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Liver Enzymes and the Development of Posttransplantation Diabetes Mellitus in Renal Transplant Recipients

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Background. Posttransplantation diabetes mellitus (PTDM) is common in renal transplant recipients (RTR), increasing the risk of graft failure, cardiovascular disease, and mortality. Early detection of a high risk for PTDM is warranted. Because liver function and liver fat are involved, we investigated whether serum liver markers are associated with future PTDM in RTR. **Methods.** Between 2001 and 2003, 606 RTR with a functioning allograft beyond the first year after transplantation were included of which 500 participants (56% men; age, 50 ± 12 years) were free of diabetes at baseline and had liver enzyme values (1 missing) available. Serum concentrations of alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase were measured at baseline at 6.0 (6.2-11.5) years posttransplantation. PTDM cases were recorded until April 2012. **Results.** During median follow-up for 9.6 years (interquartile range [IQR], 6.2-10.2) beyond baseline, 76 (15.2%) patients developed PTDM. Comparing the highest to the lower tertiles, higher liver enzyme activities were significantly related to incident PTDM for ALT (hazard ratio [HR], 2.22; IQR, 1.42-3.48), for GGT (HR, 2.93; IQR, 1.87-4.61), and for alkaline phosphatase (HR, 1.78; IQR, 1.13-2.80). The associations of ALT and GGT with development of PTDM were independent of potential confounders and risk factors, including age, sex, renal function, medication use, lifestyle factors, adiposity, presence of the metabolic syndrome, fasting glucose, HbA1c, proinsulin, and cytomegalovirus status. **Conclusions.** Markers for liver function and liver fat in the subclinical range are potential markers for future PTDM, independent of other known risk factors. This may allow for early detection and management of PTDM development.

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Posttransplantation diabetes mellitus (PTDM), also known as new-onset diabetes after transplantation[1] is a common and serious metabolic complication after renal transplantation. It places renal transplant recipients (RTR) at increased risk of infections, graft failure, cardiovascular disease, and early mortality.[2-4] To improve long-term outcomes in RTR, it is of great importance to identify modifiable risk factors for PTDM to effectively develop strategies to reduce PTDM risk.

Although transplantation-specific risk factors for PTDM are the chronic exposure to immunosuppressive medication and cytomegalovirus (CMV) infection,[5] PTDM and type 2 diabetes mellitus share similar pathophysiology. Components of the metabolic syndrome, such as increased waist circumference, hyperlipidemia, and hypertension, enhance the development of PTDM. The metabolic syndrome[6] is very common after renal transplantation, and partly explained by the use of immunosuppressive medication and post transplantation weight gain.[7,8] In type 2 diabetes mellitus, impaired liver function and liver fat play a role in the pathogenesis of PTDM.[9-11] Liver enzymes like alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (AP) are strongly related to impaired cardiometabolic health and can be considered as markers for the hepatic component of the metabolic syndrome. In RTR, GGT and AP strongly predict total and cardiovascular

TABLE 1.

Demographics and baseline characteristics according to sex-stratified tertiles of ALT, GGT, and total AP

