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RESEARCH ARTICLE

Modifiable clinical and lifestyle factors are associated with elevated alanine aminotransferase levels in newly diagnosed type 2 diabetes patients: results from the nationwide DD2 study

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Abstract

Background Current literature lacks data on markers of non-alcoholic fatty liver disease (NAFLD) in newly diagnosed type 2 diabetes mellitus (T2DM) patients. We therefore, conducted a cross-sectional study to examine modifiable clinical and lifestyle factors associated with elevated alanine aminotransferase (ALT) levels as a marker of NAFLD in new T2DM patients.

Methods Alanine aminotransferase levels were measured in 1026 incident T2DM patients enrolled in the nationwide Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort. We examined prevalence of elevated ALT (>38 IU/L for women and >50 IU/L for men) and calculated prevalence ratios associated with clinical and lifestyle factors using Poisson regression. We examined the association with other biomarkers by linear regression.

Results The median value of ALT was 24 IU/L (interquartile range: 18–32 IU/L) in women and 30 IU/L (interquartile range: 22–41 IU/L) in men. Elevated ALT was found in 16% of incident T2DM patients. The risk of elevated ALT was increased in patients who were <40 years old at diabetes debut [adjusted prevalence ratio (aPR): 1.96, 95% confidence interval (CI): 1.15–3.33], in those with alcohol overuse (>14/>21 drinks per week for women/men) (aPR: 1.60, 95% CI: 1.03-2.50), and in those with no regular physical activity (aPR: 1.42, 95% CI: 1.04–1.93). Obesity and metabolic syndrome *per se* showed no association with elevated ALT when adjusted for other markers, whereas we found positive associations of ALT with increased C-peptide ($\beta = 0.14$, 95% CI: 0.06–0.21) and fasting blood glucose ($\beta = 0.07$, 95% CI: 0.03–0.11).

Conclusions Among newly diagnosed T2DM patients, several modifiable clinical and lifestyle factors are independent markers of elevated ALT levels.

Keywords type 2 diabetes mellitus; alanine aminotransferase; markers; lifestyle factors; DD2 study; non-alcoholic fatty liver disease

Introduction

Type 2 diabetes mellitus (T2DM) is associated with non-alcoholic fatty liver disease (NAFLD), and up to 50–60% of prevalent T2DM patients may either have or develop NAFLD [1–3]. Metabolic syndrome is a possible link between T2DM

and NAFLD, as several components of metabolic syndrome that are closely related to T2DM [4], such as abdominal obesity, insulin resistance, and dyslipidemia, are also commonly associated with NAFLD [5–8]. T2DM patients with NAFLD may have increased morbidity and mortality. Compared with T2DM patients without NAFLD, Adams *et al*., reported a 2.2-fold [95% confidence interval (CI): 1.1–4.2] higher overall mortality in T2DM patients with NAFLD, with a mean follow-up of 10.9 years [9]. Vice versa, NAFLD patients with T2DM are at higher risk of progression to non-alcoholic steatohepatitis [10], fibrosis, and cirrhosis [11–13] compared with patients without T2DM. Mechanisms behind the adverse outcome of NAFLD in T2DM patients may relate to activation of inflammatory pathways, increased oxidative stress, free fatty acid lipotoxicity, and mitochondrial dysfunction [10,14].

In epidemiological studies of the general population, elevated alanine aminotransferase (ALT) is a commonly used marker of NAFLD, although its sensitivity and specificity are suboptimal [15]. After excluding individuals with chronic viral hepatitis and excessive alcohol consumption, most of the remaining cases with elevated ALT indicate NAFLD [15,16], and ALT remains an early, simple, and non-invasive marker of liver injury in everyday clinical practice [17]. Population-based data on ALT elevation and associated factors including other biomarkers in newly diagnosed T2DM patients are very sparse. In a study from six diabetes care hospitals in Italy, Forlani *et al*. found increased triglyceride level and large waist circumference to be independent predictors of elevated ALT in T2DM patients [odds ratio (OR): 1.57, 95% CI: 1.43–1.84, and OR: 1.47, 95% CI: 1.17–1.85, respectively] [18].

