% (n=298) had Pre-DM, 16% (n=362) developed PTDM, 5% (n=118) developed transient hyperglycemia (t-HG) post-LT, and 65% (n=1,431) never developed DM (no DM). Baseline clinical characteristics of patients with PTDM was similar to that of patients with pre-DM. Incidence of PTDM peaked during first-year (87%) and plateaued thereafter. On multivariate analysis (Bonferroni-corrected), non-alcoholic fatty liver disease and the use of Tacrolimus and Sirolimus use were independently associated with PTDM development. Both Pre-DM and PTDM patients had satisfactory and comparable glycaemic control throughout the follow-up period. Those who developed t-HG seems to have a unique characteristic compared to others. Overall, 9%, 5%, and 8% developed end-stage renal disease (ESRD), major cardiovascular event (mCVE), and de novo cancer, respectively. Both Pre-DM and PTDM did not adversely affect patient survival, re-LT, or de novo cancer. The risks of ESRD and mCVE were significantly higher in patients with Pre-DM followed by PTDM and no DM.

Conclusions: In this largest non-registry study, patients with pre-DM and PTDM share similar baseline clinical characteristics. Pre-DM increases the risk of ESRD and mCVE; however, patient survival was comparable to those with PTDM and without diabetes. Understanding the impact of PTDM would need prolonged follow-up.

INTRODUCTION

Diabetes Mellitus (DM) poses an increasing challenge for patients and health care systems worldwide [1]. It leads to significant morbidity and mortality through several macrovascular and microvascular complications [2, 3]. Further, DM is also associated with an increased risk of several cancers [4]. DM is an important risk factor for the development of non-alcoholic fatty liver disease (NAFLD), which is a leading cause of cirrhosis, liver cancer, and liver transplantation (LT). Further, the presence of DM increases the risk of death in patients with liver disease [5].

LT remains the only treatment option that improves the dismal prognosis of end-stage liver disease. Advances in perioperative management and immunosuppressive treatment has led to excellent long-term outcomes with 1-, 5-, and 10-year post-LT survival rates of >90%, 85%, and 70%, respectively [6-8]. However, post-transplant immunosuppression remains associated with a number of side effects including new-onset diabetes mellitus known as post-transplant diabetes mellitus (PTDM). PTDM was first described over 50 years ago [9], but has only more recently gained recognition as a major complication with the potential for serious consequences [10]. The reported incidence of PTDM after all solid organ transplantation ranges from 2% to 53% [10, 11]. PTDM has been reported in 7 – 28% of LT recipients LT [12]. While the pathophysiology of PTDM is not fully understood, pancreatic β-cell dysfunction, impaired insulin secretion (associated with the use calcineurin inhibitors and mammalian target of rapamycin complex 1 inhibitors), postoperative weight gain and hepatitis C virus infection (associated with insulin resistance) are all thought to play a role [13, 14].

The impact of PTDM and pre-existing DM on outcomes following LT is limited and equivocal. While some studies demonstrate a negative impact of PTDM on patient and graft survival [15-20], others were not able to confirm this [21-23]. Further, these studies have usually not compared the impact of PTDM to that of pre-existing DM [15, 17, 18, 22], and many had limited follow-up duration potentially leading to underestimating of a potential impact [16, 18, 21, 22]. In addition, most of these studies have been reported from East Asia [15, 17, 18, 21, 23], and therefore may not represent the experience in the West. The aim of the present study is to assess the impact of pre-existing DM and PTDM on long-term outcomes of LT recipients in the Toronto transplant program, one of the largest in North America.

MATERIALS & METHODS

Patient selection and data collection

This was a retrospective analysis of prospectively collected data from a single, high-volume liver transplant center. All adult patients who received a liver transplant in the Toronto liver transplant program for any indication between January 1990 and December 2015 were eligible for inclusion. The following patients were excluded from the study: (a) patients who underwent combined solid organ transplantation, and (b) patients who were transplanted in other centers and subsequently followed up in the Toronto program.

Demographic and clinical data were extracted from electronic transplant database (OTTR: Transplant Care Platform 6, OTTR Chronic Care Solutions, Omaha, NE), and supplemented with manual chart review.

For the purposes of this study, patients were categorised into four groups based on diabetic status: those without DM before and after transplantation (no DM); those with pre-transplant DM (Pre-DM); those who developed PTDM; and those who developed transient hyperglycemia (t-HG). Main analysis included no DM, Pre-DM, and PTDM groups. t-HG group was compared to the other three groups separately but was not included in the main analysis.

The study was approved by the Toronto University Health Network Research Ethics Board (REB 16-5023).