Tertiles of liver enzymes	ALT			GGT			Total AP		
Range	(4-15)	(14-22)	(20-157)	(8-19)	(18-29)	(26-337)	(21-64)	(60-85)	(78-224)
General and lifestyle									
Age, y	50 ± 13	50 ± 12	51 ± 11	47 ± 13	52 ± 12[a]	52 ± 11[a]	50 ± 12	51 ± 12	50 ± 13
Male sex, n (%)	92 (56)	99 (57)	90 (55)	95 (56)	90 (56)	93 (56)	94 (56)	91 (56)	96 (58)
Current smoker, n (%)	80 (49)	71 (41)	52 (32)[a]	77 (45)	58 (36)	68 (41)	67 (40)	67 (41)	69 (41)
Alcohol consumption									
Abstainers, n (%)	74 (45)	73 (42)	80 (49)	79 (46)	72 (45)	75 (46)	66 (39)	79 (48)	82 (49)
1-7 units/wk, n (%)	63 (38)	74 (43)	58 (36)	69 (40)	60 (38)	64 (39)	77 (46)	52 (32)[a]	66 (40)
> 7 units/wk, n (%)	27 (17)	22 (13)	25 (15)	22 (13)	26 (16)	25 (15)	24 (14)	32 (19)	19 (11)
Physical activity, METS	95 [17-271]	125 [34-358]	182 [44-335][a]	137 [35-304]	121 [26-302]	144 [26-337]	167 [29-328]	125 [18-350]	112 [35-284]
BMI, kg/m²	24.5 ± 3.9	26.1 ± 3.7[a]	26.6 ± 4.5[a]	24.5 ± 3.8	25.8 ± 3.8[a]	27.0 ± 4.4[ab]	24.9 ± 4.0	26.0 ± 4.0[a]	26.4 ± 4.3[a]
Waist circumference, cm	92 ± 13	97 ± 12[a]	98 ± 14[a]	92 ± 13	97 ± 11[a]	100 ± 14[a]	93 ± 13	96 ± 12	99 ± 14[ab]
Metabolic parameters									
Metabolic syndrome, n (%)	84 (51)	106 (61)	104 (64)[a]	85 (50)	94 (59)[a]	114 (69)[a]	83 (49)	92 (56)	119 (71)[a,b]
Total cholesterol, mmol/L	5.6 ± 1.0	5.6 ± 1.0	5.7 ± 1.2	5.4 ± 1.0	5.7 ± 1.0	5.7 ± 1.2	5.5 ± 1.0	5.8 ± 1.2	5.7 ± 1.0
HDL cholesterol, mmol/L	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.0 ± 0.3
LDL cholesterol, mmol/L	3.6 ± 0.9	3.6 ± 0.9	3.5 ± 1.2	3.6 ± 0.9	3.6 ± 0.9	3.5 ± 1.2	3.6 ± 0.9	3.7 ± 1.1	3.5 ± 1.0
Triglycerides, mmol/L	1.8 [1.3-2.3]	1.9 [1.3-2.6]	2.1 [1.5-2.9][ab]	1.7 [1.2-2.3]	1.9 [1.4-2.6][a]	2.1 [1.6-3.0][a]	1.8 [1.3-2.3]	1.8 [1.3-2.4]	2.2 [1.6-2.9][ab]
Use of statins, n (%)	59 (36)	91 (53)[a]	88 (54)[a]	70 (41)	87 (54)[a]	80 (49)	76 (45)	82 (50)	80 (48)
Systolic blood pressure, mmHg	150 ± 23	153 ± 24	150 ± 20	148 ± 21	155 ± 23[a]	150 ± 21	150 ± 21	150 ± 23	154 ± 22
Diastolic blood pressure, mmHg	89 ± 10	90 ± 10	90 ± 9	90 ± 10	90 ± 9	89 ± 10	90 ± 10	89 ± 10	91 ± 10
Antihypertensive use, n (%)	139 (85)	153 (88)	135 (83)	145 (85)	134 (84)	145 (88)	141 (83)	142 (87)	144 (86)
HbA1c, %	6.3 ± 0.8	6.2 ± 0.9	6.4 ± 0.9	6.1 ± 0.8	6.3 ± 0.8	6.4 ± 0.9[ab]	6.2 ± 0.9	6.3 ± 0.7	6.4 ± 0.9
HbA1c, mmol/mol	45 ± 8.4	45 ± 9.5	46 ± 9.5	43 ± 8.8	45 ± 8.9	47 ± 9.6[ab]	44 ± 9.5	45 ± 8.1	46 ± 9.7
Fasting glucose, mmol/L	4.6 ± 0.6	4.6 ± 0.6[a]	4.6 ± 0.7	4.5 ± 0.6	4.4 ± 0.6	4.7 ± 0.7[ab]	4.5 ± 0.6	4.6 ± 0.6	4.5 ± 0.7
HOMA IR	1.8 [1.3-2.8]	2.0 [1.5-2.8]	2.4 [1.6-3.3][ab]	1.9 [1.5-2.6]	2.0 [1.4-2.9]	2.4 [1.6-3.6][ab]	2.0 [1.4-3.0]	2.0 [1.5-2.9]	2.2 [1.6-3.1]
Renal parameters									
Creatinine clearance, mL/min	55 [40-73]	63 [48-80][a]	62 [50-77][a]	59 [41-79]	61 [50-77]	59 [46-76]	59 [45-77]	60 [44-75]	61 [49-79]
Proteinuria, g/24 h	0.3 [0.1-0.5]	0.2 [0.0-0.5]	0.2 [0.0-0.4]	0.2 [0.0-0.5]	0.2 [0.0-0.5]	0.2 [0.0-0.5]	0.2 [0.0-0.5]	0.2 [0.0-0.5]	0.2 [0.0-0.5]
Immunosuppression, n (%)									
Cyclosporine	99 (60)	113 (65)	103 (63)	101 (59)	110 (69)	101 (61)	91 (54)	99 (60)	125 (75)[ab]
Tacrolimus	26 (16)	17 (10)	30 (18)[b]	25 (15)	16 (10)	31 (19)[b]	27 (16)	19 (12)	27 (16)
Azathioprine	67 (41)	56 (32)	41 (25)[a]	70 (41)	48 (30)[a]	45 (27)[a]	70 (41)	55 (34)	39 (23)[ab]
Mycophenolate mofetil	68 (42)	75 (43)	69 (42)	67 (39)	74 (46)	69 (42)	68 (40)	73 (45)	71 (43)
Prednisolone dose, mg/d	9.1 ± 1.5	9.3 ± 1.1	9.2 ± 1.5	9.0 ± 1.4	9.3 ± 1.4	9.3 ± 1.3	9.1 ± 1.4	9.3 ± 1.2	9.2 ± 1.4
CMV positive, n (%)	40 (24)	40 (23)	28 (17)	38 (22)	30 (19)	40 (24)	40 (24)	36 (22)	32 (19)