With the increasing worldwide prevalence of diabetes, the burden of NAFLD is expected to increase [19,20]. Knowledge about modifiable clinical and lifestyle factors that are markers of asymptomatic NAFLD in T2DM is crucial for early detection and intervention and thus to improve future prognosis of T2DM patients with NAFLD. Investigation of phenotypes and biomarkers associated with NAFLD may foster our understanding of disease processes leading to NAFLD in T2DM and ultimately form the basis for preventing NAFLD. In the present nationwide study, we aimed to examine the prevalence of elevated ALT in newly diagnosed T2DM patients and the clinical and lifestyle factors associated with such elevation.

Materials and methods

Study design

We conducted this study using information from the Danish Centre for Strategic Research in Type 2 Diabetes (DD2), a nationwide cohort that began enrolling newly diagnosed T2DM patients from general practitioners and hospital specialist outpatient clinics throughout Denmark in November 2010 [21]. The implementation and logistics of the DD2 project have been described in detail recently [22]. In brief, data recorded in the DD2 database include each patient's civil registration number (CPR number), and detailed interview and clinical examination data provided by general practitioners or hospital physicians for each DD2 patient at time of enrolment. Blood samples (fasting) are obtained from each patient, either on the day of the interview or later [23].

We extracted additional clinical data from the Danish Diabetes Database for Adults (DDDA) for a subcohort of DD2 patients included in the DDDA at present [21]. Additionally, a complete hospital contact history of each DD2 participant was obtained by linkage with the Danish National Patient Register (DNPR), covering all Danish hospitals [24]. The DNPR contains information on discharges from all hospitalizations in Danish non-psychiatric hospitals since 1977 and all hospital outpatient and emergency department visits since 1995. It includes data on dates of admission and discharge and up to 20 discharge diagnoses coded by physicians according to the International Classification of Diseases, 8th revision (ICD-8) until 31 December 1993 and the 10th revision (ICD-10) thereafter [25]. Moreover, complete data on filled medication prescriptions for each DD2 participant were obtained from the Danish National Database of Reimbursed Prescriptions [23,26]. The unique CPR number, provided to each Danish resident at birth or upon immigration, made it possible to link data between various health registers [27].

Data on ALT elevation

From the DD2 biobank, we extracted information on ALT levels, measured by the photometric method using the COBAS-6000 analyser produced by Roche Diagnostics GmbH, Mannheim, Germany. The technique to measure ALT was in accordance with the recommendation from European Committee for Clinical Laboratory Standards [28]. The gender-specific upper reference interval (>38 IU/L for women and >50 IU/L for men) corresponded to elevated ALT levels [29].

Data on other biomarkers

From the DD2 biobank, we also extracted information on the following biomarkers: C-reactive protein levels, measured using the particle-enhanced immunoturbidimetric method (Tina-quant C-reactive Protein Gen.3, Roche Diagnostics); amylase levels, measured using an enzymatic colorimetric method (Pancreas α-amylase); C-peptide

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levels, measured using the ADVIA Centaur C-Peptide assay (Siemens Healthcare Diagnostics Ltd, Frimley, Camberley, UK); and fasting blood glucose levels, analysed using a enzymatic hexokinase method (Gluco-quant Glucose/HK, Roche Diagnostics).

Data on demographic, lifestyle, and clinical factors

From the DD2 database, we extracted data on age, gender, waist circumference, and self-reported lifestyle factors including alcohol intake, physical activity, and weight gain since the age of 20 years [22]. We obtained complete data on antihypertensive and insulin treatment from the Danish National Database for Reimbursed Prescriptions. For a subcohort of DD2 patients, we could link additional data from the DDDA on tobacco smoking, body mass index (BMI), blood pressure, glycosylated haemoglobin A_{1c}, plasma lipids, including total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels, as described in detail by Thomsen *et al.* [21]. We identified patients with metabolic syndrome using the International Diabetes Federation criteria for clinical diagnosis of metabolic syndrome, defined as fulfilling three or more of the following criteria: raised fasting glucose (>7 mmol/L); HDL cholesterol <1.0 mmol/L for men and <1.3 mmol/L for women; triglycerides ≥1.7 mmol/L or treatment with fibrates (C10AB) or nicotinic acid (C10AD); central obesity (waist circumference ≥94 cm for men and ≥80 cm for women); and hypertension at enrolment (anti-hypertensive treatment or systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg) [30].