Diagnosis of PTDM

PTDM was diagnosed, based on the 2003 International Consensus Guidelines [24]. LT recipients who were not diabetic prior to transplantation but developed symptoms of DM with an elevated random plasma glucose (≥11.1 mmol/l) or an elevated fasting plasma glucose (≥7.0 mmol/l) or an elevated 2-hour plasma glucose (≥11.1 mmol/l) during an oral glucose tolerance test were diagnosed to have PTDM. In addition, those who were initiated on hypoglycemic agents for more than 14 continuous days post-transplant were also considered to have PTDM. Glucose levels are monitored daily (pre- and post-meal) during the immediate post-transplant period. Following discharge, random glucose level is regularly checked in LT recipients at their out-patient clinic appointments and those with elevated glucose levels are referred to their family physicians for further investigations.
Those who diagnosed with DM range hyperglycaemia based on the above criteria, but became euglycemic without the need for hypoglycemic agents within 6 months of LT were categorised as t-HG.

Immunosuppression regimen

All patients received intravenous Methylprednisolone followed by an oral course of Prednisone and Tacrolimus immediately following LT; Basiliximab (before that thymoglobulin) induction was used to delay introduction of calcineurin inhibitors in some cases (e.g. patients with renal failure). Oral Prednisone dose followed a standard tapering regimen and was stopped at 3 months post-LT. Tacrolimus was substituted with Cyclosporine in case of Tacrolimus intolerance or neurotoxicity. Mycophenolate mofetil was added if calcineurin inhibitor (Tacrolimus or Cyclosporine) dose reduction was required. Long-term dual immunosuppressant regimen (calcineurin inhibitor or Sirolimus, and Mycophenolate mofetil) was the standard of care for patients transplanted for

autoimmune liver diseases, acute liver failure, and inborn metabolic disorders. Long-term low-dose Prednisone was part of the immunosuppressant regimen for patient with a history of recurrent acute cellular rejection or chronic rejection. Further, the target trough levels of Cyclosporine, Tacrolimus, and Sirolimus remained unchanged over the study period. However, the use of immunosuppressants evolved during the 25-year study period. In brief, these include introduction of Tacrolimus as the primary immunosuppressant in 2001/2002, prior to which Cyclosporine remained the primary immunosuppressant and the use of Sirolimus since 2005 as the primary immunosuppressant in those who were intolerant to calcineurin inhibitors.

For the purposes of this study, only those patients who were taking an immunosuppressant continuously for a minimum of 6 months were considered to have a positive exposure to that immunosuppressant.

Endpoints

Patient survival was the primary endpoint. Patient survival was defined as the time from LT to death from any cause. Patients who were lost to follow-up, or moved to another center for ongoing care, were censored at the time of their last clinic visit. In the event of more than one LT, cases were censored at the time of the second LT.

Re-LT and development of end-stage renal disease (ESRD), major cardiovascular events (mCVE), and de novo cancer were secondary endpoints. ESRD was defined as GFR <15 ml/min/1.73m^2 for 6 months or more, requirement of renal replacement therapy, or renal transplantation. Myocardial infarction and stroke were considered as mCVE.

Data analysis

Continuous variables were presented as median and interquartile range (IQR). Categorical variables were presented as number and percentage. Normality of distributions was assessed using histograms and confirmed using Shapiro-Wilk test. Kruskal-Wallis, Mann-Whitney and Chi-square test were used as appropriate to compare basic demographics of the four study groups. A p-value of <0.05 was considered significant.

Predictors of PTDM was investigated using univariate and multivariate analysis. Univariate analysis was performed using Mann-Whitney test and Chi-square for continuous and categorical variables, respectively. Variables with a p-value <0.10 were included in the multivariate logistic regression model. Variables were considered to have independent association only if the p-value reached Bonferroni-corrected level of significance.

Patient survival analysis for all-cause mortality (primary outcome) was performed using the Kaplan-Meier method. The probability of re-LT and developing ESRD, mCVE, and de novo cancer were investigated using a competing risk model. Death was considered as a competing event and the cumulative incidence of re-LT, ESRD, mCVE, and de novo cancer were calculated using Gray's test.

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY) for univariate and multivariate prediction of PTDM, GraphPad prism 5 (San Diego, CA) for Kaplan-Mayer analysis, and visualisation and R statistical software, version 3.4.1 (http://cran.r-

project.org/) for competing risk analysis. The R-package "cmprsk" version 2.2-7 was used to compare the cumulative incidence and conduct a regression analysis of competing risks.