Tertile ranges are given in U/L. Data are represented as mean ± SD or median [IQR]. Categorical variables are presented as number (%).

[a] Tertile significantly different from tertile 1, $P = <0.05$.

[b] Tertile significantly different from tertile 2, $P = <0.05$.

METS, metabolic equivalents; LDL, low-density lipoproteins; HOMA, homeostasis model assessment.

mortality, independent of the metabolic syndrome.[12] To date the association with liver enzymes and PTDM has not been studied. Because the hepatic component of the metabolic syndrome can be an important modifiable risk factor for PTDM, the present study prospectively investigates the association between the liver enzymes ALT, GGT, and AP and PTDM development after renal transplantation.

MATERIALS AND METHODS

Subjects

In this prospective cohort study, all adult RTR who survived with a functioning allograft beyond the first year after transplantation were considered eligible to participate. Baseline data, obtained at least 1 year after transplantation, were collected between August 2001 and July 2003 at a median of 6.0 (interquartile range [IQR], 2.6-11.5) years after transplantation.[12] For the current study, 105 recipients with existing diabetes (defined as fasting plasma glucose ≥ 7.0 and/or antidiabetic medication) at baseline were excluded. All ethnicities (3.6% non-white) were included. Baseline visits were postponed until symptoms had resolved in patients with fever or other signs of infection (eg, complaints of upper respiratory tract infection or urinary tract infection). Patients with overt congestive heart failure and patients diagnosed with cancer other than cured skin cancer were not considered eligible for the study. Liver enzyme analyses were not available for 1 patient resulting in a total of 500 patients for analysis. The institutional review board approved the study protocol (METc 2001/039). The study was performed per the declaration of Helsinki and the declaration of Istanbul.

Endpoint of the Study

Care-based data about the development of PTDM after baseline were retrieved from patient files of all RTR until April 2012. PTDM was defined based on criteria outlined by the International PTDM consensus guideline.[13] PTDM was defined as having a fasting plasma glucose level of 7.0 mmol/L or greater or use of oral hypoglycemic agents or insulin therapy for 30 consecutive days or longer. HbA1c was not included in the PDTM criteria because it is not routinely tested in nondiabetic RTR.

Measurements and Definitions

Patient characteristics including age, sex, date of transplantation, and immunosuppressive medication were extracted from the Groningen Renal Transplant Database and patients' medical records. Information on cardiovascular disease history, smoking, alcohol consumption, and physical activity was obtained using a self-report questionnaire.[14] At baseline, body mass index (BMI) was calculated as weight in kilograms, divided by height in meters squared. Hip circumference was measured at the widest point at the level of the trochanter major, and waist circumference was measured at the point halfway between the spina iliaca and the lower rib using a plastic tape measure. Blood pressure was measured as the average of 3 automated measurements with 1-minute intervals after a 6-minute rest in supine position. (Omron M4; Omron Europe B.V., the Netherlands). Blood was drawn after an overnight fasting period. Serum creatinine concentrations were determined using the Jaffe method, and serum triglycerides were determined with the glycerol-3-phosphate oxidase-p-aminophenazone method. Total