We obtained a complete hospital contact history for all participants' major coexisting diseases as included in the Charlson comorbidity index (CCI) since 1977 using DNPR [31]. The CCI includes 19 different disease categories and takes into account the number and the seriousness of comorbid diseases. The CCI includes the categories mild liver disease (including chronic viral hepatitis, alcoholic or toxic liver disease, NAFLD, fibrosis, and cirrhosis) and moderate to severe liver disease (including acute hepatitis with hepatic coma, hepatic failure, portal hypertension, and oesophageal varices). On the basis of hospital diagnosis codes [32], we computed a CCI score for each person [33], defining three comorbidity levels: low (score of 0), medium (score of 1–2), and high (score of 3+). Diabetes was excluded from the CCI because it constituted the index disease of our study cohort. We separately ascertained any previous diagnoses of acute or chronic viral hepatitis, any mild or severe liver disease in the CCI, cancer, and cardiovascular diseases.

Patient registration and sample collection for the DD2 cohort have been approved by the National Committee on Health Research Ethics (Denmark) (record number S-20100082) and the Danish Data Protection Agency (record number 2008-58-0035). After receiving detailed oral and written information approved by the National Committee on Health Research Ethics (Denmark), patients volunteer to participate in the DD2 study. Participants sign a written informed consent document.

Statistical analysis

We calculated the median ALT value and prevalence of elevated ALT level (>38 IU/L for women and >50 IU/L for men) according to the demographic, clinical, and lifestyle characteristics defined earlier. We then calculated crude and adjusted prevalence ratios (aPRs) with 95% CIs of ALT elevation associated with presence of each factor, using Poisson regression analysis with robust error variance. Three sequential cumulative adjustment models were used to calculate aPRs: in model 1, we adjusted for age and gender; in model 2 we adjusted for age, gender, and central obesity; and in model 3, we adjusted for age, gender, central obesity, comorbidity level, physical activity, and alcohol consumption. Similarly, for the patient subgroup with available DDDA data ($n = 520$), we used three models to calculate aPRs for elevated ALT: model 1 was adjusted for age and gender; model 2 was adjusted for age, gender, and BMI; and model 3 was adjusted for age, gender, BMI, smoking, blood pressure, physical activity, lipid levels, and alcohol consumption.

To examine if there was a linear relation between increasing ALT levels and increasing levels of specific biomarkers, rather than using cut points, we performed linear regression analysis. In all linear regression analyses, normal distribution was approximated by log transforming the variables. We used multiple linear regression analysis to examine the association of each explanatory variable (different biomarkers) with the response variable (ALT level). To examine the effect of different confounders, we used sequential cumulative adjustment models in introducing the confounders. In the first step, we only adjusted for age and gender (model 1); in the second step, we adjusted for age, gender, and waist circumference (model 2); and in the third step, we adjusted for age, gender, waist circumference, C-reactive protein, C-peptide, HDL cholesterol, total cholesterol, triglycerides, and fasting blood glucose (model 3). All analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina).

Results

Serum ALT levels were measured in 1026 newly diagnosed T2DM patients. Of these, 166 (16%) had elevated

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ALT levels (>38 IU/L for women and >50 IU/L for men). The median value of ALT was 24 IU/L (interquartile range 18–32 IU/L) in women and 30 IU/L (interquartile range 22–41 IU/L) in men.