RESULTS

Demographics and clinical characteristics

The study included 2,209 patients, who underwent LT between January 1990 and December 2015 and fulfilled the eligibility criteria. The median age at LT was 54 years (IQR 47 – 60) and 67% (n=1,480) were males (table 1). The median duration of post-LT follow-up was 6.7 years (IQR 2.9 – 11.3).

Of the 2,209 patients, 13% (n=298; median follow-up 6.3 years [IQR 2.7 – 10.4]) had Pre-DM, 16% (n=362; median follow-up 8.9 years [IQR 4.9 – 12.7]) developed PTDM, 5% (n=118; median follow-up 6.4 years [IQR 1.6 – 10.8]) developed t-HG, and 65% (n=1,431; median follow-up 6.1 years [IQR 2.8 – 10.9]) remained free of DM. The demographic characteristics at LT of all four groups are summarised in table 1.

Incidence, risk factors, and characteristics of PTDM

Of the 1,911 patients who were not diabetic at LT, 362 (19%) developed PTDM following LT. The vast majority were diagnosed to have PTDM during the first year after LT (87%, n=314); the occurrence of PTDM plateaued thereafter – 8 per year at 2 years, 3 per year at 5 years, and 2 per year at 10 years post-LT. Further, the era of transplantation did not impact the development of PTDM – 21% (n=77) of 374 patients who were transplanted in 1990–1999, 19% (n=171) of 916 patients who were transplanted in 2000–2009, and 18% (n=114) of 621 patients who were transplanted in 2010–2015 developed PTDM (p=0.66).

In comparison to no DM group, the following parameters were associated with the development of PTDM (table 2). On univariate analysis, age, male sex, BMI, non-alcoholic fatty liver disease as the etiology of liver disease, pre-LT dyslipidemia, cold ischemia time, Roux-en-Y biliary anastomosis, and the use of Cyclosporin, Sirolimus, and Prednisone for immunosuppression. In addition to the above parameters, pre-LT hypertension (p=0.07) and the use of Tacrolimus (p=0.06) and Mycophenolate (p=0.07) were included in the multivariate analysis (total of 13 parameters); the Bonferroni-corrected level of significance in the multivariate analysis was p <0.0038. On multivariate (Bonferroni-corrected) analysis, non-alcoholic fatty liver disease as the etiology of liver disease (OR 2.03, 95%CI 1.31 – 3.16; p=0.002) and the use of Tacrolimus (OR 2.76, 95%CI 1.78 – 4.30; p<0.001) and Sirolimus (OR 2.72, 95%CI 1.67 – 4.42, p <0.001) as immunosuppression were independently associated with the development of PTDM.

The clinical characteristics of PTDM patients at LT were similar to Pre-DM patients but were different to that of no DM patients. Compared to no DM group, PTDM patients were older (p<0.0001), predominantly male (p=0.001), with higher BMI (p<0.0001), and were likely to have non-alcoholic fatty liver disease as the etiology of liver disease (p <0.0001). In contrast, PTDM patients had marked similarities with Pre-DM patients at LT – age (p=0.40), sex (p=0.09), BMI (p=0.99), and non-alcoholic fatty liver disease etiology (p=0.54). Interestingly, hepatitis C etiology did not differ between PTDM and no DM (p=0.21) or Pre-DM (p=0.23) groups.

Both PTDM and Pre-DM patients had optimal and comparable glycemic control during the post-LT period. The overall median glycosylated hemoglobin (HbA1c) of PTDM and Pre-DM were 6.7% (IQR 6.4 – 7.0) and 6.9% (IQR 6.7 – 7.5), respectively. Similarly, the yearly median HbA1c ranged between 6.0% – 7.1% and 6.3% – 8.1%, respectively (figure 1).

Primary outcome (patient survival)

Over a third (n=782, 35%) of the total study population died following LT. The 1-, 5-, 10-, and 20-year patient survival rates were 90%, 79%, 64%, and 23% in the Pre-DM group, 97%, 88%, 74%, and 22% in the PTDM group, and 90%, 79%, 68%, and 34% in the no-DM group, respectively. Overall, there was no difference in patient survival between the three study groups (p=0.30, figure 2). A summary of the major causes of death are shown in Supplementary Table 1.