cholesterol was determined using the CHOD PAP method (MEGA AU 510; Merck Diagnostica, Darmstadt, Germany). High-density lipoproteins (HDL) cholesterol was determined with the CHOD PAP method on a Technikon RA-1000 (Bayer Diagnostics B.V., Mijdrecht, the Netherlands) and low-density lipoproteins cholesterol was calculated using the Friedewald formula.[15] Plasma glucose was determined by the glucose-oxidase method (YSI 2300 Stat plus; Yellow Springs, OH). HbA1c was determined by high performance liquid chromatography (VARIANT HbA1c Program with Bio-Rad CARIANT Hb Testing System, Bio-Rad, Hercules, CA) in all RTR at baseline for study purposes and at clinical indication during follow-up. Serum high sensitive C-reactive protein was assessed with a high sensitivity C-reactive protein enzyme-linked immunosorbent assay assay as described before.[16] At baseline, uniform measurement of ALT, GGT, and AP in serum was performed using IFCC-method (37°C). Serum bone-specific AP kit was used for assessment of the bone-specific part of AP activity (MetraTM Bone AP assay (Quidel Corporation, San Diego, CA). Nonbone AP was calculated by subtracting bone AP from total AP. University Medical Center Groningen laboratory ranges for the liver enzymes are less than 45 U/L for ALT in men, less than 34 U/L for ALT in women, less than 55 U/L for GGT in men, less than 38 U/L for GGT in women, less than 115 U/L for AP in men, and less than 98 for AP in women. Although aspartate aminotransferase was measured too, it is not a candidate marker for liver fat and was not related to PTDM in our study (hazard ratio [HR], 1.46; 95% confidence interval [CI], 0.92-2.33) nor in the literature,[9] and therefore, it was not further investigated. Homeostasis model assessment was calculated as [glucose (in millimoles per liter) × insulin (in microunits per milliliter)]/22.5.[17,18] Metabolic syndrome was defined according to the National Cholesterol Education Program Expert Panel, that is, presence of 3 or more of the following components: waist circumference, greater than 102 cm in men and greater than 88 cm in women; serum triglycerides, 1.70 mmol/L or greater; serum HDL cholesterol, less than 1.03 mmol/L in men and less than 1.29 mmol/L in women; blood pressure, 130/85 mm Hg or higher or use of antihypertensive medication; and fasting plasma glucose, 6.1 mmol/L or greater or use of antidiabetic medication.

Statistical Analyses

Data were analyzed using SPSS version 22 (SPSS Inc., Chicago, IL), Stata IC version 11 (2009, StataCorp, College Station, TX), and GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA). Data are expressed as mean ± standard deviation or as median (interquartile range) if not normally distributed. Baseline characteristics were compared for sex-stratified tertiles of ALT, GGT, and AP. Categorical variables were compared using χ^2 test, and Kruskal-Wallis 1-way analysis of variance for continuous variables. The association between liver enzymes and the development of PTDM was analyzed using Kaplan-Meier analyses with log-rank test. To rule out competing risk, a composite endpoint of the first occurrence of PTDM or death was studied using sensitivity analysis. Finally, we tested for potential nonlinearity of the associations of ALT, GGT, and AP with PTDM using fractional polynomial regression analysis.[19] To meet the proportionality of hazards assumption, the first and second tertiles of liver enzymes were combined

and used as reference in the Cox-regression analyses. Multivariable Cox-regression analyses were applied to adjust the association between ALT, GGT, bone AP and nonbone AP with PTDM for age (model 2), which was also included in models 3 to 8. We adjusted for kidney function (model 3), use of immunosuppressive medication (model 4), lifestyle factors (model 5), BMI, waist circumference, metabolic syndrome (model 6), fasting glucose, HbA1c, proinsulin (model 7), and CMV status (model 8).

Because sex-specific tertiles were used, no additional adjustment for sex was made. Patients were censored at date of last follow-up or death.

RESULTS

Baseline measurements were taken at a median (IQR) of 6.0 (2.6-11.5) years posttransplantation. The median (IQR) baseline enzyme activities of ALT, GGT, and AP were 17.5 U/L (13.0-24.0 U/L), 22.0 U/L (17.0-36.0 U/L), and 70.0 U/L (56.3-88.8 U/L), respectively, with bone- and nonbone AP, respectively, accounting for 42.9% ± 12% and 57.1% ± 12% of total AP activity. Patients with higher levels of liver enzymes ALT, GGT, and AP more often had metabolic syndrome at baseline (Table 1). When looking at the individual components of the metabolic syndrome, higher liver enzyme concentrations were associated mainly to higher waist circumference and higher triglycerides. Associations with HDL-cholesterol, fasting glucose, and systolic and diastolic blood pressure were less clear. Although fasting glucose and HbA1c were higher in the highest tertile of GGT, no clear associations were found for ALT and AP. Furthermore, patients with higher liver enzymes used azathioprine less often, and creatinine clearance was higher in the 2 highest tertiles of ALT.