Table 1 shows demographic, clinical, and lifestyle characteristics according to ALT levels, and the corresponding prevalence ratios, crude, and for adjustment models 1–3, with 95% CI. The prevalence of elevated ALT was increased among those who were younger than 60 years at diabetes debut: model 3 aPR: 1.96 (95% CI: 1.15–3.33) in those aged <40 years and 1.68 (95% CI: 1.24–2.26) in those aged 40–59 years, as compared with those aged >60 years at debut. We found a substantially higher prevalence of elevated ALT in patients who reported consumption of more than 14/21 alcoholic drinks per week for women/men (model 3 aPR: 1.60, 95% CI: 1.03–2.50) compared with those who consumed less than the recommended maximal limit. T2DM patients with no regular physical exercise had significantly higher prevalence of elevated ALT (model 3 aPR: 1.42, 95% CI: 1.04–1.93) as compared with patients with regular physical exercise, and importantly, this association was not weakened by adjustment for central obesity, alcohol consumption, and comorbidity (Table 1). Weight gain of more than 30 kg since 20 years of age was associated with elevated ALT in crude analyses (prevalence ratio: 1.10, 95% CI: 0.83–1.46) but lost association after adjustment for age, gender, physical activity, alcohol consumption, and comorbidity (aPR: 0.98, 95% CI: 0.74–1.30). Central obesity *per se* also showed a very modest and non-significant association with ALT elevation (model 3 aPR: 1.21, 95% CI: 0.66–2.24).

Characteristics of the 520 DD2 patients also included in the DDDA database are shown in Table 2. As for central obesity, the association between obesity (BMI > 30) and ALT elevation was weak. A higher prevalence of elevated ALT levels was seen in patients with increased blood pressure (model 3 aPR: 1.38, 95% CI: 0.72–2.64). Patients with the metabolic syndrome did not have elevated ALT in crude analyses (prevalence ratio: 0.86, 95% CI: 0.49–1.50), or when adjusting for other factors (Table 2).

In the linear regression analysis, increasing C-peptide (adjusted β model 3: 0.14, 95% CI: 0.06-0.21) and fasting blood glucose (adjusted β model 3: 0.07, 95% CI: 0.03−0.11) showed a positive association with ALT, after adjusting for age, gender, waist circumference, and all other biomarker levels (Table 3).

Discussion

These early results from the nationwide DD2 study demonstrate that among newly diagnosed T2DM patients, 16% have elevated ALT levels of >38 IU/L for women and >50 IU/L for men. Several important potentially modifiable factors, including alcohol overuse, low physical activity, and high C-peptide and fasting blood glucose, at T2DM debut are associated with elevated ALT.

We found a much higher prevalence of elevated ALT levels (16%) in T2DM patients in our study compared with previously reported prevalences of elevated ALT in the general Danish adult population (i.e. 4.6% in women and 9.8% in men) [34]. The observed prevalence is similar to that reported among prevalent T2DM patients (16%) in Italy [18]. The results of our population-based study partly corroborate findings from the few existing studies on elevated ALT among T2DM patients [7,18,35], showing that poor glucose control and dyslipidemia are markers of elevated ALT levels. Also, in accordance with current knowledge considering NAFLD, a hepatic manifestation of the metabolic syndrome, we found that some metabolic syndrome components, including insulin resistance, increased fasting blood glucose, and high blood pressure, tended to be associated with ALT elevation, whereas MS as a whole was not [36,37]. A recent review identified morbid obesity as a risk factor for NAFLD, in the general population [38], yet in our T2DM population, central obesity *per se* was rather weakly associated with ALT elevation when adjusted for other markers, and obesity *per se* was not associated with ALT. The fact that ALT elevation was common also in the minor group of T2DM patients without obesity or metabolic syndrome may suggest that other factors associated with T2DM *per se* are at play in increasing ALT. Of potential importance for preventive efforts, we found lack of regular physical activity to be clearly associated with increased ALT, even after adjustment for obesity and a range of other factors.