Secondary outcomes

Re-LT

Overall, 6% (n=126) underwent re-LT. Of the 298 patients with Pre-DM, 4% (n=12) underwent re-LT, 32% (n=94) died without undergoing re-LT, and 64% (n=192) were alive without requiring re-LT. The 1-, 5-, 10-, and 20-year cumulative incidence of re-LT were 3%, 4%, 5%, and 5%, respectively. Of the 362 patients with PTDM, 6% (n=23) underwent re-LT, 35% (n=127) died without undergoing re-LT, and 59% (n=212) were alive without requiring re-LT. The 1-, 5-, 10-, and 20-year cumulative incidence of re-LT were 2%, 3%, 4%, and 10%, respectively. Similarly, of the 1,431 patients with no DM, 6% (n=91) underwent re-LT, 33% (n=467) died without undergoing re-LT, and 61% (n=873) were alive without requiring re-LT. The 1-, 5-, 10-, and 20-year cumulative incidence of re-LT were 3.1%, 4.6%, 6.6%, and 9.3%, respectively. The cumulative incidence of re-LT was similar between the three study groups (p=0.50).

ESRD

Overall, 9% (n=194) developed ESRD following LT. Of the 298 patients with Pre-DM, 12% (n=35) developed ESRD, 28% (n=82) died without developing ESRD, and 61% (n=181) were alive and free of ESRD. The 1-, 5-, 10-, and 20-year cumulative incidence of ESRD were 3%, 7%, 13%, and 39%, respectively. Of the 362 patients with PTDM, 10% (n=38) developed ESRD, 32% (n=117) died without developing ESRD, and 57% (n=207) were alive and free of ESRD. The 1-, 5-, 10-, and 20-year cumulative incidence of ESRD were 1%, 4%, 8%, and 22%, respectively. Similarly, of the 1,431 patients with no DM, 7.6% (n=109) developed ESRD, 31.2% (n=447) died without developing ESRD, and 61.1% (n=875) were alive and free of ESRD (median follow up 8.0 years). The 1-, 5-, 10-, and 20-year cumulative incidence of ESRD were 2%, 4%, 7%, and 15%, respectively. The cumulative incidence of developing ESRD was significantly higher in Pre-DM patients compared to no DM patients (p=0.003) but was similar to that of PTDM patients (p=0.08). However, the cumulative incidence of developing ESRD was similar in patients with PTDM and no DM (p=0.27).

mCVE

Overall, 5% (n=103) developed mCVE following LT. Of the 298 patients with Pre-DM, 7% (n=21) experienced a mCVE, 32% (n=94) died without ever experiencing a mCVE, and 61% (n=183) were alive and free of mCVE. The 1-, 5-, 10-, and 20-year cumulative incidence of mCVE were 2%, 3%, 8%, and 15%, respectively. Of the 362 patients with PTDM, 6% (n=21) experienced a mCVE, 34% (n=123) died without ever experiencing a mCVE, and 60% (n=218) were alive and free of mCVE. The 1-, 5-, 10-, and 20-year cumulative incidence of mCVE were 1%, 2%, 4%, and 10%, respectively. Similarly, of the 1,431 patients with no DM, 4% (n=57) experienced a mCVE, 33% (n=475) died without ever experiencing a mCVE, and 63% (n=899) were alive and free of mCVE. The 1-, 5-, 10-, and 20-year

cumulative incidence of mCVE were 1%, 2%, 4%, and 7%, respectively. The cumulative incidence of developing mCVE was significantly higher in Pre-DM patients compared to no DM patients (p<0.01) but was similar to that of PTDM patients (p=0.11). However, the cumulative incidence of developing mCVE was similar in PTDM and no DM patients (p= 0.95).

De novo cancer

Overall, 8% (n=183) developed de novo cancer following LT. Of the 298 patients with Pre-DM, 9% (n=26) developed de novo cancer, 31% (n=93) died without developing de novo cancer, and 60% (n=179) were alive and free of de novo cancer. The 1-, 5-, 10-, and 20-year cumulative incidence of de novo cancer were 2%, 7%, 10%, and 13%, respectively. Of the 362 patients with PTDM, 9% (n=33) developed de novo cancer, 31% (n=112) died without developing de novo cancer, and 60% (n=217) were alive and free of de novo cancer. The 1-, 5-, 10-, and 20-year cumulative incidence of de novo cancer were 2%, 5%, 8%, and 14%, respectively. Similarly, of the 1,431 patients with no DM, 8% (n=116) developed de novo cancer, 31% (n=450) died without developing de novo cancer, and 60% (n=865) were alive and free of de novo cancer. The 1-, 5-, 10-, and 20-year cumulative incidence of de novo cancer were 1%, 4%, 7%, and 15%, respectively. Unlike with ESRD and mCVE, the cumulative incidence of developing de novo cancer in no DM patients was similar to that of Pre-DM (p=0.43) and PTDM (p=0.95) patients. The distribution of different types of cancers within each group is summarised in Supplementary Table 2.