During a follow-up time of 9.6 years (6.2-10.2) beyond baseline, 76 subjects (15.2%) developed PTDM, and 137 RTR (27.4%) died. The cumulative incidence of PTDM at 2, 4, 6, 8, and 10 years after baseline was 3.2, 5.6%, 8.4%, 13.4%, and 14.6%, respectively. Incidence of PTDM was 45 (16%) of 281 in men versus 31 (14%) of 219 in women. RTR with metabolic syndrome at baseline were more likely to develop PTDM (Figure 1A; HR, 3.77; 95% CI, 2.11-6.74) than those without, with 62 cases (30.1%) versus 14 cases (6.8%), respectively (Figure 1A). The risk of PTDM increased particularly in the highest tertiles of ALT, GGT, and AP, whereas tertiles 1 and 2 were comparable for all enzymes investigated (Figures 1B-D). In Cox-regression analyses, GGT was most strongly related to PTDM (Table 2). Adjustment for age (model 2), and additional adjustments for kidney function (model 3), immunosuppressive medication (model 4), or lifestyle (model 5) did not materially influence the association. Further adjustment for BMI, waist circumference and presence of the metabolic syndrome (model 6), fasting glucose, HbA1c, and proinsulin (model 7), and CMV status (model 8) slightly weakened the associations for ALT and GGT. Associations of total AP with PTDM disappeared after adjustments applied in models 6, 7, and 8, and of nonbone AP after adjustments applied in models 6 and 7. Since AP is mostly produced in the liver, as well as in other organs, but also in bone, we analyzed bone- and nonbone AP separately. The nonbone fraction of total AP showed a stronger association with PTDM than the total AP, but was no longer associated to PTDM after adjustment for BMI, waist circumference, and metabolic syndrome (model 6), as well as for fasting glucose, HbA1c, proinsulin (model 7).

Furthermore, ethnicity also did not change the associations of liver enzymes with incident PTDM.

Additionally, possible interactions between liver enzymes, metabolic syndrome, and waist circumference were investigated, but none were found. A slightly nonlinear pattern was observed (Figures 2A-C). The figure illustrates how the risk of PTDM can already be increased at liver enzyme levels in the subclinical range.
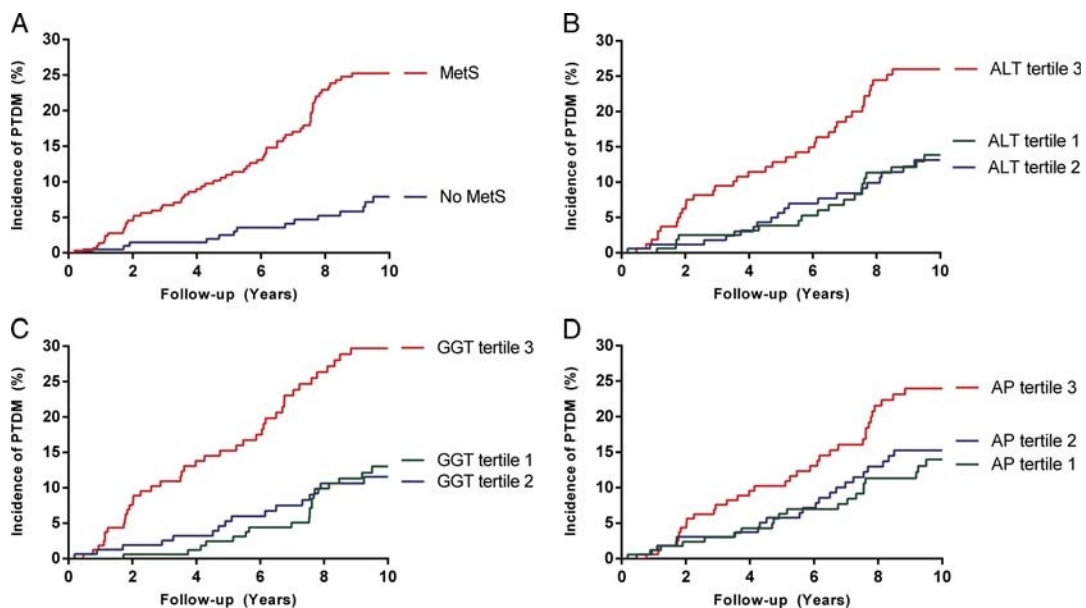
FIGURE 1. Kaplan-Meier curves for incident PTDM. Presence or absence of metabolic syndrome and PTDM (panel A), ALT and PTDM (panel B), GGT and PTDM (panel C), total AP and PTDM (panel D).