The pathophysiology underlying NAFLD in T2DM patients is not well understood. Recent studies have demonstrated that hepatic and peripheral insulin resistance plays an important role by causing increased production of reactive oxygen species and up-regulation of the pro-inflammatory cascade via lysosomal destabilization [39,40]. These changes ultimately lead to enhanced hepatic gluconeogenesis, inefficient free fatty acid metabolism, and impaired triglyceride transport, and furthermore to hepatic steatosis [41]. On the other hand, fat accumulation in the liver may subsequently decrease insulin sensitivity and increase blood glucose. Hepatic steatosis in turn may lead to steatohepatitis, fibrosis, and cirrhosis through ill-defined pathways. Prospective follow-up studies are needed to improve the understanding of the mechanism and temporal sequence of ALT elevation and NAFLD in T2DM patients in order to improve the management of these patients.

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Table 1. Characteristics of 1026 newly diagnosed type 2 diabetes mellitus patients in the Danish-population-based Danish Centre for Strategic Research in Type 2 Diabetes study and prevalence ratios of elevated serum alanine aminotransferase levels

	ALT levels[a]		Prevalence ratios (95% CI) of elevated ALT level			
	Normal (n = 860)	Elevated (n = 166)	Crude	Model 1[b]	Model 2[b]	Model 3[b]
Gender						
Men	495 (85)	89 (15)	0.87 (0.66-1.16)	0.85 (0.65–1.13)	0.86 (0.65–1.13)	0.80 (0.60–1.05)
Women	365 (83)	77 (17)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Age						
Age <40 years	41 (74)	14 (26)	2.10 (1.27–3.47)	2.10 (1.26–3.49)	2.03 (1.21–3.40)	1.96 (1.15–3.33)
Age 40–59 years	327 (80)	84 (20)	1.68 (1.26–2.26)	1.68 (1.25–2.26)	1.66 (1.24–2.23)	1.68 (1.24–2.26)
Age ≥60 years	492 (88)	68 (12)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Alcohol consumption						
≤14/21 alcoholic drinks/week for women/men	803 (84)	149 (16)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
>14/21 alcoholic drinks/week for women/men	57 (77)	17 (23)	1.47 (0.94–2.28)	1.58 (1.01–2.48)	1.59 (1.01–2.48)	1.60 (1.03–2.50)
Physical activity						
Regular	341 (87)	50 (13)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
None	519 (82)	116 (18)	1.43 (1.05–1.94)	1.48 (1.09–2.00)	1.46 (1.08–1.98)	1.42 (1.04–1.93)
Weight gain since 20 years of age						
<30 kg	522 (84)	96 (16)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
≥30 kg	338 (83)	70 (17)	1.10 (0.83–1.46)	1.01 (0.76–1.34)	1.01 (0.76–1.34)	0.98 (0.74–1.30)
Central obesity[c]						
No	69 (88)	9 (12)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Yes	790 (83)	157 (17)	1.44 (0.76–2.70)	1.30 (0.70–2.41)	1.30 (0.70–2.41)	1.21 (0.66–2.24)
Insulin treatment	50 (71)	20 (29)	1.87 (1.25–2.79)	1.75 (1.17–2.60)	1.76 (1.18–2.61)	1.61 (1.06–2.42)
Antihypertensive treatment	612 (85)	110 (15)	0.83 (0.62–1.11)	1.00 (0.73–1.38)	0.98 (0.71–1.36)	0.93 (0.67–1.28)
CCI score[d] of 0	591 (84)	112 (16)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
CCI score[d] of 1–2	224 (84)	43 (16)	1.01 (0.73–1.40)	1.17 (0.84–1.62)	1.16 (0.83–1.61)	1.14 (0.82–1.59)
CCI score[d] of 3+	45 (80)	11 (20)	1.23 (0.71–2.15)	1.44 (0.83–2.51)	1.44 (0.83–2.51)	1.42 (0.80–2.52)
Any previous cancer	60 (84)	11 (16)	0.96 (0.54–1.67)	1.16 (0.66–2.02)	1.16 (0.67–2.02)	0.91 (0.47–1.79)
Any previous cardiovascular disease	172 (86)	29 (14)	0.87 (0.60–1.26)	1.07 (0.73–1.57)	1.06 (0.72–1.55)	1.07 (0.73–1.58)
Previous viral hepatitis	0 (0)	1 (100)	—	—	—	—
Any previous mild liver disease	6 (54)	5 (46)	2.86 (1.47–5.56)	2.62 (1.42–4.84)	2.65 (1.46–4.82)	2.21 (1.13–4.32)
Any previous severe liver disease	2 (100)	0 (0)	—	—	—	—