Transient hyperglycaemia

One hundred and eighteen patients fulfilled the diagnostic criteria of t-HG. All achieved a state of euglycemia without the need for anti-diabetic medications within 6 months of LT.

t-HG patients were unique in their baseline clinical characteristics. The age of t-HG patients at LT was no different from that of patients with Pre-DM (p=0.06), PTDM (p=0.25), and no DM (p=0.11). However, BMI of t-HG patients at LT was significantly higher than that of no DM patients (p=0.03) but was similar to that of Pre-DM (p=0.50) and PTDM (p=0.54) patients. Similarly, t-HG patients were predominantly males compared to no DM patients (p=0.01) but were not different from Pre-DM (p=0.47) or PTDM (p=0.60) patients. Yet, the prevalence of NAFLD being the underlying etiology of liver disease was similar between t-HG and no DM (p=0.36) patients but was significantly lower compared to that of Pre-DM (p=0.0006) and PTDM (p=0.002) patients. More interestingly, the prevalence of hepatitis C being the underlying etiology of liver disease was significantly higher in t-HG patients compared to no DM (p=0.002) and PTDM (p=0.04) patients but similar to that of Pre-DM (p=0.25) patients.

Outcomes of patients with t-HG were not analysed due to small number of events.

DISCUSSION

This study represents the largest reported non-registry long-term experience on DM (Pre-DM and PTDM) following LT to date. Further, it represents a multi-ethnic Western population, whereas previous literature predominantly involves studies of monoethnic cohorts from East Asia. Additionally, this study explores the risk factors of PTDM and the impact of Pre-DM and PTDM on renal, cardiovascular, and de novo cancer outcomes. Neither the Pre-DM nor the PTDM appear to impact patient survival, re-transplantation, or development of de novo cancer. However, not surprisingly, the risk of developing ESRD and mCVE was significantly higher in patients with Pre-DM. Although not statistically significant, the impact of PTDM on ESRD and mCVE was numerically between that of Pre-DM and no DM.

A quarter of the patients who were not diabetic at the time of LT developed PTDM, which is on par with the previously reported 19 – 35% [15, 17, 18, 25-28]. A few previous studies however have reported a lower incidence rate (6 – 8%) [21, 29] and the reason/s for this substantial difference is not apparent. Further, the occurrence of PTDM was considerably high during the early post-transplant period in this study and previous studies [18, 21, 26, 27]. In addition, the demographic and clinical characteristics at the time of LT of patients with Pre-DM and patients who later developed PTDM were similar. This remarkable similarity and the onset of PTDM immediately following LT raises important questions including whether PTDM is merely the clinical manifestation of a previously subclinical prediabetic state and whether those at risk of developing PTDM may potentially be identified pre-transplant with rigorous investigations such as oral glucose tolerance test or glucose clamp studies. The above notions are perhaps supported by the findings of a retrospective study [30], which suggest a twofold increase in the development of PTDM with every 10mg/dl increase in pre-transplant fasting plasma glucose level. However, prospective studies are needed for the validation of these hypotheses.

A wide variety of recipient and donor factors, etiology and severity of liver disease, and surgical and post-transplant management factors have been suggested to play a role in the development of PTDM [15, 17, 18, 21, 25-33], with limited consistency in common independent risk factors. The possible explanations for such discrepancy between studies include difference in cohort characteristics, cohort size, follow-up duration, and factors included in the analysis. A recent meta-analysis involving over 4,500 patients from 19 retrospective studies identified male sex, body mass index, hepatitis C etiology, pre-LT impaired fasting glucose, and use of tacrolimus as independent risk factors of PTDM [34]. However, this meta-analysis failed to include recipient age, non-alcoholic fatty liver disease etiology, and donor factors in the analysis, which have been shown to increase the risk of PTDM development. Although we included a range of factors that were shown to be associated with the development of PTDM in our analysis, Bonferroni correction was undertaken to mitigate the problem of multiple comparisons. In our study, NAFLD etiology and the use of Sirolimus and Tacrolimus were the only independent factors associated with the development of PTDM. Sirolimus inhibits insulin-mediated inactivation of hepatic glycogenolysis [35], causes a reduction in human pancreatic ductal cells, and inhibits glucose-stimulated insulin secretion [36], which potentially explains its role in the development of PTDM. Similarly, reduced insulin secretion and increased insulin resistance have been proposed as potential mechanisms of Tacrolimus-related PTDM [37]. On the other hand, the independent association between NAFLD etiology and PTDM, may be due to the subclinical pre-diabetic status of these individuals (as discussed above).