Association of sex-stratified tertiles of ALT, GGT, total AP, and nonbone AP with incident PTDM

	Reference (tertiles 1–2)	ALT, tertile 3	P	GGT, tertile 3	P	Total AP, tertile 3	P	Nonbone, AP tertile 3	P
Model 1 (crude)	1.00	2.22 (1.42-3.48)	0.001	2.93 (1.87-4.61)	<0.001	1.78 (1.13-2.80)	0.012	2.22 [1.40.3.43]	0.001
Model 2 (age)	1.00	2.21 [1.41-3.47]	0.001	2.94 [1.87-4.62]	<0.001	1.79 (1.14-2.82)	0.012	2.21 [1.40-3.50]	0.001
Model 3 (renal function)	1.00	2.26 [1.43-3.57]	<0.001	2.97 [1.88-4.69]	<0.001	1.81 [1.15-2.85]	0.010	2.21 [1.40-3.49]	0.001
Model 4 (medication)	1.00	2.14 [1.36-3.37]	0.001	2.81 [1.78-4.45]	<0.001	1.84 [1.15-2.93]	0.011	2.28 [1.44-3.63]	<0.001
Model 5 (lifestyle)	1.00	2.50 [1.51-4.15]	<0.001	2.90 [1.76-4.79]	<0.001	1.99 [1.21-3.26]	0.007	2.54 [1.54-4.20]	<0.001
Model 6 (BMI, waist circumference, M.S.)	1.00	1.74 [1.10-2.77]	0.018	2.08 [1.29-3.35]	0.003	1.28 [0.81-2.04]	0.292	1.51 [0.94-2.41]	0.089
Model 7 (fasting glucose, HbA1c, proinsulin)	1.00	1.87 (1.18-2.98)	0.008	2.56 [1.61-4.05]	<0.001	1.53 [0.95-2.46]	0.077	1.35 [0.83-2.19]	0.230
Model 8 (CMV positive)	1.00	1.89 [1.11-3.23]	0.019	2.59 [1.52-4.41]	<0.001	1.68 [0.98-2.86]	0.059	2.52 [1.47-4.33]	0.001

Model 1, univariate analysis.

Model 2, model 1+ adjusted for age.

Model 3, model 2+ creatinine clearance and proteinuria.

Model 4, model 2+ use of immunosuppressive medication (cyclosporine, tacrolimus, azathioprine, and daily prednisolone dose).

Model 5, model 2+ smoking, alcohol consumption, and physical activity.

Model 6, model 2+ BMI, waist circumference and presence of the metabolic syndrome.

Model 7, model 2+ fasting glucose, HbA1c, proinsulin.

Model 8, model 2+ CMV status.

Values are mean (95% CI).