ALT, alanine aminotransferase; CI, confidence interval; CCI, Charlson comorbidity index.
[a]ALT levels: normal, ≤38 IU/L (women) and ≤50 IU/L (men); elevated, >38 IU/L (women) and >50 IU/L (men).
[b]Model 1 adjusted for age and gender; model 2 adjusted for age, gender, and central obesity ('weight gain since 20 years of age' was not adjusted for central obesity); and model 3 adjusted for age, gender, central obesity ('weight gain since 20 years of age' was not adjusted for central obesity), comorbidity, physical activity, and alcohol consumption (except when stratified by the variable itself).
[c]Central obesity = waist circumference >94 (men) and >80 (women).
[d]CCI score was calculated without diabetes.

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Table 2. Characteristics of 520 newly diagnosed type 2 diabetes mellitus patients in the Danish Diabetes Database for Adults dataset and prevalence ratios of elevated serum alanine aminotransferase levels

	ALT levels[a]		Prevalence ratio (95% CI) of elevated[a] ALT level			
	Normal ($n = 424$)	Elevated ($n = 96$)	Crude	Model 1[b]	Model 2[b]	Model 3[b]
Smoking (daily or occasionally)						
No	308 (82)	69 (18)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Yes	87 (82)	19 (18)	0.98 (0.62–1.55)	0.95 (0.60–1.48)	0.96 (0.60–1.52)	0.83 (0.47–1.46)
BMI						
$\leq$30 kg/m^2	156 (82)	35 (18)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
$>$30 kg/m^2	191 (76)	48 (20)	1.10 (0.74–1.62)	0.95 (0.64–1.40)	0.95 (0.64–1.40)	1.03 (0.68–1.57)
Blood pressure						
Systolic BP $<$130 or diastolic BP $<$80	73 (84)	14 (16)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Systolic BP 130+ or diastolic BP 80+	320 (80)	91 (20)	1.22 (0.72–2.05)	1.22 (0.72–2.05)	1.39 (0.77–2.50)	1.38 (0.72–2.64)
Metabolic syndrome[c]						
No	41 (79)	11 (21)	Ref (1.00)	Ref (1.00)	Ref (1.00)	Ref (1.00)
Yes	383 (82)	85 (18)	0.86 (0.49–1.50)	0.90 (0.51–1.58)	0.74 (0.39–1.40)	0.82 (0.46–1.45)

ALT, alanine aminotransferase; CI, confidence interval; BMI, body mass index; BP, blood pressure.

[a]ALT levels: normal, $\leq$38 IU/L (women) and $\leq$50 IU/L (men); elevated, $>$38 IU/L (women) and $>$50 IU/L (men).

[b]Model 1 adjusted for age and gender; model 2 adjusted for age, gender, and BMI (metabolic syndrome is not adjusted for BMI); and model 3 adjusted for age, gender, smoking, BMI, blood pressure, physical activity, lipid levels, and alcohol consumption (except when stratified by variable itself, and Metabolic syndrome is not adjusted for BMI, blood pressure, and lipid levels).

[c]Metabolic syndrome was defined as fulfilling three or more of the following criteria: raised fasting glucose ($>$7 mmol/L); HDL cholesterol $<$1.0 mmol/L for men and $<$1.3 mmol/L for women; triglycerides $\geq$1.7 mmol/L or treatment with fibrates or nicotinic acid; central obesity (waist circumference $\geq$94 cm for men and $\geq$80 cm for women); and hypertension. Metabolic syndrome not adjusted for BMI in model 2 and blood pressure, BMI, and lipid levels in model 3 because these are part of the syndrome.