Interestingly, neither the Pre-DM nor the PTDM adversely impacted patient survival and re-transplantation. However, despite the optimal HbA1c control in the post-LT period, Pre-DM adversely affected renal and cardiovascular outcomes. The possible reasons for this are the presence of micro and macrovascular end-organ damage even prior to the formal diagnosis of DM [38] and the questionable reliability of HbA1c as a marker of glycemic control [39]. On the other hand, the impact of PTDM on ESRD and mCVE was in between that of Pre-DM and no DM. Given that the atherosclerosis which leads to both micro and macrovascular complications require prolonged duration of DM, we suspect that PTDM would have an adverse impact on renal and cardiovascular outcomes that is comparable to that of Pre-DM in the long term. These findings enhance our understanding of Pre-DM and PTDM and their effect on the quality and quantity of life after LT, which is helpful especially given the paucity of such data to date. However, given that the use of Tacrolimus was an independent risk factor for PTDM, the development of ESRD especially in patients with PTDM may have be confounded by the use of Tacrolimus. On the other hand, as we enter an era where recurrent hepatitis C after LT becomes obsolete, and de novo/recurrent NAFLD increase in incidence, we are likely to see changes in the patterns of incidence and impact of PTDM.

The literature on the impact of both Pre-DM and PTDM, on outcomes in other solid organ transplantation has been limited and contradictory. In a South Korean study of 176 kidney transplant recipients without diabetes at transplantation, PTDM was associated with the increased incidence of cardiovascular disease but did not compromise graft or patient survival [40]. In a single-centre study from Portugal involving 648 kidney transplant recipients, PTDM was shown to have no impact on renal dysfunction, cardiovascular events, graft, and patient survival [41]. Similarly, in a study from South Africa involving 111 kidney transplant recipients, PTDM did not impact graft or patient survival [42]. However, in a multicentre study involving over 2000 kidney transplant recipients from five North American and two European centres, PTDM was independently associated with graft survival; this study failed to investigate the impact of PTDM on patient survival or other outcomes [43]. In a registry study involving more than 9,000 lung transplant recipients, both Pre-DM and PTDM were shown to impact patient survival adversely [44]. Literature on the impact of Pre-DM and PTDM on outcomes heart transplant recipients were not available.

Interestingly, those who developed transient hyperglycaemia (t-HG patients) had unique demographic and clinical characteristics, with hepatitis C being the most common underlying etiology of liver disease in these patients; prevalence of NAFLD was at its lowest despite increased BMI. Whether hepatitis C played a crucial role in the development of transient hyperglycemia during the immediate post-LT period and why the hyperglycemic state did not continue beyond the first 3 months remain open to speculation.

Our study has its own strengths and limitations. It is the largest non-registry study to date. Unlike the national registry studies, single-center studies, such as ours, have the advantage of consistency with respect to the use of immunosuppression and the management of DM. Our study also has the advantage of representing the longest duration of post-LT follow-up, with up to 20 years after transplantation; previous studies have been restricted with follow-up and have been limited by relatively small patient numbers. Further, ours is the first non-registry study to investigate the impact on the development ESRD, major cardiovascular events, and de novo cancer in liver transplant

recipients with Pre-DM, PTDM and no DM, in the long term. On the other hand, the retrospective nature of our study is a natural limitation, due to the risk of bias inherent to study design when compared to a prospective trial. One such limitation is the lack of data on the duration of DM in Pre-DM patients, which has limited our ability to distinguish those who developed DM secondary to cirrhosis from those with DM that is unrelated to cirrhosis. Interestingly, neither the BMI at listing nor the change in BMI during the first year of transplantation were associated with development of PTDM. This, however, needs to be interpreted with caution because of the limitations in the accuracy of BMI measurement in the pre-transplant population (e.g. due to fluid retention). Further, though our study represents one of the longest experiences, it may still not be adequate to capture the 'true' impact of PTDM on outcomes.

In conclusion, this is the largest non-registry study to investigate the impact of Pre-DM and PTDM on LT outcomes. The baseline (at LT) clinical characteristics of patients who later developed PTDM were similar to that of patients with Pre-DM, suggesting that the genetic susceptibility may be similar between the two clinical entities. Pre-DM seem to pose the highest risk towards the development of ESRD and mCVE following LT, despite satisfactory HbA1c control. This emphasises the urgent need for better investigation/s of glycaemic control to minimize the long-term impact of DM. Although PTDM appears to have a lesser impact than that of Pre-DM, this needs to be interpreted with caution as the pathogenesis of macrovascular and microvascular complications requires prolonged duration of DM.

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TABLES

Table 1: Demographic characteristics at liver transplantation of all patients together and of individual groups based on their state of diabetes mellitus.