Median [IQR] steroid dose in subjects who developed PTDM was no different from those who did not develop PTDM (10.0 [7.5-10.0] vs 10.0 [7.5-10.0], respectively). Of immunosuppressive calcineurin inhibitor drugs, use of tacrolimus was significantly associated with development of PTDM (HR, 1.82; 95% CI, 1.06-3.13; P = 0.029),
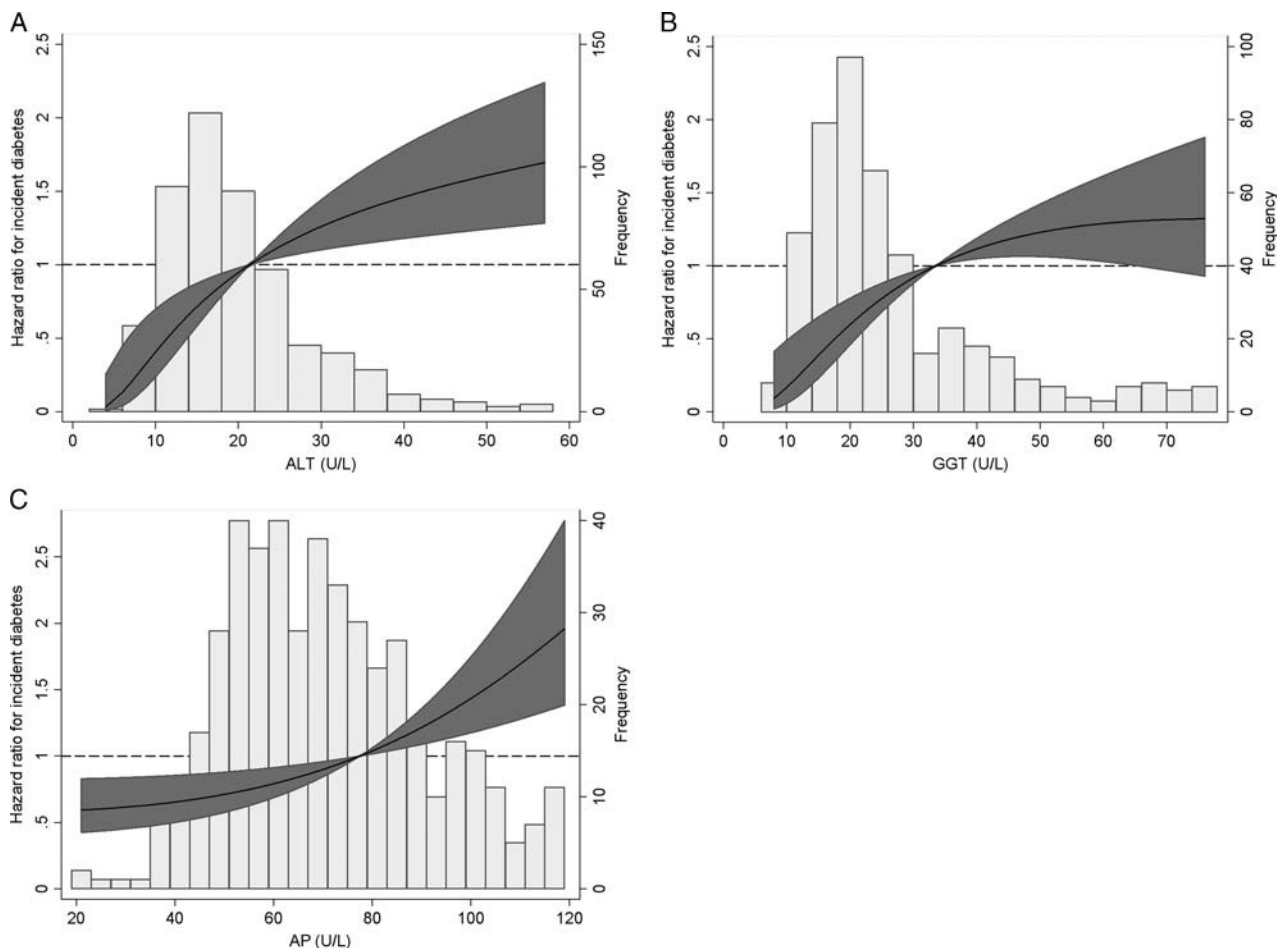
FIGURE 2. Fractional polynomial graph of the hazard ratio for PTDM per concentration serum concentrations of ALT (panel A), GGT (panel B) and total AP (panel C) in U/L.

independent of age, sex and fasting glucose, whereas use of cyclosporin was not independently associated (HR, 0.78; 95% CI, 0.49-1.23; $P = 0.28$).

Competing risk analyses using log2-tranformed concentrations of ALT, GGT, and AP revealed no competing risk of mortality (crude ALT Cox HR, 1.70 [1.31-2.20] vs competing risk HR, 1.82 [1.34-2.48]; crude GGT Cox HR, 1.48 [1.21-1.82] vs competing risk HR, 1.42 [1.10-1.82], crude total AP Cox HR, 2.11 [1.39-3.20] vs competing risk HR, 2.13 [1.36-3.34], crude nonbone AP Cox HR, 2.82 [1.28-6.21] vs competing risk HR, 3.03 [1.18-7.82]).

DISCUSSION

This study shows that elevated serum concentrations in the subclinical range of ALT and GGT and to lesser extent AP were associated with incident PTDM in RTR, independent of known risk factors for type 2 diabetes mellitus as well as medication use. These data indicate that impaired liver function is important already in the early stages of PTDM pathogenesis.

Regarding the high prevalence of metabolic syndrome and other mortality risk factors in our sample, it is possible that death might prevent occurrence of PTDM. However, in competing risk analyses, we found no competing risk of death for ALT and total AP.