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Table 3. Linear regression analysis of biomarkers associated with log10 alanine aminotransferase levels in newly diagnosed type 2 diabetes mellitus patients in the Danish-population-based Danish Centre for Strategic Research in Type 2 Diabetes study

	β coefficient and 95% confidence interval of log10 (ALT)			
	Crude	Model 1[a]	Model 2[a]	Model 3[a]
Log10 (CRP)	0.126 (−0.012 to 0.264)	0.131 (−0.013 to 0.274)	−0.019 (−0.155 to 0.117)	−0.048 (−0.246 to 0.149)
Log10 (amylase)	−0.031 (−0.098 to 0.036)	−0.24 (−0.094 to 0.047)	−0.009 (−0.081 to 0.062)	−0.021 (−0.121 to 0.080)
Log10 (C-peptide)	0.226 (0.171–0.282)	0.247 (0.188–0.305)	0.166 (0.114–0.219)	0.136 (0.061–0.210)
Log10 (fasting blood glucose)	0.093 (0.069–0.117)	0.077 (0.052–0.101)	0.071 (0.046–0.096)	0.071 (0.032–0.110)
Log10 (HbA_{1c})	0.032 (0.004–0.061)	0.022 (−0.007 to 0.052)	0.020 (−0.10 to 0.049)	0.004 (−0.025 to 0.034)
Log10 (total cholesterol)	0.042 (0.002–0.081)	0.049 (0.008–0.090)	0.048 (0.007–0.090)	0.022 (−0.017 to 0.062)
Log10 (HDL cholesterol)	−0.050 (−0.096 to 0.004)	0.015 (−0.030 to 0.060)	0.030 (−0.014 to 0.074)	0.036 (−0.004 to 0.077)
Log10 (triglycerides)	0.134 (0.039–0.230)	0.087 (−0.011 to 0.186)	0.050 (−0.047 to 0.146)	−0.041 (−0.125 to 0.043)

ALT, alanine aminotransferase; CRP, C-reactive protein; HbA_{1c}, haemoglobin A_{1c}; HDL, high-density lipoprotein.
[a]Model 1 adjusted for age and gender; model 2 adjusted for age, gender, and waist circumference; model 3 adjusted for age, gender, waist circumference, CRP, C-peptide, HDL cholesterol, total cholesterol, triglycerides, and fasting blood glucose (except when stratified by the variable itself).

The DD2 study cohort will be a valuable resource for such follow-up studies in the future.

The main strength of our study is its comprehensive and detailed assessment of lifestyle, clinical, and biomarkers based on the DD2 database and biobank, with close to 100% completeness for demographic and clinical characteristics [23]. Linkage with other population-based health registries provided detailed clinical information on patients with T2DM. ALT may be regarded a suboptimal marker for liver disease, yet it has been demonstrated that ALT has high sensitivity (62.5%) in predicting steatosis (liver fat deposits, a surrogate marker for early NAFLD), compared with aspartate transaminase (18%) and gamma glutamyl transferase (20%) [42].

This study also has some limitations. The DD2 project is still in its initial phase, and the current cohort likely represents patients whose newly diagnosed T2DM is more advanced than average in Denmark, as initial enrolment has mainly relied on hospital outpatient clinics [23]. Also, not all DD2 patients can currently be linked to the DDDA database for additional data, because of delayed enrolment into this quality database [21]. Finally, the cross-sectional design leads to some uncertainty as to whether elevated ALT preceded or followed some of the clinical and metabolic factors that we examined in this study.

In conclusion, 20% of newly diagnosed T2DM patients in Denmark have elevated ALT levels indicating NAFLD. In T2DM patients, several potentially modifiable clinical and lifestyle factors, including high alcohol consumption, lack of regular physical exercise, and high C-peptide and fasting blood glucose levels, are associated with elevated ALT. These findings among newly diagnosed T2DM patients may aid early identification of patients with high risk of NAFLD, who may benefit from lifestyle and pharmacological interventions to prevent later liver and cardiovascular diseases.

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Conflicts of interest

The authors have no conflicts of interest.

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