	All patients (n=2,209) median (IQR)/number (%)	**Pre-DM group (n=298)** median (IQR)/number (%)	**No DM group (n=1,431)** median (IQR)/number (%)	**PTDM group (n=362)** median (IQR)/number (%)	**t-HG group (n=118)** median (IQR)/number (%)
Age (years)	54 (47 – 60)	57 (50 – 61)	53 (45 – 59)	56 (49 – 61)	53 (49 – 60)
Male sex	1480 (67%)	232 (78%)	899 (63%)	261 (72%)	88 (75%)
BMI (kg/m²)	26.4 (23.3 – 30.3)	27.4 (24.4 – 31.4)	25.9 (22.8 – 29.8)	27.4 (24.4 – 31.8)	27.0 (24.6 – 30.4)
BMI >30	503 (23%)	89 (30%)	284 (20%)	98 (27%)	32 (27%)
ΔBMI at 1st year	-0.29 (-2.36 – 1.88)	-0.04 (-2.25 – 2.45)	-0.26 (-2.26 – 1.77)	-0.32 (-2.67 – 1.78)	-1.45 (-3.95 – 0.94)
Etiology					
NAFLD	224 (10%)	54 (18%)	105 (7%)	59 (16%)	6 (5%)
HCV	752 (34%)	118 (40%)	453 (32%)	127 (35%)	54 (46%)
ALD	333 (15%)	51 (17%)	202 (14%)	69 (19%)	16 (14%)
HBV	227 (10%)	24 (8%)	153 (11%)	31 (9%)	19 (16%)
AIH	73 (3%)	11 (4%)	46 (3%)	13 (4%)	3 (3%)
PBC	136 (6%)	8 (3%)	112 (8%)	13 (4%)	3 (3%)
PSC	196 (9%)	13 (4%)	154 (11%)	21 (6%)	8 (7%)
Other	268 (12%)	19 (6%)	206 (14%)	29 (8%)	9 (8%)
MELD score	16 (11 – 23)	15 (11 – 22)	16 (11 – 24)	17 (12 – 23)	15 (10 – 25)
Immunosuppression exposure†					
Tacrolimus	1499 (68%)	215 (72%)	950 (66%)	263 (73%)	71 (60%)
Cyclosporin	763 (35%)	97 (33%)	478 (33%)	150 (41%)	38 (32%)
Sirolimus	157 (7%)	27 (9%)	78 (5%)	44 (12%)	8 (7%)
Prednisone	450 (20%)	61 (20%)	285 (20%)	82 (23%)	22 (19%)
Mycophenolate	1029 (47%)	149 (50%)	650 (45%)	184 (51%)	46 (39%)

Please refer to the text for comparison between the individual groups. †a minimum of 6 months continuous exposure to an immunosuppressant was considered a positive exposure to that particular immunosuppressant.

Abbreviations: BMI body mass index; ΔBMI change in BMI; AIH autoimmune hepatitis; ALD alcohol-related liver disease; HBV hepatitis B; HCV hepatitis C; MELD model for end-stage liver disease; NAFLD non-alcoholic fatty liver disease; PBC primary biliary cholangitis; PSC primary sclerosing cholangitis.

Table 2: Predictors of post-transplant diabetes mellitus (PTDM) in liver transplant recipients – univariate and multivariate logistic regression

	Univariate analysis	Multivariate analysis		
	p value	Odds ratio	95% CI	p value
Age at listing*	<0.0001	-	-	0.051
Male sex*	0.001	1.56	1.11 – 2.20	0.01
BMI at listing*	<0.0001	1.03	1.00 – 1.06	0.03
ΔBMI at 1st year post-LT	0.99			
Etiology – NAFLD*	<0.0001	2.03	1.31 – 3.16	**0.002**
Etiology – HCV	0.21			
Hypertension*	0.07	2.40	1.19 – 4.82	0.01
Dyslipidemia*	0.02	-	-	0.18
Donor age	0.81			
Cold ischemia time*	0.01	-	-	0.13
Warm ischemia time	0.23			
Duct anastomosis - Roux-en-Y*	0.00002	0.60	0.41 – 0.87	0.007
Immunosuppression - Tacrolimus*	0.06	2.76	1.78 – 4.30	**<0.001**
Immunosuppression – Cyclosporin*	0.009	1.68	1.07 –2.63	0.02
Immunosuppression – Sirolimus*	<0.0001	2.72	1.67 – 4.42	**<0.001**
Immunosuppression - Prednisone*	0.02	1.47	1.06 – 2.02	0.02
Immunosuppression - Mycophenolate*	0.07	-	-	0.77

Only those who were not diabetic at the time of transplantation (i.e. not including those with pre-transplantation diabetes) were included in this analysis. Parameters with a p-value <0.10 on univariate analysis were included in the multivariate analysis and these parameters are indicated by an asterisk (*). The Bonferroni-corrected level of significance in this analysis was p <0.0038. **Abbreviations**: BMI body mass index; ΔBMI change in BMI; HCV hepatitis C; LT liver transplantation; NAFLD non-alcoholic fatty liver disease.