Regarding medication use, it could either mask the predictive potential of the liver enzymes or the effect would be stronger due to compromised liver function in this population. However, the role of medication use seems minor, whereas tacrolimus is related to future PTDM as expected by its effect on reducing beta-cell insulin release.[16,17] Despite the fact that use of tacrolimus was related to liver enzyme concentrations in our sample and that its use was associated with development of PTDM, adjustment for use of tacrolimus did not materially change associations of liver enzymes with development of PTDM. Cyclosporine is related to elevations in liver enzymes, but was not related to the development of type 2 diabetes in our study. Thus, although medication may affect either liver enzyme concentration or diabetes development, it does not alter the role of liver function markers in PTDM development in the present study.

Similarly, we found no evidence that CMV status alters the role of liver function markers in PTDM development, except for total AP.

Although levels of AP have been associated with cardiovascular disease and mortality, it has been less studied in relation to type 2 diabetes. It has been shown long ago that nonbone AP can be elevated in patients with type 2 diabetes mellitus.[19] In the Mexico City diabetes study, it was shown that elevated levels of total AP were related to future type 2 diabetes mellitus, but not independently of other predictors of diabetes.[20] Similarly, in the present study, total AP is related to future diabetes until adjusted for BMI, waist circumference, and metabolic syndrome (model 6). The association of total AP with future diabetes also lost significance when it was adjusted for fasting glucose, HbA1c, and proinsulin (model 7), and when it was adjusted for CMV status (model 8). Findings were similar for nonbone AP, of which the associations with future development of diabetes remained significant in all models, except the model in which

the association was adjusted for BMI, waist circumference, and metabolic syndrome (model 6), and the model in which the association was adjusted for fasting glucose, HbA1c, and proinsulin (model 7). Bone AP was not associated with PTDM. The pathway that is most likely underlying our study outcomes is obesity driven as BMI and waist circumference were markedly higher in the second and third tertile of ALT, GGT, and AP suggesting an important role of abdominal fat in deterioration of liver function. Weight gain is common after renal transplantation, particularly in the abdominal region.[8,21] Increased abdominal fat can enhance the hepatic influx of free fatty acids, increasing hepatic fat storage.[22-25] Hepatic fat storage is associated with increased gluconeogenesis[26] and increased hepatic insulin resistance[27-29] as well as peripheral insulin resistance. Ultimately, this may provoke β-cell insufficiency.[30]

The strength of this study is its prospective design, long duration, and high completeness of follow-up. Information concerning patient status was obtained by closely monitoring the patients at regular check-ups at our clinic. Our study population is a cross-section of all the RTR that visited our outpatient clinic, giving a variation of RTR with different times after transplantation, also including stable RTR late after transplantation. This variation in time after transplantation improves the generalizability to the total RTR population. The fact that we have no data on liver fat imaging and on insulin resistance may be considered limitation of our study. Some degree of survivor bias at baseline may be present in our study, because median time after transplantation is long. Future studies could investigate whether liver enzymes also predict PTDM earlier after transplantation, allowing earlier intervention or prevention. Furthermore, liver enzymes were only measured in the baseline samples. The use of a single measurement of liver enzymes may induce regression dilution bias, thus it is possible that our results underestimated the strength of the associations.[31]

Furthermore, the potential value of ALT, GGT, and AP needs to be investigated for their predictive ability in other centers and/or multicenter studies.

In summary, ALT and GGT in the subclinical range were significantly associated with incident PTDM independent of well-known risk factors. Importantly, the liver enzymes were associated with BMI and waist circumference, supporting the notion that the increased abdominal fat causes hepatic fat accumulation, reducing hepatic insulin sensitivity.[27-29]

The outcomes of our study have important implications for posttransplantation care, because it shows that even mild elevations in liver enzymes could reflect early impairments in hepatic glucose metabolism because of hepatic fat accumulation. Increased liver enzymes in RTR, particularly when coexisting with increased waist circumference, should be an incentive to target lifestyle factors, such as physical activity and dietary habits to prevent or even reverse hepatic fat deposition.[32] Indeed, lifestyle interventions leading to a reduction in bodyweight and/or increased physical activity have consistently shown to reduce liver fat and improve glucose control.[32–37] Another opportunity for management and prevention of PTDM lies in the choice of the immunosuppressive agents. Immunosuppressive regimens could be tailored to the individual patient on the basis of predictive criteria for the development of PTDM.

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