FIGURE LEGENDS

Figure 1 – Glycosylated Hemoglobin levels
Comparison of annual glycosylated hemoglobin (HbA1c) levels in patients with pre-existing diabetes mellitus and patients who developed post-transplant diabetes mellitus (PTDM).

Figure 2 – Patient Survival
Kaplan-Meier estimates of patient survival time following liver transplantation in patients who never developed diabetes mellitus (no DM; green line); patients with pre-transplant diabetes mellitus (Pre-DM; blue line); and patients who developed post-transplant diabetes mellitus (PTDM; red line).

SUPPLEMENTARY TABLES

Supplementary Table 1: Distribution of major causes of death in patients with pre-transplant diabetes, post-transplant diabetes, and no diabetes

Cause of death	Pre-DM group (n=298) number (%)	No DM group (n=1,431) number (%)	PTDM group (n=362) number (%)
Cancer†	23 (7.7%)	120 (8.4%)	39 (10.8%)
Graft failure‡	17 (5.7%)	84 (5.9%)	20 (5.5%)
Sepsis/Infection*	17 (5.7%)	56 (3.9%)	21 (5.8%)
MSOF	13 (4.4%)	80 (5.6%)	16 (4.4%)
Cerebrovascular event	7 (2.3%)	20 (1.4%)	7 (1.9%)
Cardiac – heart failure	2 (0.7%)	11 (0.8%)	3 (0.8%)
Cardiac – MI	5 (1.7%)	14 (1.0%)	3 (0.8%)
ESRF	4 (1.3%)	7 (0.5%)	3 (0.8%)
Other	1 (0.3%)	29 (1.2%)	1 (0.3%)
Unknown§	12 (4.0%)	85 (5.9%)	21 (5.8%)

Of the total study population (n=2,209), 782 died following transplantation. This includes 101 deaths in the pre-transplant diabetes (pre-DM) group, 134 deaths in the post-transplant diabetes (PTDM) group, 506 deaths in the no diabetes 9no DM) group, and 41 in the transient hyperglycemia group. †cancer deaths included those who died of recurrence of hepatocellular carcinoma. ‡graft failure deaths were due to causes such as recurrence of primary liver disease (e.g. hepatic C, autoimmune liver diseases, alcoholic recidivism), vascular complications, and graft rejection. *sepsis/infection deaths included acute infections (e.g. pneumonia) and chronic infections (e.g. invasive fungal infections. §unknown cause of death also included those where the cause of death was documented as 'cardiac arrest' or 'respiratory arrest' or 'cardiorespiratory arrest/failure'. **Abbreviations**: DM diabetes mellitus; PTDM post-transplant diabetes mellitus; MSOF multi system organ failure; MI myocardial infarction; ESRF end stage renal failure.

Supplementary Table 2: Distribution of different types of de novo cancers in patients with pre-transplant diabetes, post-transplant diabetes, and no diabetes

Type of cancer	Pre-DM group (n=298) number (%)	No DM group (n=1,431) number (%)	PTDM group (n=362) number (%)
Upper GI	1 (0.3%)	3 (0.2%)	0 (0.0%)
Pancreato-biliary	2 (0.7%)	7 (0.5%)	1 (0.3%)
Colon	4 (1.3%)	12 (0.8%)	3 (0.8%)
Lung	2 (0.7%)	8 (0.6%)	3 (0.8%)
PTLD	3 (1.0%)	22 (1.5%)	3 (0.8%)
Hematological (excl. PTLD)	1 (0.3%)	5 (0.3%)	1 (0.3%)
Renal (KUB) tract	3 (1.0%)	13 (0.9%)	4 (1.1%)
Prostate	3 (1.0%)	24 (1.7%)	4 (1.1%)
Skin	1 (0.3%)	2 (0.1%)	3 (0.8%)
Breast	2 (0.7%)	2 (0.1%)	2 (0.6%)
Central nervous system	0 (0.0%)	2 (0.1%)	0 (0.0%)
Cancer of unknown primary	2 (0.7%)	9 (0.6%)	3 (0.8%)
Other	2 (0.7%)	7 (0.5%)	6 (1.7%)

Abbreviations: DM diabetes mellitus; PTDM post-transplant diabetes mellitus; GI gastrointestinal; PTLD post-transplant lymphoproliferative disease; KUB kidney, ureter, and bladder.